

**GEORGIA DOT RESEARCH PROJECT 17-10
FINAL REPORT**

**ENHANCING EXTENSION RECOMMENDATIONS
FOR IMPROVING HERBICIDE RESISTANCE
MANAGEMENT ON GEORGIA ROADSIDES**



Georgia Department of Transportation

**OFFICE OF PERFORMANCE-BASED
MANAGEMENT AND RESEARCH
600 W PEACHTREE STREET NW
ATLANTA, GA 30308**

1. Report No.: FHWA-GA-20-1710		2. Government Accession No.: NA		3. Recipient's Catalog No.: NA	
4. Title and Subtitle: Enhancing Extension Recommendations for Improving Herbicide Resistance Management on Georgia Roadsides			5. Report Date: June, 2020		
			6. Performing Organization Code: NA		
7. Author(s): Patrick McCullough, Donn Shilling			8. Performing Organ. Report No.: 17-10		
9. Performing Organization Name and Address: University of Georgia 1109 Experiment Street Griffin, GA 30223			10. Work Unit No.: NA		
			11. Contract or Grant No.:		
12. Sponsoring Agency Name and Address: Georgia Department of Transportation Office of Performance-based Management and Research 600 W Peachtree St. NW Atlanta, GA 30308			13. Type of Report and Period Covered: Final; August, 2017- June, 2020		
			14. Sponsoring Agency Code: NA		
15. Supplementary Notes: Prepared in cooperation with the U.S. Department of Transportation, Federal Highway Administration.					
16. Abstract: The presence of Italian ryegrass on Georgia roadsides reduces motorist site visibility and increases maintenance costs for the DOT. Herbicide selection is limited on roadsides due to economics and the susceptibility of roadside turfgrasses to injury. Consequently, agronomists often apply the same chemistries every year without rotating herbicide modes of action. These spray programs promote the development of herbicide resistance in weed populations and may compromise the safety and sustainability of roadsides throughout Georgia. Research was conducted to evaluate the extent of herbicide resistance to glyphosate and acetolactate synthase (ALS)-inhibitors in areas it is most commonly found on Georgia roadsides. These are the two chemistries that have been primarily used for controlling ryegrass on roadsides for several decades. Twenty-eight populations were sampled on routes that had sprayed herbicides for ryegrass control in the winters of 2018 and 2019. Plants that were not controlled by the current programs were grown in the greenhouse, seed was harvested, and new plants were screened for resistance. In dose response, experiments approximately one- third of the ryegrass plants exhibited resistance or enhanced tolerance levels to glyphosate compared to known susceptible populations and the majority of other plants collected on roadsides. In hydroponic assays, approximately 20% of the ryegrass populations were resistant to ALS-inhibitor herbicides. Glyphosate-resistant ryegrass was controlled by Envoy (clethodim) and Piper (flumioxazin + pyroxasulfone) when applied alone or in combinations with Esplanade (indaziflam). The majority of the ryegrass sampled in roadsides was susceptible to glyphosate and ALS-inhibitors. Nonetheless, resistance was detected in several populations that could warrant rotation to other chemistries to help delay the spread of these biotypes on roadsides.					
17. Key Words: herbicide, glyphosate, ryegrass, resistance			18. Distribution Statement: NA		
19. Security Classification (of this report): Unclassified		20. Security Classification (of this page): Unclassified		21. Number of Pages: 37	22. Price: Free

GDOT Research Project No. RP 17-10

**Enhancing Extension Recommendations for Improving Herbicide Resistance
Management on Georgia Roadsides**

Final Report

By
Dr. Patrick McCullough
Associate Professor

Dr. Donn Shilling
Professor

Contract with
Georgia Department of Transportation

In cooperation with
U.S. Department of Transportation
Federal Highway Administration

June 2020

The contents of this report reflect the views of the author(s) who is (are) responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the Georgia Department of Transportation or the Federal Highway Administration. This report does not constitute a standard, specification, or regulation.

(This page intentionally left blank)

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF PICTURES	vii
EXECUTIVE SUMMARY	viii
ACKNOWLEDGEMENTS	ix
*INTRODUCTION	1
*OBJECTIVE	6
*PROCEDURES.....	7
*FINDINGS	12
*CONCLUSIONS.....	23
*REFERENCES	26

LIST OF TABLES

Table		Page
1.	Location information for annual ryegrass plants collected for herbicide screenings.....	8
2.	Statistics from regression analysis conducted for ryegrass plants treated with glyphosate in dose-response experiments in 2018-2019.....	14
3.	Statistics from regression analysis conducted for ryegrass plants treated with glyphosate in dose-response experiments in 2019-2020.....	16
4.	Resistance confirmations to ALS-inhibitors from hydroponic assays.....	19
5.	Control of glyphosate-resistant and susceptible biotypes at 28 days after treatments of various herbicides with different modes of action in the greenhouse.....	21

LIST OF FIGURES

Figure		Page
1.	Injury of ryegrass biotypes to glyphosate in dose-response experiments, 2018-2019.....	13
2.	Injury of ryegrass biotypes to glyphosate in dose-response experiments, 2019-2020.....	15

LIST OF PICTURES

Picture	Page
1. Italian ryegrass growing on a roadside in May 2018.....	2
2. Segregation of herbicide-resistant and susceptible biotypes after a broadcast of application of sulfonylurea herbicide	4
3. Establishment of new ryegrass from seed harvested off of plants collected on roadsides	9
4. Hydroponic screening of Italian ryegrass biotypes in a greenhouse experiment	10
5. Ryegrass plants treated with a glyphosate in various concentrations in a dose-response experiment.....	17
6. Response of glyphosate-resistant and susceptible ryegrass biotypes to herbicides in a greenhouse experiment	22

EXECUTIVE SUMMARY

The presence of Italian ryegrass on roadsides reduces site visibility for motorists and increases maintenance costs for the Georgia DOT. Herbicide applications are the most economical method to control Italian ryegrass to enhance roadside quality. Agronomists using the same herbicides every year without rotating modes of action promote the onset of herbicide resistance over time. Understanding the extent of resistance in ryegrass is critical for improving the sustainability of roadside management programs for Georgia. Research was conducted to evaluate resistance to glyphosate and acetolactate synthase (ALS)-inhibitors in areas where ryegrass is most prevalent on Georgia roadsides. These chemistries were the focus of the research because of their extensive use for controlling ryegrass on roadsides for several decades. Twenty-eight populations were sampled on routes that had sprayed herbicides for ryegrass control in the winters of 2018 and 2019. Plants that were not controlled by the current programs were grown in the greenhouse, seed was harvested, and new plants were screened for resistance. In dose response, experiments approximately one-third of the ryegrass plants exhibited resistance or enhanced tolerance levels to glyphosate compared to known susceptible populations and the majority of other plants collected on roadsides. In hydroponic assays, approximately 20% of the ryegrass populations were resistant to ALS-inhibitor herbicides. Glyphosate-resistant ryegrass was controlled by Envoy (clethodim) and Piper (flumioxazin + pyroxasulfone) when applied alone or in combinations with Esplanade (indaziflam). From this work, the majority of ryegrass sampled was susceptible to glyphosate and ALS-inhibitors. However, resistance was detected in several populations that could warrant the rotation to other herbicide modes of action to delay the spread of these biotypes on roadsides.

