Weed Technology

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Annual bluegrass cross resistance to prodiamine and pronamide in the southern United States

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Research Article

Cite this article: Osburn AW, Bowling RG, Unruh JB, McKeithen C, Hathcoat D, Bagavathiannan M (2024) Annual bluegrass cross resistance to prodiamine and pronamide in the southern United States. Weed Technol. **38**(e76), 1–9. doi: 10.1017/wet.2024.43

Received: 8 October 2023
Revised: 28 April 2024
Accepted: 29 May 2024

Associate Editor:
Barry Brecke, University of Florida

Nomenclature:
Indaziflam; prodiamine; pronamide; annual bluegrass, *Poa annua* L.

Keywords:
Turf weed; microtubule inhibitor; herbicide resistance; dose-response; integrated weed management

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Abstract

Annual bluegrass is one of the most problematic weeds in the turfgrass industry, exhibiting both cross-resistance and multiple-herbicide resistance. Prodiamine, pronamide, and indaziflam are commonly used preemergence herbicides for the control of this species on golf courses in the southern United States. There have been increasing anecdotal reports of annual bluegrass populations escaping control with these herbicides, but resistance has yet to be confirmed. To evaluate the response of annual bluegrass to three herbicides, populations were collected from golf courses, athletic fields, and landscape areas in Texas and Florida, and a dose-response assay was conducted on populations that were suspected to be resistant to and known to be susceptible to prodiamine, pronamide, and indaziflam. The suspected-resistant populations showed survival to prodiamine at 32 times the recommended field rate (both populations from Florida and Texas) of 736 g ai ha^{-1}, and to pronamide at 32 times (the Florida populations) or 16 times (the Texas populations) the recommended field rate of 1,156 g ha^{-1}. In contrast, the known susceptible populations attained 100% mortality at rates as low as 46 and 578 g ha^{-1}, respectively, from applications of prodiamine and pronamide. For indaziflam, the suspected-resistant populations showed reduced sensitivity up to the recommended field rate of 55 g ha^{-1}, but they were controlled when treated with a rate twice that of the field rate. Overall, annual bluegrass populations with resistance to prodiamine and pronamide, and reduced sensitivity to indaziflam (at the recommended field rate) were confirmed from golf courses in Florida and Texas. In the presence of herbicide-resistant annual bluegrass populations, especially to commonly used herbicides such as prodiamine and pronamide, turfgrass managers should adopt integrated management strategies and frequently rotate herbicide sites of action, rather than relying solely on microtubule-assembly inhibitors or cellulose biosynthesis inhibitors, to control this species.

Introduction

Annual bluegrass (*Poa annua* L.) is an invasive and problematic weed in turfgrass systems worldwide (Christians 2006; Tutin 1957). This species is highly pervasive and has successfully colonized all seven continents, including the maritime regions around Antarctica (Molina-Montenegro et al. 2012). A recent survey revealed that *Poa* species (including annual bluegrass) are the most troublesome weeds in turfgrass systems; however, this weed has been difficult to control for decades (Van Wychen 2020). Although annual bluegrass occurs worldwide, it is especially ubiquitous and difficult to control in warm-season turfgrass systems in the southern United States due to its high seed production and destructive growth habit (Goss and Zook 1971). Specifically, annual bluegrass produces unsightly seed heads, reduces the aesthetic value of turfgrass, negatively affects the quality of playing surfaces, and leaves behind bare patches in the summer months (Bingham et al. 1969; Hall and Carey 1992).

To control annual bluegrass infestations and prevent these issues, turfgrass managers have commonly applied preemergence herbicides that include microtubule assembly inhibitors and cellulose biosynthesis inhibitors. The frequent use of herbicides with little to no rotation of chemistry can lead to rapid development of herbicide resistance (Brosnan et al. 2020a; Holt and LeBaron 1990; Manalil et al. 2011). Because annual bluegrass predominately exhibits a winter annual life cycle in the southern United States due to its high sensitivity to heat, drought, and disease pressure (Cordukes 1977; Inguagiato et al. 2009; Slavens et al. 2011; Smiley et al. 2005; Walsh et al. 1999), preemergence herbicides are commonly applied to golf courses in the fall to prevent seedling emergence. Postemergence herbicides are subsequently applied on an as-needed basis to control any seedlings that escape preemergence herbicide applications.

Prodiamine, pronamide, and indaziflam are herbicides used on warm-season turfgrass found within agroclimatic zones 9a and 9b (USDA-ARS 2023), especially on the golf courses in these zones.

Prodiamine and pronamide are both microtubule-assembly inhibitors and are classified by the Weed Science Society of America (WSSA) as Group 3 herbicides, belonging to the dinitroaniline and benzamide chemical families, respectively (Shaner 2014a). Prodiamine prevents cell division in the roots and shoots of recently germinated grasses and small-seeded broadleaf weeds and has no postemergence activity (McElroy and Martins 2013). Whereas pronamide affects plants similarly to prodiamine, it is in the benzamide chemical family, exhibits postemergence activity, and has been used to control weeds (including annual bluegrass) that have become resistant to dinitroaniline herbicides (Delyé et al. 2004; Isgrigg et al. 2002; Vaughn et al. 1987). Indaziflam is a cellulose biosynthesis inhibitor (WSSA Group 29) in the alkylazine chemical family (Shaner 2014a). Indaziflam is a unique herbicide due to its low use rate compared with other preemergence herbicides and has a dual effect as a preemergence and early postemergence herbicide on annual grassy weeds in warm-season turfgrasses (McElroy and Martins 2013). Additionally, indaziflam can control annual bluegrass, which has been documented to exhibit resistance to prodiamine (Brosnan et al. 2014). The frequent use of an herbicide and the overreliance on a single herbicide site of action (SOA) can result in the evolution of reduced sensitivity or resistance in weed species (Shaner 2014b).