ACKNOWLEDGEMENTS

Special thanks to VJ Mantripragada, David Westbury, Seth Williams, Dr. Diego Gomez de Barreda, and Dr. Jialin Yu for technical support with this research.

INTRODUCTION

The presence of Italian ryegrass (*Lolium multiflorum* L.) on Georgia roadsides reduces motorist site visibility and increases mowing costs for the Georgia DOT (GDOT). Seed germinates from September to November when soil temperatures drop below 70° F. Ryegrass seedlings mature in fall, overwinter in a vegetative state, and resume active growth in spring. Italian ryegrass grows well under cool conditions when roadside grasses are dormant or have limited competitive growth. Plants exhibit erect growth that reaches approximately three feet in height upon maturity (Picture 1). Consequently, ryegrass may interfere with the growth of roadside grasses during spring transition and early summer.

Italian ryegrass is a prolific seed producer that contributes to annual infestations. The seedhead is a long spike with at least ten alternating florets. The florets contain long awns that are not present on perennial ryegrass (*Lolium perenne*). Plants typically produce seedheads by March in most parts of Georgia. Seed dispersed in late spring can remain dormant in soil for years. Another key characteristic to help identify ryegrass, without seedheads, is the clasping auricles at the junction of the leaf sheath and blade. The auricle may help practitioners identify ryegrass from other weedy grasses, such as tall fescue.

Mowing and Cultural Control

Modifications to management programs will help reduce Italian ryegrass establishment in fall. For example, mowing before seedhead formation can suppress ryegrass growth and inhibit the production of viable seed in spring. However, this method is cost prohibitive for managing over 700,000 acres of roadside vegetation in Georgia. Modifications in cultural practices that are applicable to other cropping systems, such as

pastures and forages, are often not applicable for most roadside managers. Therefore, an integrated strategy consisting of timely mowing, controlling summer weeds, and promoting roadside grass competitive growth can help reduce ryegrass populations.



Picture 1. Italian ryegrass growing on a roadside in May 2018.

The importance of herbicides for ryegrass control

Practitioners may use preemergence herbicides for preventing the establishment of ryegrass in fall. Dinitroanilines, or WSSA Group 3 herbicides, include oryzalin (Surflan, others), pendimethalin (Pendulum, others) and prodiamine (ProClipse, others). These herbicides inhibit microtubule assembly during cell division of young roots and shoots after germination that prevents ryegrass establishment (Senseman 2007). Indaziflam (Esplanade) inhibits cellulose biosynthesis and offers an alternative mode of action to DNA herbicides for ryegrass control in warm-season grasses. Applications should be timed

when soil temperatures drop below 70° in fall. Ideally, treatments should be applied prior to a rainfall to enhance soil incorporation and herbicide activation. However, due to the costs of applications and scheduling with DOT applications, preemergence herbicides are rarely used alone for ryegrass control and treatments are often applied with postemergence herbicides in late fall.

The optimum timing for postemergence control of ryegrass on roadsides is when plants are less than 6 inches in height in early winter. Bermudagrass managers have used acetolactate synthase (ALS) inhibitors for several decades due to the selectivity and safety to roadside turf. These herbicides include Escort (metsulfuron), Oust (sulfometuron), Pastora (nicosulfuron + metsulfuron), Derigo (thiencarbazone + foramsulfuron + iodosulfuron), and Matrix (rimsulfuron). Bahiagrass managers have traditionally used Oust for postemergence ryegrass control, but most other ALS-inhibitors cause unacceptable injury. Postemergence herbicides are more effective in early winter, compared to spring timings, because of the size and maturity of plants at application. Italian ryegrass is generally susceptible to postemergence herbicides in early winter prior to the onset of freezing temperatures and before seedhead emergence. Most of these herbicides require a non-ionic surfactant at 0.25% v/v of spray solution (1 qt/100 gal) to enhance foliar uptake and spray retention.

Glyphosate (Roundup, Accord, others) is a nonselective herbicide widely used on bermudagrass roadsides for ryegrass control in winter. Moderate rates of glyphosate in bermudagrass, such as 0.125 to 0.25 lb ae/acre, generally do not affect spring transition when applied in winter. However, glyphosate use in spring could cause delayed green-up and growth inhibition to bermudagrass.

Herbicide resistance and concerns about roadside management in Georgia

A major limitation to postemergence control of ryegrass is herbicide resistance (Heap 2020). Resistance of ryegrass species to ALS-inhibitors and glyphosate has been confirmed throughout the world due to overuse of these herbicides (Feng et al. 1999; Nandula et al. 2008; Shaner 1999). Resistance develops from selection pressure caused by repeated use of the same herbicide or mode of action over years. Genetic variation among biotypes in a ryegrass population contribute to differential levels of susceptibility to herbicides through altered target-site binding or enhanced degradation (Simarmata and Penner 2008; Simarmata et al. 2003; Wiersma et al. 1989). Other resistance mechanisms for Italian ryegrass may include reduced absorption, herbicide sequestration, or overproduction of the target site enzyme (Shaner 2009; Yu et al. 2009).

As susceptible biotypes are controlled by a particular herbicide over years, resistant biotypes spread in these areas. This type of selection pressure will shift a population from susceptible to resistant biotypes over years. The potential development of resistance to these herbicides in Italian ryegrass could warrant modifications to DOT spray programs that include product rotations to delay or avoid the onset of resistance.



Picture 2. Segregation of herbicide-resistant and susceptible biotypes after a broadcast of application of sulfonylurea herbicide.

Concerns about resistance in current DOT programs for ryegrass control

Glyphosate has been the primary herbicide used for ryegrass control on roadsides in Georgia for over a decade. This herbicide is nonselective and must be used during winter dormancy to minimize injury to bermudagrass and other roadside grasses. Glyphosate replaced herbicides that inhibit acetolactate synthase, or the ALS-inhibitors, due to resistance in ryegrass populations throughout the Southern U.S. While these herbicides are still in rotation for controlling other weeds, they have not been used for ryegrass control on Georgia roadsides for more than ten years.