Annual bluegrass is one of two species, the other being rigid ryegrass (*Lolium rigidum* Gaud.), that has evolved resistance to as many as 12 unique herbicide SOAs (Heap 2023). The first confirmed case of herbicide resistance in annual bluegrass was documented in France in 1978, to atrazine, a photosystem II (PS II) inhibitor (WSSA Group 5). In that case, populations from corn fields and orchards were found to be 6-fold less sensitive to the herbicide (De Prado and Menéndez 1996). The first report of an herbicide-resistant annual bluegrass population in a turfgrass setting was confirmed in Japan in 1982, in a population collected from a golf course that had been treated with simazine, which is also a PS II-inhibiting herbicide (Kumata et al. 2001). In 1998, the first case of resistance to pendimethalin and prodiamine (WSSA Group 3, dinitroanilines) was documented in an annual bluegrass population collected from a golf course in North Carolina (Isgrigg et al. 2002). Pronamide resistance by annual bluegrass was documented for the first time in a golf course population collected in Georgia, in 2016, although resistance was confirmed for the postemergence application, and not when the herbicide was applied as a preemergence control option (McCullough et al. 2016). Many instances of annual bluegrass populations developing multiple- and cross-resistances to herbicide SOAs have been reported, including a seven-way resistance in Georgia (Brosnan et al. 2017, 2020b), five-way resistance found in Victoria, New South Wales, and Southern Australia provinces in Australia (Barua et al. 2020), and three-way resistance in Texas (Singh et al. 2021). Indaziflam resistance was first documented in 2019 in Tennessee, and is also the first documented report of evolved indaziflam resistance found by any plant to date (Brosnan et al. 2020b).

Although preemergence herbicides are commonly used on turfgrass, little research has occurred to determine whether annual bluegrass populations in Texas and Florida express resistance (including cross-resistance) to prodiamine and pronamide or multiple resistance to Group 3 and Group 29 herbicides. If annual bluegrass were to evolve cross-resistance to pronamide and prodiamine, fewer preemergence options would be available for control, especially on golf course putting greens, for which very few preemergence herbicides are labeled for use. The development of multiple SOA resistances for Group 3 and Group 29 herbicides would eliminate many of the commonly used preemergence options available for annual bluegrass control on turfgrass. Documenting herbicide-resistant annual bluegrass populations in new areas could further demonstrate to end users the need to adopt integrated pest management tactics and implement more robust herbicide programs that include SOA rotation.

The objectives of this research were to 1) survey annual bluegrass populations from four major turfgrass systems (golf courses, athletic fields, sod production facilities, and public lawn care areas) from Florida and Texas for preemergence herbicide resistance to three commonly used herbicides, prodiamine, pronamide, and indaziflam; and 2) determine the level of preemergence herbicide resistance by annual bluegrass populations exhibiting cross-resistance or multiple resistance to prodiamine, pronamide, and indaziflam.

Materials and Methods

Plant Material

Annual bluegrass plants from Texas and Florida were collected between fall 2019 and spring 2020 from four major turfgrass systems: golf courses, sod production farms, athletic fields, and public lawn areas (i.e., city parks and public green spaces). Sites were selected for this survey in one of two ways: Either turfgrass managers were contacted directly and asked whether difficult-to-control annual bluegrass populations were present on their property; or 2) public sites were randomly selected and scouted for annual bluegrass populations. Surveys of annual bluegrass populations followed the methodology described by Rutland et al. (2023). In Texas, populations were primarily collected from turfgrass systems around the greater Houston metropolitan area due to southern Texas being within agroclimatic zone 9a, while in Florida, sample populations were collected in a state-wide survey since most of the state includes zones 9a and 9b. In each collection site, approximately 15 to 20 individual annual bluegrass plants were carefully uprooted from a discrete area (e.g., roughs, fairways, collar regions, baseball infields). Golf course putting greens were excluded from sampling in this study primarily to avoid destruction to high-value surfaces. A total of 46 populations (23 from Florida and 23 from Texas) were selected for this evaluation from the various turfgrass systems. Samples were consolidated into a labeled 3.79-L plastic bag and placed on ice for transport to the greenhouse facilities at Texas A&M University, in College Station. Annual bluegrass populations were tiller-propagated into individual cone-tainers filled with potting soil (Pro-Mix LP15; Premier Tech Horticulture, Quakertown, PA) and maintained under greenhouse conditions (28/22 C with a 16/8-h photoperiod) until maturity and senescence. Populations were spaced in the greenhouse such that cross-pollination among the populations was avoided as much as possible. Mature seed was harvested from each plant, pooled among the individuals within a population, and dried in an oven at 50 C for 48 h. To break dormancy, seeds were subjected to a cold treatment (−20 C) for 3 wk and then kept at room temperature before being used in the preliminary screening evaluation, which was then followed by dose-response assays.

https://doi.org/10.1017/wet.2024.43 Published online by Cambridge University Press

Table 1. Herbicides and their recommended field rate used for herbicide screening and dose-response assays of annual bluegrass from Texas and Florida turfgrass systems.[a]

No.	Herbicide common name	Trade name	WSSA Group	Chemical family	1× Rate	Manufacturer
					g ai ha^{-1}	
1	Prodiamine	Barricade 4FL	3	Dinitroaniline	736	Syngenta Crop Protection, Greensboro, NC
2	Pronamide	Kerb	3	Benzamide	1,156	Corteva AgroSciences LLC, Indianapolis, IN
3	Indaziflam	Specticle FLO	29	Alkylazine	55	Bayer CropScience, Research Triangle Park, NC

[a]Abbreviation: WSSA, Weed Science Society of America.

Preliminary Herbicide Screening

In spring 2021, native soil (Boonville fine sandy loam, a fine, smectitic, thermic Chromic Vertic Albaqualf) was collected from a turfgrass field located in College Station, Texas. The soil was mixed with calcined clay (Turface Athletics, Buffalo Grove, IL) at a 3:2 (native soil: calcined clay) ratio to prevent cracks and hardening of native soil between watering cycles. The pot size was 8.89 cm diam × 8.26 cm tall.

The experiment was conducted following a randomized complete block design with four replications (one pot per replication) for each treatment. Each replication contained one of each population for each of the four treatments: a label-recommended (1×) field rate of prodiamine, pronamide, and indaziflam, and a nontreated control (Table 1). In each pot, 15 seeds were hand-sown, lightly covered with the soil mixture, and sprayed immediately with the respective herbicide treatment. The applications were made using a DeVries track sprayer (DeVries Manufacturing, Inc., Hollandale, MN) mounted with a TeeJet XR80015 nozzle, calibrated to deliver 140.3 L ha^{-1} at 4.83 kph with 276 kPa. Treated pots were then placed back into the greenhouse and lightly watered to a depth of 1.5 cm to ensure proper activation of each herbicide. The pots were irrigated as necessary throughout the experiment. Seedling emergence was recorded at 7, 14, and 21 d after treatment (DAT). Plants that survived the preliminary screening were grown under greenhouse conditions and seeds were collected and pooled per population once senescence occurred. Seed pooling among the plants within each population was necessary to collect enough seeds to conduct the dose-response assay.

Dose-Response Assay

Dose-response assays were conducted for each herbicide for the population with the highest survival rate identified in the preliminary screening and, for those that had sufficient seed availability (Tables 2 and 3). The selected populations were TX-05-GC-15 (survived all three herbicides), FL-05-GC-20 (survived applications of prodiamine and pronamide), and FL-05-GC-14 (survived an application of indaziflam); known susceptible populations from Texas (TX-05-LC-Cemetary; susceptible to all three herbicides) and Florida (FL-05-AF-16, and FL-05-GC-15; susceptible to prodiamine/pronamide, and susceptible to indaziflam, respectively) were used for comparison purposes (Tables 2 and 3). The progeny from FL-05-GC-20 and FL-05-GC-14 used in the dose-response assay will be referred to as FL-20-1-R and FL-20-3-R, respectively, while the progeny from FL-05-AF-16 and FL-05-GC-15 will be referred to as FL-20-2-S and FL-20-4-S, respectively. The progeny from TX-05-GC-15 used in the dose-response assay will be referred to as TX-20-1-R and the progeny from TX-05-LC-Cemetary will be referred to as TX-20-2-S.

Seven doses of each herbicide were used for the putative resistant (0.5, 1, 2, 4, 8, 16, and 32× the recommended label rate) and susceptible (0.03125, 0.0625, 0.0125, 0.25, 0.5, 1, and 2×) populations. The treatments were arranged in a randomized complete block design with four replications (one pot per replication) and repeated twice in time. The pot size and potting mix used were the same as those used in the preliminary screening. A total of 10 seeds were planted in each pot and sprayed immediately with the respective herbicide using a track sprayer described above. Treated pots were placed into the greenhouse and watered to activate the herbicides. All treatments received regular watering to provide adequate growing conditions. A nontreated standard for each population was used to compare differences in seedling emergence in the absence of the herbicide. Seedling emergence was recorded 7, 14, 21, and 28 DAT. Herbicide resistance was confirmed based on the R/S (LD_{50}) ratio between the putative resistant (R) and susceptible (S) populations (Burgos et al. 2013).

Statistical Analysis

An ANOVA for herbicide screening and dose-response data was conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Because there were no treatment-by-run interactions, data were pooled across the two experimental runs for final analysis.

For the dose-response study, annual bluegrass survival was regressed against herbicide dose using a three-parameter logistic regression equation in SigmaPlot (version 14; Systat Software, Inc., San Jose, CA) that provided the best fit to the data:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \quad [1]$$

where LD_{50} is the dose that caused a 50% reduction in seeding emergence.

R/S ratios were calculated by dividing the LD_{50} of the putative-resistant population by the LD_{50} of the known susceptible population for each herbicide treatment.