The current ryegrass control program that was adopted by GDOT several years ago includes glyphosate (Accord XRT) plus a preemergence herbicide indaziflam (Esplanade). The use of Esplanade significantly improves the residual control of annual weeds on roadside turf, especially when glyphosate is used for controlling established plants. An advantage of Esplanade over other preemergence herbicides is the potential for applications to provide early-postemergence control of seedling ryegrass. However, Esplanade does not control ryegrass that has matured and reached several inches in height. The spread of glyphosate resistance may be exacerbated when Accord XRT is applied with Esplanade from December through April due to limited efficacy of indaziflam on mature plants.

The development of herbicide resistance management programs is challenging for roadside managers. Agronomists have limited resources available to manage weeds and the level of herbicide resistance on roadsides has not been extensively investigated. The location of resistant weed populations could vary significantly throughout Georgia due to regional differences in management programs. These factors could all contribute to the potential for herbicide resistance development throughout Georgia roadsides. The

identification of herbicide resistant ryegrass will enable agronomists to make adjustments in control programs that will prevent further spread of these populations.

OBJECTIVE

The objective of the proposed research was to evaluate herbicide-resistance of ryegrass on roadsides managed by GDOT and determine herbicide alternatives for programs that could control and delay the further spread of resistant populations.

PROCEDURES

Evaluation of glyphosate resistance. Experiments were conducted to evaluate the differential tolerance levels to glyphosate for ryegrass populations collected on Georgia roadsides. Plants were collected on roadsides in late winter and spring in 2018 and 2019 that were displaying no visual signs of control from herbicide applications made to the roadsides (Table 1). Approximately fifteen plants were collected per location. Plants were placed in plastic pots with 20-cm diameters and 30-cm depths filled with potting soil and placed in a greenhouse on the UGA Griffin Campus set for approximately 25/20° C (day/night). Plants were fertilized and irrigated as needed to promote growth.

For a four to six-month period after harvesting, plants were grown in the greenhouse and seed was collected from the inflorescence. Seed was then dried in a growth chamber set for 30° C for one week and seedhead material was removed using sieves to collect seeds. The seed was then scattered over plastic flats in the greenhouse filled with sand:peat moss (85:15, Picture 3). The flats were irrigated and fertilized as needed to promote establishment of the seedlings. After reaching a multi-leaf growth stage, single plants were transplanted to plastic pots with a 3.8-cm diameter and 20-cm depths filled with the aforementioned sand:peat moss soil. Plants were then fertilized and irrigated to promote growth.

Once grasses reached about 10-cm height and were tillered, glyphosate (Roundup Pro) was applied at ten rates ranging 2 to 128 fl oz/acre. Treatments were applied at 20 gal/acre volume in a chamber using an air pressured sprayer equipped with a single, 8002E, flat-fan nozzle. Ryegrass control was visually evaluated after three weeks on a percent scale where 0 equaled no control and 100 equaled complete plant death.

Table 1. Location information for annual ryegrass plants collected for herbicide screenings.

Sample	Location	City	Collection date	Codes in Figure 1 and 2
1	I-75 Exit 205	Jackson, GA	1/26/18	RG1
2	I-75 Exit 157	Macon	1/29/18	RG2
3	5700 US Hwy 41S	Culloden, GA	1/29/18	RG3
4	Forsyth-Yatesville Rd	Yatesville, GA	1/29/18	RG4
5	Exit 198 (N) I-75	High Falls, GA	1/29/18	RG5
6	I-85 N	Fortson, GA	1/30/18	RG6
7	I-85 GA/Hwy 18	Pine Mountain, GA	1/30/18	RG7
8	I-85 N/Hwy 54	Hogansville, GA	1/30/18	RG8
9	I-85 N	Moreland, GA	1/31/18	RG9
10	I-85 N	Palmetto, GA	1/31/18	RG10
11	I-16 S/Sgoda Rd	Macon, GA	1/31/18	RG11
12	I-16 S/GA Hwy-358	Danville, GA	1/31/18	RG12
13	I-16 W	Dublin, GA	3/8/18	RG13
14	Hwy-520	Augusta, GA	3/8/18	RG14
15	I-75 Exit 64	Tifton, GA	3/25/19	RG1
16	I-75 Exit 80	Sycamore, GA	3/25/19	RG2
17	I-75 Exit 109	Vienna, GA	3/25/19	RG3
18	I-75 Exit 127	Henderson, GA	3/25/19	RG4
19	I-75 Exit 185	Forsyth, GA	3/25/19	RG5
20	I-75 Exit 205	Jackson, GA	3/25/19	RG6
21	I-20 Exit 92	Convington, GA	3/26/19	RG7
22	I-20 Exit 105	Rutledge, GA	3/26/19	RG8
23	I-20 Exit 121	Buckhead, GA	3/26/19	RG9
24	I-20 Exit 138	Union Point, GA	3/26/19	RG10
25	I-20 Exit 148	Crawfordville, GA	3/26/19	RG11
26	I-20 Exit 130	Greensboro, GA	3/26/19	RG12
-	-	Commercial seed	n/a	#4



Picture 3. Establishment of new ryegrass from seed harvested off of plants collected on roadsides.

The experimental design was a randomized complete block with five replications. Regression analysis was performed with the Linear and Nonlinear Regression Procedures in SAS. Data was plotted on figures, and regressed against the following equations:

$$y = \beta_0 + * \beta_1 (1 - (\exp(-\beta_2 * x)))$$

where β_0 is the lower asymptote, β_1 is the maximum predicted response, β_2 is the slope, and x is the glyphosate rate in g ai/ha. The equation was selected that described the relationship of plant response with herbicide concentrations.

Hydroponic assays for ALS-resistance. Ryegrass seed was planted in greenhouse flats filled with sand:peat (85:15) and irrigated as needed to promote germination. Seedlings were fertilized and irrigated in flats until developing 3 to 5 leaves. Individual plants were then removed from the flats and soil was washed from roots. Plants were then placed in a 6-L hydroponic tank filled with half-strength Hoagland nutrient solution. Fifteen holes measuring 1.5-cm each were drilled in the lids of the tanks and plants roots were placed through the holes to facilitate suspension in the nutrient solution. Fifteen plants were placed in each tank that included three biotypes with five replications. An aquarium pump provided oxygen to the solution. After one week, a sulfonylurea herbicide, flazasulfuron (Katana 25WG), was spiked in the tanks at 0 or 1 μM . Plants were grown for one week after treatments and then control was visually evaluated as 0 (no response) or 1 (controlled). This methodology has shown separation of resistant grass biotypes from susceptible ones.