Results and Discussion

Prescreen Evaluation

Of the initial 23 populations from Florida treated with a recommended field rate of prodiamine, pronamide, and indaziflam, 87% survived at least one of the treatments. Survival was defined by a treated plant emerging through the herbicide treatment, reaching maturity, and producing seed (Tables 2 and 3). Eight populations (35%) exhibited the potential for cross-resistance to prodiamine and pronamide, whereas 10 populations (43%) exhibited the potential for multiple resistance to Group 3 and Group 29 herbicides. Due to seed limitations, FL-05-GC-24 was not selected for the dose-response assay despite exhibiting the

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Table 2. Florida annual bluegrass populations from three major turfgrass systems and the survival rate when treated with the recommended 1× field rate of prodiamine, pronamide, and indaziflam.[a]

No.	Turf system	Sample ID	Progeny ID[b]	Survival		
				%		
				Prodiamine	Pronamide	Indaziflam
1	Athletic field	FL-05-AF-03&GC-12		6.25	3.13	0.00
2	Athletic field	FL-05-AF-04		0.00	2.17	0.00
3	Athletic field	FL-05-AF-11		0.00	0.00	0.00
4	Athletic field	FL-05-AF-14		0.00	2.70	0.00
5	Athletic field	FL-05-AF-16[c]	FL-20-2-S	0.00	0.00	2.04
6	Athletic field	FL-05-AF-17		0.00	0.00	3.85
7	Athletic field	FL-05-AF-19		0.00	0.00	8.57
8	Athletic field	FL-05-AF-20		0.00	2.22	4.44
9	Golf course	FL-05-GC-11		15.79	7.89	7.89
10	Golf course	FL-05-GC-13		0.00	2.86	11.43
11	Golf course	FL-05-GC-14[c]	FL-20-3-R	9.38	0.00	25.00
12	Golf course	FL-05-GC-15[c]	FL-20-4-S	13.36	0.00	0.00
13	Golf course	FL-05-GC-19		35.90	0.00	0.00
14	Golf course	FL-05-GC-20[c]	FL-20-1-R	100.00	13.04	0.00
15	Golf course	FL-05-GC-22		11.11	2.78	8.33
16	Golf course	FL-05-GC-24		11.63	6.98	13.95
17	Golf course	FL-05-GC-26		18.92	5.41	10.81
18	Golf course	FL-05-GC-27		22.73	2.27	0.00
19	Golf course	FL-05-GC-29		8.89	2.22	4.44
20	Golf course	FL-05-GC-30		13.16	0.00	18.42
21	Golf course	FL-05-GC-31		0.00	0.00	0.00
22	Lawn care operation	FL-05-LC-05		0.00	3.13	3.13
23	Lawn care operation	FL-05-LC-07		0.00	0.00	0.00

[a]The 1× field rates are as follows: prodiamine, 736 g ai ha^{-1}; pronamide, 1,156 g ha^{-1}; and indaziflam, 55 g ha^{-1}.
[b]Populations that were selected for advancement to the dose-response assay.
[c]Progeny used for dose-response assay and characterization of herbicide sensitivity.

Table 3. Texas annual bluegrass populations from four major turfgrass systems and the survival rate when treated with the recommended 1× field rate of prodiamine, pronamide, and indaziflam.[a]

No.	Turf system	Sample ID	Progeny ID[b]	Survival		
				%		
				Prodiamine	Pronamide	Indaziflam
1	Athletic field	TX-04-AF-2		0.00	0.00	0.00
2	Athletic field	TX-04-AF-5		0.00	0.00	0.00
3	Golf course	TX-04-GC-5		7.69	2.56	7.69
4	Lawn care operation	TX-04-LC-1		0.00	2.17	2.17
5	Lawn care operation	TX-04-LC-2		0.00	0.00	0.00
6	Sod Farm	TX-04-SP-6		0.00	7.69	0.00
7	Athletic field	TX-05-AF-4		0.00	0.00	21.28
8	Athletic field	TX-05-AF-5		0.00	0.00	0.00
9	Athletic field	TX-05-AF-cricket field		0.00	0.00	0.00
10	Golf course	TX-05-GC-13		4.76	2.38	4.76
11	Golf course	TX-05-GC-15[c]	TX-20-1-R	23.53	23.53	35.29
12	Golf course	TX-05-GC-sand hill		16.67	0.00	13.89
13	Lawn care operation	TX-05-LC-1		0.00	6.82	0.00
14	Lawn care operation	TX-05-LC-2		0.00	6.67	4.44
15	Lawn care operation	TX-05-LC-cemetary[c]	TX-20-2-S	0.00	4.08	0.00
16	Lawn care operation	TX-05-LC-5		0.00	0.00	5.41
17	Lawn care operation	TX-05-LC-10		0.00	0.00	12.50
18	Lawn care operation	TX-05-LC-Hempstead Park		0.00	0.00	0.00
19	Sod Farm	TX-05-SP-26		0.00	0.00	0.00
20	Sod Farm	TX-05-SP-20		0.00	0.00	1.59
21	Sod Farm	TX-05-SP-19		0.00	9.38	0.00
22	Sod Farm	TX-05-SP-18		0.00	13.79	10.34
23	Sod Farm	TX-05-SP-17		0.00	8.00	0.00

[a]The 1× field rates are as follows: prodiamine, 736 g ai ha^{-1}; pronamide, 1,156 g ha^{-1}; and indaziflam, 55 g ha^{-1}.
[b]Progeny used for dose-response assay and characterization of herbicide sensitivity.
[c]Populations that were selected for advancement to the dose-response assay.

greatest frequency of survival to all three herbicides. Thus, progeny from FL-05-GC-20 and FL-05-GC-14 were advanced for the dose-response assay due to the greatest likelihood of cross-resistance to prodiamine and pronamide and resistance to indaziflam, respectively (Table 2). Known susceptible populations were determined based on their lack of survival when treated with each herbicide. Progeny from FL-05-AF-16 was advanced as the known susceptible for prodiamine and pronamide and progeny

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from FL-05-GC-15 were advanced as the known susceptible for indaziflam (Table 2).