Picture 4. Hydroponic screening of Italian ryegrass biotypes in a greenhouse experiment.

Evaluation of herbicide alternatives for controlling glyphosate-resistant ryegrass. A greenhouse experiment was conducted to evaluate the efficacy of glyphosate alternatives for controlling Italian ryegrass. Susceptible and resistant biotypes to glyphosate were seeded in greenhouse flats. Seed was then scattered over plastic flats in the greenhouse filled with sand:peat moss (85:15). The flats were irrigated and fertilized as needed to promote establishment of the seedlings. After reaching a multi-leaf growth stage, single plants were transplanted to plastic pots with a 3.8-cm diameter and 20-cm depths filled with the aforementioned sand:peat moss soil. Plants were then fertilized and irrigated to promote growth.

Treatments were applied to 1-tiller ryegrass and included the factorial combination two Esplanade rates, 0 or 3.5 oz/acre, applied with three herbicides, Accord XRTII at 16 oz/acre, Piper at 8 oz/acre, and Envoy Plus at 9 oz/acre. Piper and Envoy Plus offer different modes of action to glyphosate and ALS-inhibitors and have shown good efficacy in previous experiments. A nontreated check was included. Treatments were applied in 40 gal/acre volume using an air pressured sprayer equipped with a single, 8002E, flat-fan nozzle. Ryegrass control was visually evaluated after four weeks on a percent scale where 0 equaled no control and 100 equaled complete plant death. The experimental design was a randomized complete block with five replications. Data were subjected to analysis of variance and means were separated with Fisher's LSD test at $\alpha = 0.05$.

FINDINGS

Evaluation of glyphosate resistance. There was wide variability in the response to glyphosate in dose-response experiments from the populations collected throughout the state (Figure 1, Picture 5). The majority of the ryegrass plants collected in both years had comparable I_{50} levels, or glyphosate rates required to injure plants 50%, to our known susceptible biotypes (Table 2). This suggests that the majority of plants collected were susceptible to glyphosate. Three populations in the 2018 collection had I_{50} levels greater than most other biotypes, ranging about 3 to 5-fold higher. In 2019, five populations exhibited greater tolerance to glyphosate than the majority of the populations.

Most populations identified with resistance to glyphosate are from areas off of I-75 in central Georgia and I-16 south of Macon. These areas have been repeatedly sprayed with Accord XRT, Razor Pro, and other glyphosate products during the winter for over a decade. Despite the current use of glyphosate as the key herbicide for controlling established ryegrass, we did not detect significant resistance in the majority of populations. This may have occurred from skips in spray patterns or failure to apply herbicides in these areas.

Another potential limitation to collecting plants was our inability to sample in medians and shoulders on interstates. Plant collections could only be made in areas that were safe for researchers to sample such as lands adjacent to the exits. This limitation could also have prevented us in detecting resistance in plants that would be targeted for control by glyphosate in these areas.

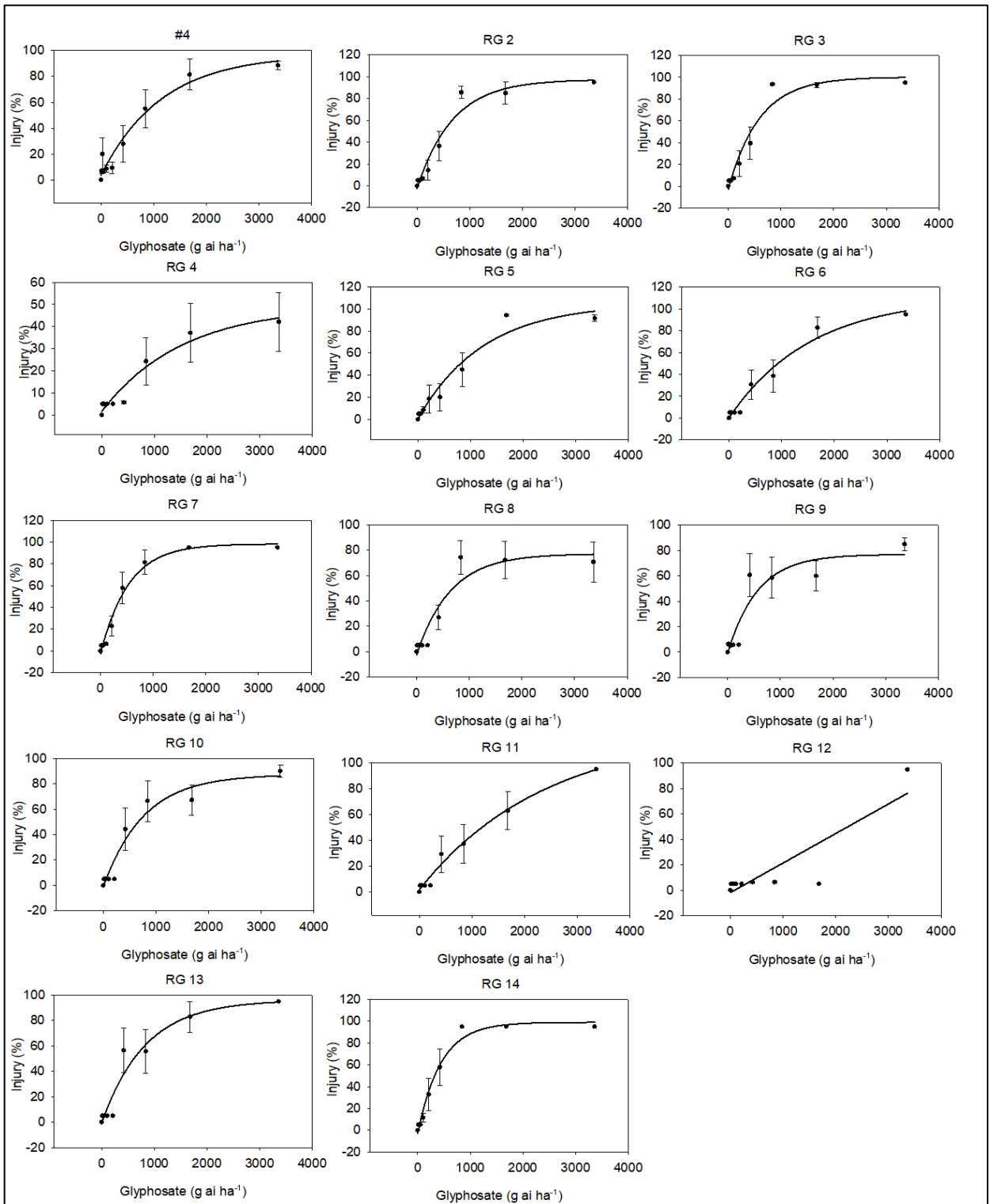


Figure 1. Injury of ryegrass biotypes to glyphosate in dose-response experiments, 2018-2019.