Among the Texas populations screened with the recommended field rate of prodiamine, pronamide, and indaziflam, 70% survived at least one of the treatments with a 2% or greater survival rate (Table 3). Three populations (13%) exhibited the potential to be cross-resistant to prodiamine and pronamide, while seven populations (30%) exhibited the potential for multiple resistance to the SOAs of herbicides in WSSA Groups 3 and 29. One population (TX-05-GC-15) exhibited the potential to be resistant to all three herbicides and had sufficient seed to advance the progeny for the dose-response assay (Table 3). A known susceptible population (TX-05-LC-Cemetary) was identified based on the lack of survival when treated with each herbicide, and progeny from this population was advanced to the dose-response assay (Table 3). Although other populations exhibited zero survival to all three herbicides, there was insufficient seed for further advancement.

Dose-Response Assay

Prodiamine

Putative-resistant annual bluegrass populations from Texas (TX-20-1-R) and Florida (FL-20-1-R) exhibited survival up to 32× the recommended field rate (23,537 g ha^{-1}) of prodiamine (Figures 1 and 2). Known susceptible populations from Texas (TX-20-2-S) and Florida (FL-20-2-S) were completely controlled at the 0.5× rate of prodiamine (368 g ha^{-1}). Dose-response curves (Figure 3) estimated the LD_{50} values for both the resistant and susceptible populations. Comparing the resistant annual bluegrass populations to the susceptible populations (R/S ratio based on LD_{50}), TX-20-1-R and FL-20-1-R were 9,587-fold and 101-fold less sensitive to prodiamine, respectively (Table 4).

Pronamide

Both known susceptible populations (TX-20-2-S and FL-20-2-S) were completely controlled at the recommended field rate of pronamide and exhibited a >95% reduction in survival to the 0.5× rate of the herbicide (578 g ha^{-1}). The putative-resistant population from Florida (FL-20-1-R) exhibited survival up to 32× (36,988 g ha^{-1}) the application rate of pronamide, while the putative-resistant population from Texas (TX-20-1-R) exhibited survival up to the 16× rate (18,494 g ha^{-1}) of the herbicide (Figures 1 and 2). Dose-response curves (Figure 4) estimated the LD_{50} of TX-20-1-R. However, due to low survivorship (<7% across all treatments), a dose-response curve could not be fit for FL-20-1-R, and LD_{50} values could not be determined. The R/S ratio (based on LD_{50}) for TX-20-1-R indicated that this population was 6-fold less sensitive to pronamide compared with the known susceptible population (Table 4).

Indaziflam

The putative-resistant and known susceptible populations exhibited similar levels of survival to indaziflam at the various treatment rates. When treated with a 2× rate (109 g ha^{-1}) of indaziflam, both the Florida putative resistant and known susceptible populations survived, albeit at low numbers, 5% and 2%, respectively (Figure 1). The putative-resistant population from Texas exhibited survivorship of up to 25% at the recommended field rate of indaziflam, whereas the known susceptible population was completely controlled at the same rate (Figure 2). While the known susceptible populations from both Texas and Florida exhibited survival up to two times the recommended field rate of indaziflam, dose-response curves (Figure 5) were able to determine the LD_{50} rates for each population. The R/S ratios demonstrated that FL-20-3-R was 9-fold less sensitive to indaziflam, whereas TX-20-1-R was 5-fold less sensitive to this herbicide.

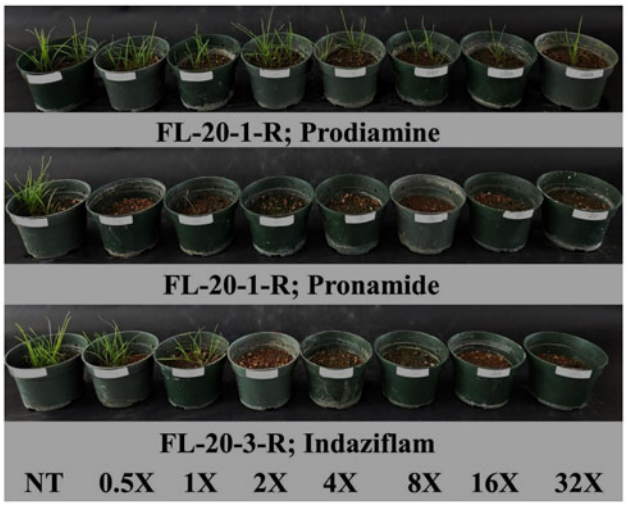

Figure 1. Response of annual bluegrass populations collected from Florida (FL-20-1-R and FL-20-2-R) when treated with seven rates of prodiamine (1× = 736 g ai ha^{-1}), pronamide (1× = 1156 g ha^{-1}), or indaziflam (1× = 55 g ha^{-1}). NT indicates nontreated samples. Photographs were taken 28 d after application.

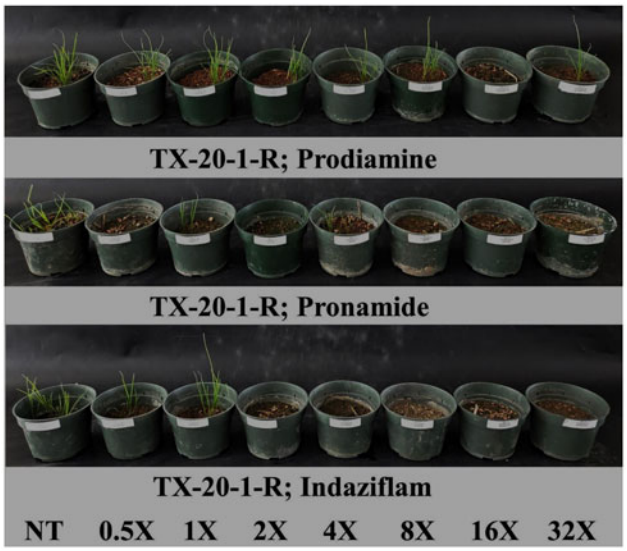

Figure 2. Response of annual bluegrass population collected from Texas (TX-20-1-R) when treated with seven rates of prodiamine (1× = 736 g ai ha^{-1}), pronamide (1× = 1156 g ha^{-1}), and indaziflam (1× = 55 g ha^{-1}). NT indicates nontreated samples. Photographs were taken 28 d after application.

To date, there have been multiple reports from around the world of cross-resistant and multiple-resistant populations of annual bluegrass to various SOAs. These reports come mostly from Australia and the United States where annual bluegrass is viewed as a problematic weed species (Barua et al. 2020; Breeden et al. 2017; Brosnan et al. 2015, 2020b; Hanson and Mallory-Smith 2000; Singh et al. 2021). These populations have shown resistance to acetolactate synthase (ALS) inhibitors (WSSA Group 2), PS II inhibitors, enolpyruvyl shikimate-3-phospate synthase (EPSPS)

https://doi.org/10.1017/wet.2024.43 Published online by Cambridge University Press

Table 4. Results of dose-response analysis of selected annual bluegrass populations collected in Texas and Florida in Spring 2020 when treated with prodiamine, pronamide, and indaziflam.

No.	Progeny ID[a]	Herbicide	Log-logistic regression equation	RMS value[b]	LD_{50}[c]	R/S ratio[d]
1	TX-20-1-R	Prodiamine	Y = 100/[1+(x-650.65)exp0.78]	40.11	650.65	9,587.02
2	TX-20-1-R	Pronamide	Y = 100/[1+(x-144.55)exp0.62]	46.73	144.55	6.05
3	TX-20-1-R	Indaziflam	Y = 100/[1+(x-12.75)exp1.2]	28.61	12.37	5.24
4	FL-20-1-R	Prodiamine	Y = 100/[1+(x-605.51)exp0.70]	13.63	605.51	101.09
5	FL-20-3-R	Indaziflam	Y = 100/[1+(x-32.59)exp3.20]	1.74	32.59	9.13

[a]Progeny used for the dose-response assay from the populations identified during the prescreen evaluation. The dose-response curve for the putative pronamide-resistant population from Florida exhibited high survival rates even at the highest dose tested; thus, a curve could not be fit.
[b]RMS indicates the residual means square value for the fitted model.
[c]LD_{50} value indicates the amount of herbicide active ingredient (g ai ha^{-1}) required to cause 50% reduced survival in the suspected resistant population.
[d]R/S ratio indicates the ratio of LD_{50} value of the suspected resistant population divided by the LD_{50} value of the known susceptible population.