Table 2. Statistics from regression analysis conducted for ryegrass plants treated with glyphosate in dose-response experiments in 2018-2019.

Populations	r ²	Regression parameters ^a			P	I ₅₀ ^b	95% CI ^c
		β ₀	β ₁	B ₂			
#4	0.65	3.6157	93.4369	0.0009	<0.0001	760	615-905
R2	0.82	-2.4253	99.8894	0.0015	<0.0001	500	415-585
R3	0.84	-2.9061	103.04	0.0017	<0.0001	420	365-475
R4	0.41	1.6752	47.4922	0.0007	<0.0001	>3360	>3360
R5	0.74	0.3852	104.5608	8.00E-04	<0.0001	800	615-985
R6	0.76	-0.0097	110.7863	0.0006	<0.0001	1000	730-1270
R7	0.84	-2.7064	101	0.0019	<0.0001	400	330-470
R8	0.63	-2.527	79.6453	1.60E-03	<0.0001	660	500-820
R9	0.59	-0.8615	77.8535	0.0018	<0.0001	600	380-820
R10	0.68	-1.1777	88.448	0.0013	<0.0001	700	485-915
R11	0.70	1.762	123.0075	0.0004	<0.0001	1300	940-1660
R12	0.77	-1.7973	177278.68	1.31E-07	<0.0001	2200	1990-2410
R13	0.69	-0.9384	96.9802	0.0012	<0.0001	650	430-870
R14	0.82	-2.9092	101.8823	0.0023	<0.0001	330	230-430

^aData were fit to the following regression equation = $y = \beta_0 + * \beta_1 (1 - (\exp(-\beta_2 * x)))$, where β_0 is the lower asymptote, β_1 is the maximum predicted response, β_2 is the slope, and x is the glyphosate rate in g ai/ha.

^bI₅₀ = glyphosate rate required to injure ryegrass 50%.

^cCI = confidence interval for the calculated I₅₀ value used to statistically separate estimates.

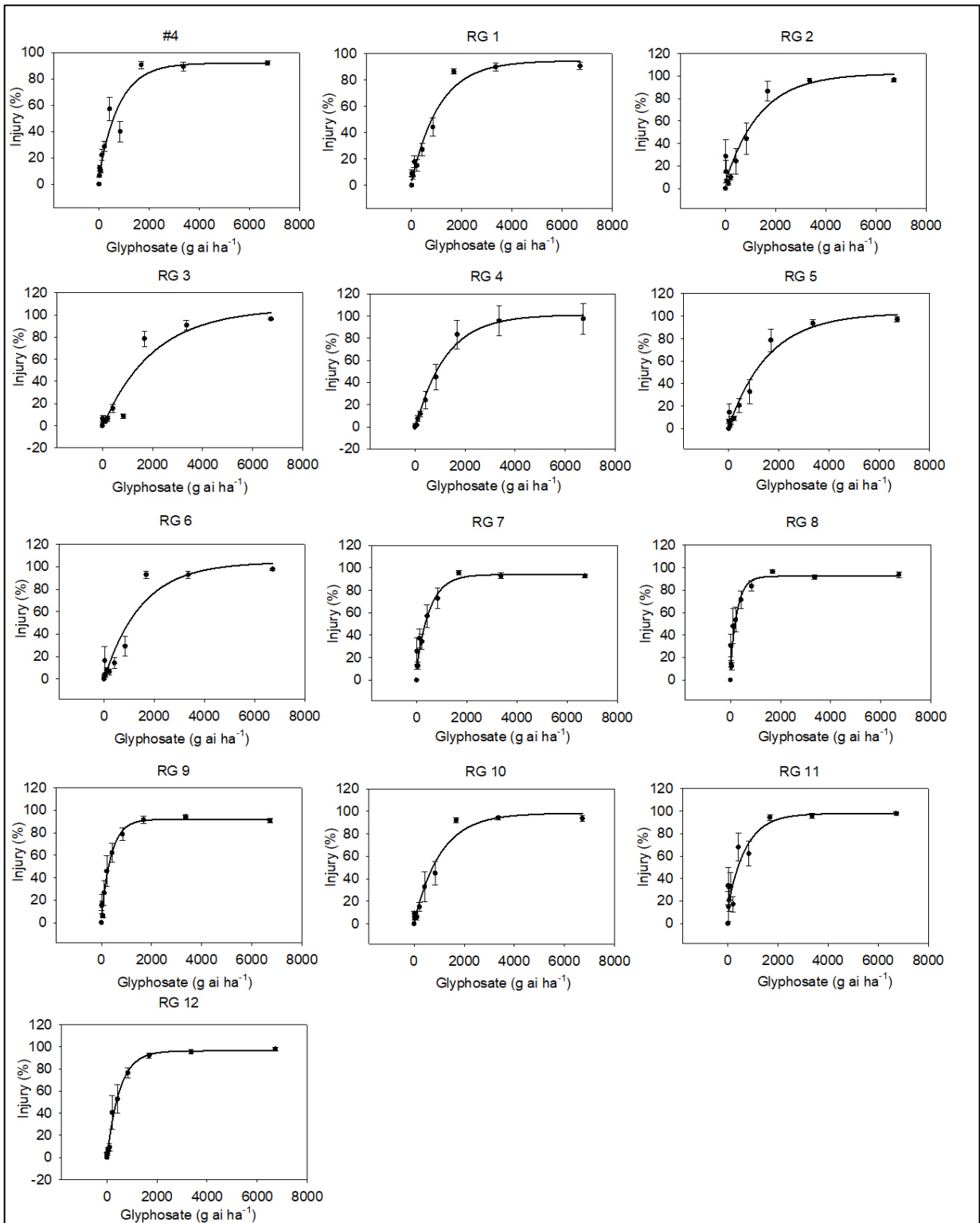


Figure 2. Injury of ryegrass biotypes to glyphosate in dose-response experiments, 2019-2020.

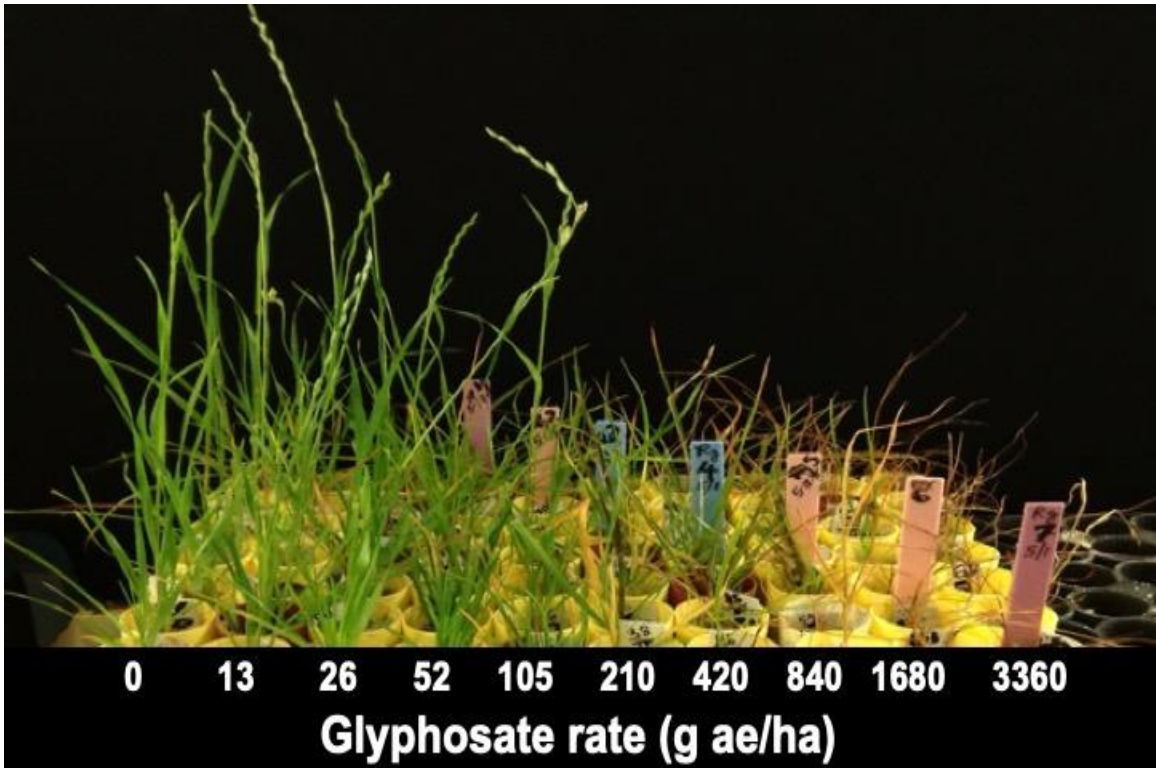
Table 3. Statistics from regression analysis conducted for ryegrass plants treated with glyphosate in dose-response experiments in 2019-2020.

Populations	r ²	Regression parameters ^a			I ₅₀ ^b	95% CI ^c
		β ₀	β ₁	B ₂		
#4	0.85	6.9768	85.0818	0.0012	600	530-670
R1	0.91	3.68E+00	90.9756	0.0009	800	731-869
R2	0.71	5.8731	96.3252	0.0007	900	630-1170
R3	0.88	-0.9661	106.8294	0.0005	1300	1165-1435
R4	0.92	-1.9415	103.0912	0.0008	900	730-1070
R5	0.85	1.7789	101.0502	6.00E-04	1100	785-1415
R6	0.84	0.1312	103.9237	0.0007	950	740-1160
R7	0.78	10.8962	83.1857	0.0019	350	320-380
R8	0.74	10.1748	82.1233	3.60E-03	180	145-215
R9	0.80	5.319	86.6192	0.0026	280	260-300
R10	0.86	1.2909	96.9935	0.0009	800	640-960
R11	0.61	14.4647	83.3997	0.0014	400	290-510
R12	0.86	-0.8725	97.179	0.002	380	320-440

^aData were fit to the following regression equation = $y = \beta_0 + \beta_1 (1 - \exp(-\beta_2 * x))$, where β_0 is the lower asymptote, β_1 is the maximum predicted response, β_2 is the slope, and x is the glyphosate rate in g ae/ha.

^bI₅₀ = glyphosate rate required to injure ryegrass 50%.

^cCI = confidence interval for the calculated I₅₀ value used to statistically separate estimates.



Picture 5. Ryegrass plants treated with a glyphosate in various concentrations in a dose-response experiment.

Evaluation of ALS-inhibitor resistance. Plants were screened hydroponically for resistance to ALS-inhibitors by spiking a sulfonyleurea, Katana (flazasulfuron), in the tanks at various concentrations. This herbicide is highly active on susceptible ryegrass biotypes and was a good indicator herbicide in pilot experiments. From these evaluations, most ryegrass plants screened were susceptible to ALS-inhibitors. This was determined based on if the majority of the replications exhibited herbicide toxicity relative to the nontreated plants.

Ryegrass with confirmed resistance to ALS-inhibitors averaged about 20% of the total populations surveyed (Table 4). These cases were scattered throughout sample sites including I-20, I-75, and I-85. There has been limited use of ALS-inhibitors for ryegrass control in the recent decade which is primarily associated with the widespread resistance throughout the southern U.S. The DOT agronomists may have limited the spread of ryegrass populations with ALS-resistance by rotating to glyphosate and indaziflam programs.

The shift in control methods may have also reduced the presence of ALS-resistant ryegrass on roadsides in Georgia. The plants collected were mostly sampled after glyphosate was sprayed by applicators. This may have controlled the ryegrass plants that were resistant to ALS-inhibitors since alternative chemistries were used in these programs. The majority of our ryegrass plants sampled were susceptible to this chemistry suggesting GDOT may have potential to incorporate products like Oust (sulfometuron) or Escort (metsulfuron) back in to winter spray programs. Since resistant biotypes are present, it would be prudent to include herbicides with alternative modes of action as tank-mix partners or in sequential programs for controlling ryegrass on roadsides.

Table 4. Resistance confirmations to ALS-inhibitors from hydroponic assays.