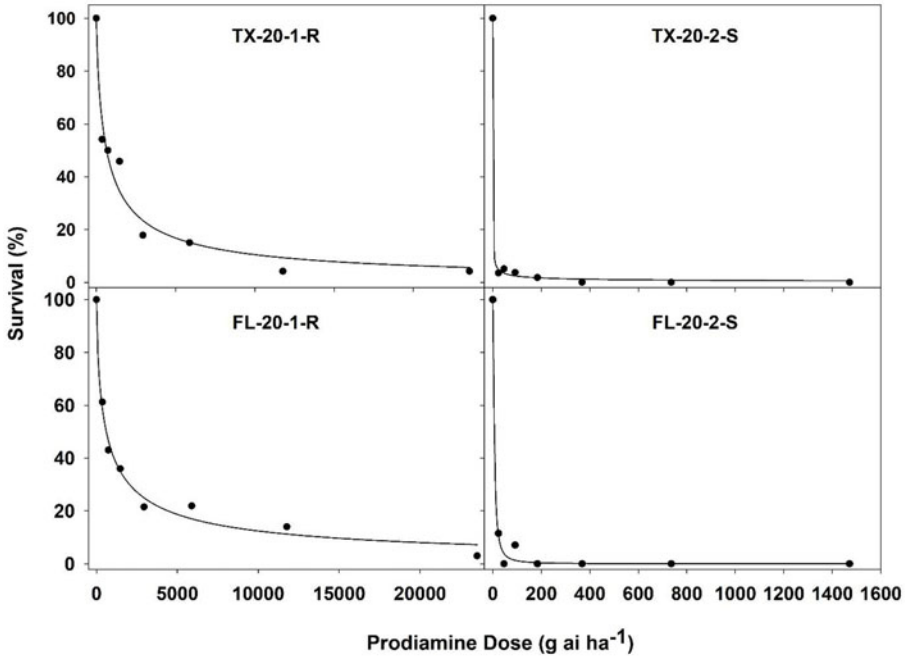

Figure 3. Dose-response of the annual bluegrass populations that exhibited putative resistance to prodiamine, collected in Texas and Florida in Spring 2020 (TX-20-1-R and FL-20-1-R), and populations collected in Texas and Florida in Spring 2020 (TX-20-2-S and FL-20-2-S) that were susceptible to prodiamine.

inhibitors (WSSA Group 9), glutamine synthase inhibitors (WSSA Group 10), microtubule assembly inhibitors, and to herbicides that inhibit cellulose biosynthesis. This research demonstrates that populations from Texas and Florida exhibit cross resistance to two commonly used preemergence microtubule assembly–inhibiting herbicides, prodiamine, a dinitroaniline herbicide, and pronamide, a benzamide herbicide. These findings expand upon the knowledge of how widespread herbicide resistance is becoming within this species.

Populations of annual bluegrass treated with prodiamine exhibited resistance to the herbicide with R/S ratios greater than 100 for the Florida population and greater than 9,000 for the Texas population, indicating that even higher application rates will not be sufficient to achieve control. Previous literature has reported that prodiamine-resistant annual bluegrass can be effectively controlled using indaziflam (Brosnan et al. 2017). Although indaziflam can be used to control annual bluegrass, this product is not labeled for use on golf course putting greens or cool-season turfgrass stands; thus, other management strategies would need to be implemented to control annual bluegrass in these settings. Moreover, overuse of indaziflam could lead to a greater incidence of resistance to this herbicide. Currently, there has only been one report of an indaziflam-resistant annual bluegrass, which originated in Tennessee in an early postemergence application scenario (Brosnan et al. 2020b). These findings, in conjunction with literature evidence, indicate that annual bluegrass can exhibit reduced sensitivity to indaziflam, thus the implementation of SOA rotations is critical to manage further evolution of indaziflam-resistant populations.

McCullough et al. (2016) first documented pronamide-resistant annual bluegrass when the product was applied postemergence. The main mechanism conferring resistance was reduced absorption and translocation, and not an altered target site. However, when treated with a preemergence application of pronamide, annual bluegrass was found to be susceptible (>92% control) to the treatment. In the current study, when treated with a preemergence application of pronamide, the population from Texas exhibited an R/S ratio of 6.05, which demonstrates that annual bluegrass can also become resistant to preemergence applications of this herbicide.

https://doi.org/10.1017/wet.2024.43 Published online by Cambridge University Press

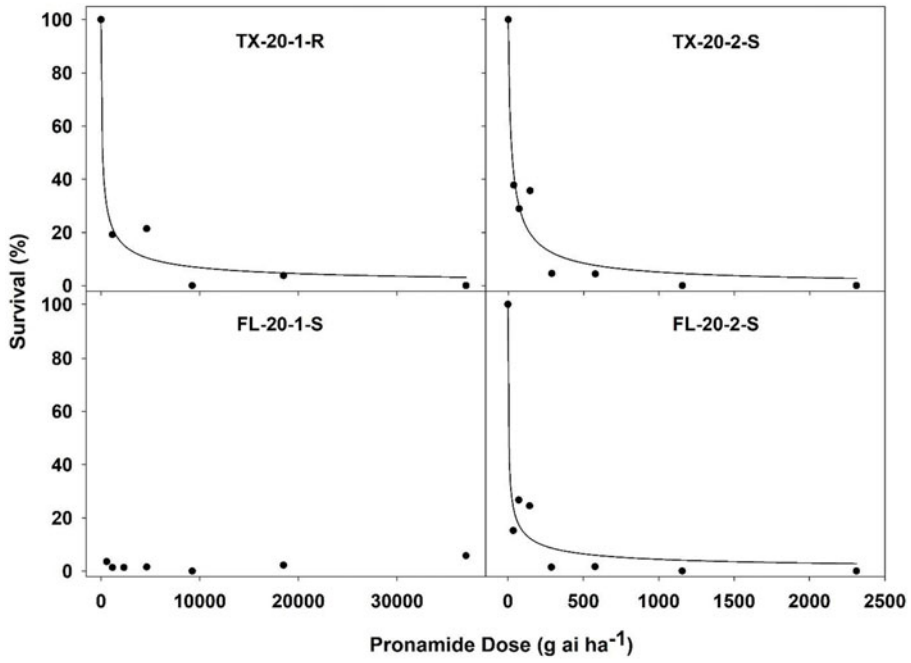

Figure 4. Dose-response of annual bluegrass populations that exhibited putative resistance to pronamide, collected in Texas and Florida in Spring 2020 (TX-20-1-R and FL-20-1-R), and populations collected in Texas and Florida in Spring 2020 (TX-20-2-S and FL-20-2-S) that were susceptible to pronamide.