Population	Location	ALS-resistance
1	I-75 Exit 205	No
2	I-75 Exit 157	No
3	5700 US Hwy 41S	No
4	Hwy-341	No
5	Exit 197 (N) I-75	Yes
6	185 N	Yes
7	185 GA/Hwy 18	No
8	I-85 N/Hwy 54	Yes
9	I-85 N	No
10	I-85 N	No
11	I-16 S/Sgoda Rd	No
12	I-16 S/GA Hwy-358	No
13	I-16 W	No
14	Hwy-520	No
15	I-75 Exit 64	No
16	I-75 Exit 80	No
17	I-75 Exit 109	No
18	I-75 Exit 127	No
19	I-75 Exit 185	No
20	I-75 Exit 205	No
21	I-20 Exit 92	No
22	I-20 Exit 105	No
23	I-20 Exit 121	Yes
24	I-20 Exit 138	No
25	I-20 Exit 148	Yes
26	I-20 Exit 130	No

Evaluation of herbicide alternatives for controlling glyphosate-resistant ryegrass.

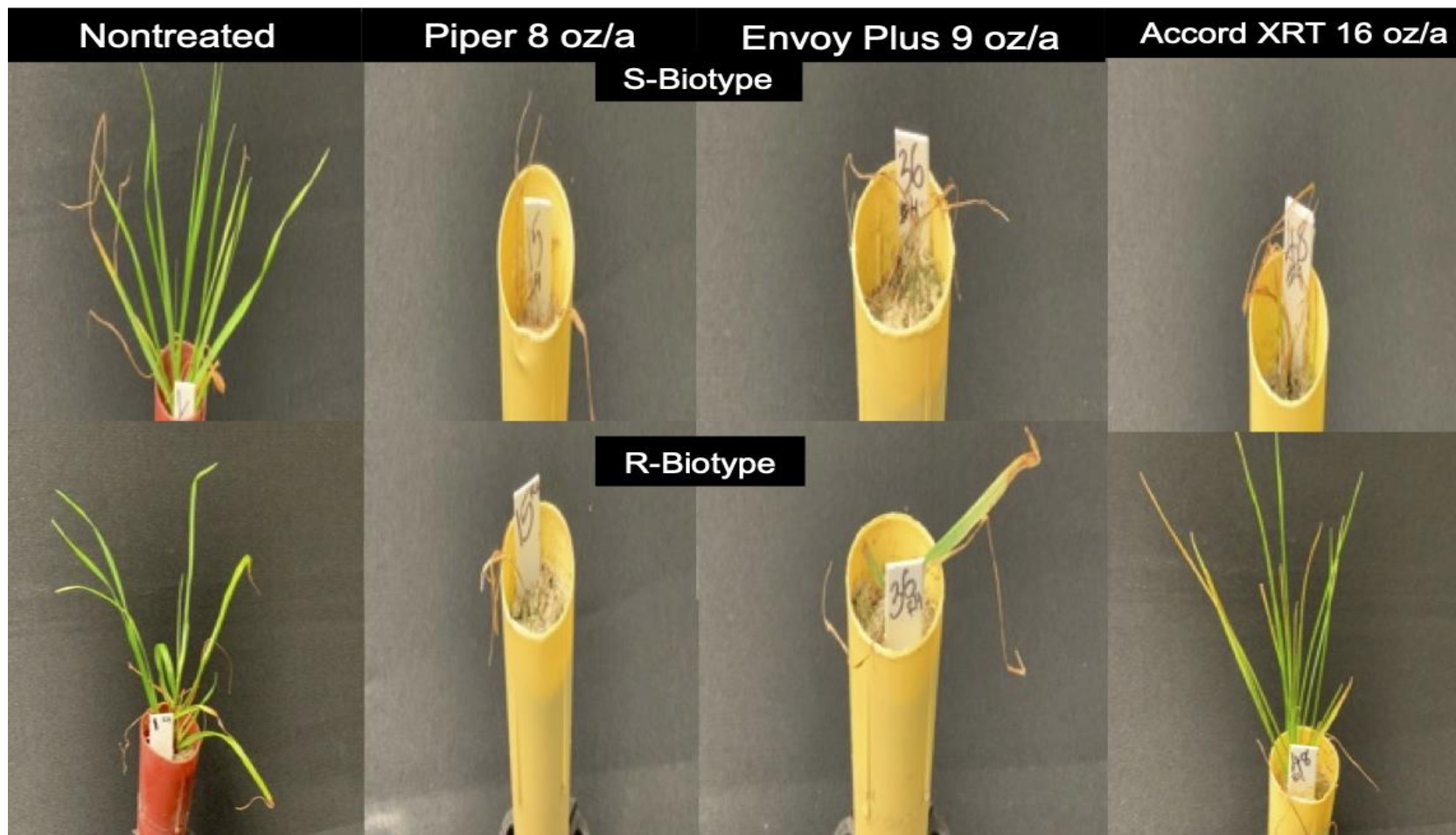
Esplanade alone at 3.5 oz/acre provided about 50% control of ryegrass that was resistant and susceptible to glyphosate (Table 5). When Esplanade was applied with Accord XRT (glyphosate), ryegrass was controlled 79%. The Esplanade + Accord XRT treatment provided similar control to Esplanade alone when applied to glyphosate-resistant ryegrass.

The current DOT spray program consists of Esplanade with Accord which can effectively control ryegrass that is susceptible glyphosate. However, the resistant biotype did not exhibit enhanced control from Esplanade when Accord was applied in the mixture. This exemplifies the limitations to only using one postemergence herbicide mode of action for controlling ryegrass populations on roadsides.

Envoy (clethodim) is an ACCase-inhibitor herbicide that effectively controls grassy weeds, including ryegrass. Envoy provided 87% and 61% control of the glyphosate susceptible and resistant biotypes in this experiment. Substituting Envoy with Esplanade improved control of both biotypes to 88% on average. Another herbicide with different modes of action to the aforementioned products, Piper (flumioxazin + pyroxasulfone), controlled both ryegrass biotypes 95% on average. There was no benefit to using Piper in combination with Esplanade or Accord for controlling ryegrass after one month. Nevertheless, the combinations could be applicable when targeting other weeds that are not controlled by these herbicides alone.

Table 5. Control of glyphosate-resistant and susceptible biotypes at 28 days after treatments of various herbicides with different modes of action in the greenhouse.

Herbicide	Active Ingredient	Product rate (oz/acre)	Ryegrass control	
			Glyphosate-susceptible	Glyphosate-resistant
			%	
Accord XRT	glyphosate	16 fl oz	61	11
Envoy	clethodim	9 fl oz	87	61
Envoy + Esplanade	clethodim + indaziflam	9 + 3.5 fl oz	86	89
Esplanade	indazilam	3.5 fl oz	50	56
Esplanade + Accord	indazilam + glyphosate	3.5 + 16 fl oz	79	48
Piper	(flumioxazin + pyroxasulfone)	8 oz	93	97
Piper + Esplanade	(flumioxazin + pyroxasulfone) + indaziflam	8 oz + 3.5 fl oz	99	86
Piper + Accord	(flumioxazin + pyroxasulfone) + glyphosate	8 oz + 16 fl oz	81	91
LSD _{0.05}			21	



Picture 6. Response of glyphosate-resistant and susceptible ryegrass biotypes to herbicides in a greenhouse experiment.

CONCLUSIONS

Controlling problem weeds is one of the greatest challenges to maintaining safe and sustainable roadside turf. Herbicides are a fundamental component of integrated weed management programs that promote the release of desirable roadside grasses and limit the establishment of invasive weeds. Italian ryegrass is the most problematic winter weed of roadsides in Georgia that must be controlled with herbicides. Failing to control ryegrass can lead to increased mowing requirements and stand thinning as populations decline in spring. Promoting bermudagrass release and density in spring by eliminating ryegrass can also reduce the potential for new weeds to emerge when populations die out in June.

Spray programs must include plans for rotating herbicides to delay the onset of resistance in Italian ryegrass. Selection pressure created by repeated use of the same herbicide over time will shift weed populations and promote the spread of resistant biotypes. Annual weeds like, Italian ryegrass, with prolific seed production are highly adaptable and prone to resistance development. Understanding the threat and spread of herbicide resistant biotypes will be important for developing programs that control established Italian ryegrass and delay the onset of resistance to current chemistries.

The GDOT agronomists have been using glyphosate as the primary postemergence herbicide for controlling ryegrass throughout Georgia for over a decade. Glyphosate has been applied during winter months to maximize selectivity and reduce potential to cause severe injury to bermudagrass and other roadside species. Although glyphosate is a nonselective herbicide, it has a single site of action which is associated with most cases of herbicide resistance. Single applications of glyphosate per year for a decade without

rotation to other chemistries has resulted in erratic levels of control that are associated with resistance.

Approximately one-third of the ryegrass plants sampled exhibited resistance or enhanced tolerance to glyphosate relative to susceptible populations. Glyphosate resistance does not appear to be a statewide problem based on the populations that we sampled in this work. However, agronomists must remain attentive that glyphosate resistance could steadily increase over time if alternative chemistries are not incorporated in spray programs.

The current use of Esplanade in ryegrass control programs will help delay the spread of populations with resistance to glyphosate or other modes of action. Contractors for the DOT are currently using Esplanade with glyphosate after ryegrass has emerged. Our greenhouse experiments show that Esplanade has some postemergence activity for controlling young ryegrass plants. This is beneficial for the DOT when treatments are made in late fall as ryegrass begins to emerge. However, erratic control may result from winter treatments if the biotypes are glyphosate resistant. This is because Esplanade does not control mature ryegrass alone when glyphosate is ineffective.

There are several chemistries than can be incorporated in GDOT spray programs to supplement or provide options for rotation over years. Envoy (clethodim) is a Group 1, ACCase-inhibitor, herbicide that is highly active on most grass species. It is injurious to bermudagrass in spring and must be used for ryegrass control in winter months, similar to glyphosate. Envoy would be an economical herbicide to combine with or substitute for glyphosate in current spray programs. A limitation to Envoy use is the failure to control broadleaf weeds. Therefore, mixtures with glyphosate or other modes of action will be

needed on roadsides if Envoy is used in winter. The Group 1 herbicides are not commonly used in DOT spray programs due to excessive injury potential to desirable grasses during the growing season. There is a high risk of injuring roadside grasses with Envoy if applications are made in late winter, but the risk levels would be comparable to winter glyphosate programs currently used by the DOT.

When screening glyphosate-resistant ryegrass, Piper alone and with Esplanade also provided effective control. Piper is a newer combination herbicide for roadside management that contains flumioxazin and pyroxasulfone. These active ingredients are different modes of action than all other herbicides used on roadsides. Piper has been previously used in certain cropping systems, such as wheat, for ryegrass control due to the widespread resistance to glyphosate and ALS-inhibitors. Piper does not control mature ryegrass at labeled use rates and agronomists need to make applications when plants are no more than three inches in height.

The identification of herbicide-resistant ryegrass on roadsides suggests that agronomists should modify spray programs over time when feasible. Rather than having contractors apply the same regimen every year, rotating from programs every one to two years could improve ryegrass control and delay the inevitable spread of herbicide resistance. If there is insufficient flexibility in spray programs for contractors, the risk for continued spread of ryegrass resistance to glyphosate, Esplanade, and other herbicides will increase. This could lead to increased costs for additional mowing throughout the state to maintain roadsides at acceptable heights and reduce ryegrass seed production. It may also lead to the spread of invasive weeds after ryegrass declines in summer that warrant additional resources required to maintain safe and sustainable roadsides.

References

- Feng P, Pratley J, Bohn J (1999) Resistance to glyphosate in *Lolium rigidum*. II. Uptake, translocation, and metabolism. *Weed Sci.* 47:412-415.
- Heap I (2020) The International Survey of Herbicide Resistant Weeds. Online at www.weedscience.com
- Nandula V, Reddy K, Poston D, Rimando A, Duke S (2008). Glyphosate tolerance mechanism in Italian ryegrass (*Lolium multiflorum*) from Mississippi. *Weed Sci* 56:344-349
- Shaner DL (1999) Resistance to acetolactate synthase (ALS) inhibitors in the United States: history, occurrence, detection, and management. *J Weed Sci Tech* 44:405-411
- Shaner DL (2009) Role of translocation as a mechanism of resistance to glyphosate. *Weed Sci* 57:118-123
- Senseman SA (2007) *Herbicide Handbook*, Ninth Edition. Weed Science Society of America.
- Simarmata M, Penner D (2008) The basis for glyphosate resistance in rigid ryegrass (*Lolium rigidum*) *Weed Sci* 56:181-188
- Simarmata M, Kaufmann JE, Penner D (2003) Potential basis of glyphosate resistance in California rigid ryegrass (*Lolium rigidum*). *Weed Sci* 51:678-682
- Wiersma PA, Schiemann MG, Condi JA, Crosby WL, Molone MM (1989) Isolation, expression, and phylogenetic inheritance of an acetolactate synthase gene from *Brassica napus*. *Mol Gen Genet.*219:413-420

Yu Q, Heping H, Powles SB (2008) Mutations of the ALS gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. *Pest Mang Sci* 64:1229-1236

Yu Q, Abdallah I, Han H, Owen M, Powles S (2009) Distinct non-target site mechanisms endow resistance to glyphosate, ACCase, and ALS-inhibiting herbicide in multiple herbicide-resistant *Lolium rigidum*. *Planta* 230:713-723