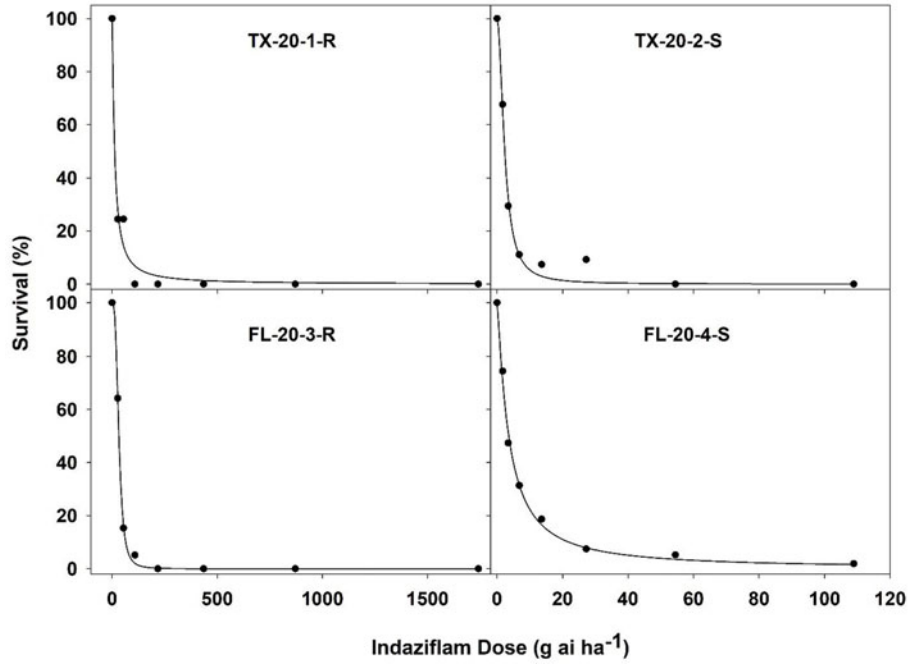

Figure 5. Dose-response of annual bluegrass populations that exhibited putative resistant to indaziflam, collected in Texas and Florida in Spring 2020 (TX-20-1-R and FL-20-3-R), and populations collected in Texas and Florida in Spring 2020 (TX-20-2-S and FL-20-4-S) that were susceptible to indaziflam.

If annual bluegrass is not controlled with a preemergence herbicide or a preemergence herbicide program is not implemented, several postemergence control options are available, especially for treating bermudagrass (*Cynodon dactylon* L.) (Hanson and Mallory-Smith 2000; Toler et al. 2007). However, there are many documented instances of annual bluegrass populations exhibiting resistance to the three most commonly-used postemergence herbicide SOAs: ALS (McElroy et al. 2013), PS II (De Prado and Menéndez 1996; Kumata et al. 2001), and microtubule assembly–inhibiting herbicides (Isgrigg et al. 2002). A relatively new postemergence control option available to turfgrass managers is methiozolin (SOA is unknown but it is in isoxaline chemical family) (Brabham et al. 2020), which has been shown to be effective at controlling herbicide-resistant annual bluegrass but is mainly limited for use on warm-season and cool-season golf courses (Brosnan et al. 2017).

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Practical Implications

Findings from this study confirm resistance to two common microtubule assembly–inhibiting herbicides, prodiamine and pronamide, which are used in annual bluegrass control on warm-season turfgrass, further demonstrating the widespread epidemic of herbicide resistance in annual bluegrass in managed turfgrass systems. As herbicide-resistant weeds become more common, delaying the onset of further herbicide resistance will be crucial in managing problematic weeds. To our knowledge, this is the first report of cross-resistance to prodiamine and pronamide when applied as a preemergence herbicide to Texas and Florida golf courses. Resistance of annual bluegrass to prodiamine, pronamide, and indaziflam was not detected on Florida and Texas athletic fields, sod farms, or lawn care sites sampled for this study. This may be attributed to the fact that golf courses notoriously use greater herbicide inputs, particularly pronamide and indaziflam, compared to other industries. While these populations exhibited reduced sensitivity to indaziflam (WSSA Group 29), and complete control was achieved at twice the recommended field rate, this may be the early stages of resistance evolution. Indaziflam resistance is a relatively new development, and annual bluegrass is the first species with documented resistance to this herbicide. This research further demonstrates the widespread herbicide resistance epidemic in annual bluegrass in managed turfgrass systems as we see the reduced efficacy of many of the products used to control annual bluegrass. Turfgrass managers should implement a diversified approach to weed management and use tank mixes with different SOAs when applicable and rotate the SOAs as frequently as possible. Additional research still needs to be done to characterize the extent of herbicide resistance in this species across the United States and to understand the underlying mechanisms that are conferring resistance, especially to indaziflam.

Acknowledgments. We thank all the research personnel at Texas A&M University and the University of Florida who assisted with the collection of plant material, greenhouse maintenance, and data collection.

Funding. Funding was provided by award 2018-51181-28436 from the U.S. Department of Agriculture–National Institute of Food and Agriculture Specialty Crop Research Initiative.

Competing Interests. The authors declare they have no competing interests.

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