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Biology and Management of Glyphosate-Resistant and Susceptible Italian Ryegrass Phenotypes from a Segregating Population

Sushila Chaudhari[1], David L. Jordan[1], Alan C. York[1], Katherine M. Jennings[2], Charles W. Cahoon[1], Aman Chandi[3], and Abigail B. Haselton[1]

1. Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA

2. Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695, USA

3. DuPont Crop Protection, Newark, DE 19711, USA

Abstract: Glyphosate-resistant (GR) Italian ryegrass is a major weed in both annual and perennial crops in the United States. A segregating population of GR Italian ryegrass collected in North Carolina during 2009, and vegetative propagation (cloning) technique was used to identify and isolate GR and glyphosate-susceptible (GS) phenotypes to compare: level of resistance following glyphosate application; early season interference from GR and GS phenotypes with barley, oat, cereal rye, and wheat; effect of drought stress on GR and GS phenotypes; and response of both phenotypes to POST herbicides. The GR50 (glyphosate rate providing 50% reduction in tiller dry biomass) was 3.2 times greater with the GR phenotype compared to GS phenotype. Interference with crop growth did not differ between GR and GS phenotypes. Although tiller count and biomass of Italian ryegrass grown with the crops did not differ between phenotypes, height reduction was greater for the GR phenotype compared with the GS phenotype. Response of Italian ryegrass and wheat to drought stress did not differ by Italian ryegrass phenotype. Control of Italian ryegrass by herbicides was similar when comparing GR and GS phenotypes. These results indicate that in the absence of glyphosate selection pressure, resistance to glyphosate does not influence the basic biology and management of GR and GS Italian ryegrass.

Key words: evolved resistance, herbicide resistance, Italian ryegrass, weed management

1. Introduction

Glyphosate is a broad-spectrum, nonselective herbicide that has been used POST to control a wide range of annual and perennial plant species in transgenic crops, plantation crops, no-tillage systems, and non-agricultural situations [1]. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway in plants, thus preventing biosynthesis of essential aromatic amino acids [2]. As a consequence of extensive and exclusive use of glyphosate, resistance evolution in a number of weed species, including Italian ryegrass, has been confirmed worldwide [3]. In 2003, a population of Italian ryegrass in Oregon was the first in the United States to be confirmed resistant to glyphosate [4]. Since then, GR Italian ryegrass has been documented in seven states, including North Carolina [3]. Italian ryegrass populations appear to have independently evolved more than one mechanism for glyphosate resistance, including an altered target site [5, 6], amplification of the EPSPS gene [7], over-expression of EPSPS [8], and reduced glyphosate translocation [9, 10].

Italian ryegrass is one of the most common and troublesome weeds in cereal grain production system

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Corresponding author: David Lindley Jordan, Ph.D., Professor of Crop Science; research areas/interests: pest management, agronomy, weed science. E-mail: david_jordan@ncsu.edu.

throughout the southeastern United States [11]. This winter annual weed is highly competitive with wheat, and can reduce tillering, cause lodging, interfere with harvesting, and contaminate harvested grain [12, 13]. Italian ryegrass control prior to planting in conservation or no-tillage systems is important to ensure adequate crop stand establishment and to minimize early season competition with crops [14]. Glyphosate has been used historically as a preplant burndown in reduced-tillage crops in North Carolina to control Italian ryegrass. However, the control of glyphosate-resistant (GR) Italian ryegrass poses a challenge to producers utilizing conservation or no-tillage systems [14].

Due to widespread distribution of GR Italian ryegrass, it is important to quantify the effect of glyphosate-resistance traits on Italian ryegrass biology and management. Fitness costs associated with glyphosate resistance have been reported in rigid ryegrass (*Lolium rigidum* Gaudin) [15], annual ryegrass [16], and perennial ryegrass (*Lolium perenne* L.) [17]. Pedersen et al. [15] reported no differences in vegetative growth or competitiveness of the GR and GS rigid ryegrass population; however, a reduction of 4 to 18% in seed number was reported in GR rigid ryegrass plants when subjected to no or low competition from wheat in comparison to GS plants. Wakelin and Preston [16] reported fitness cost associated with GR annual ryegrass that resulted in a reduction in the frequency of GR individuals over time in the absence of glyphosate selection. Glyphosate-resistant perennial ryegrass plants showed a reduction in height, leaf blade area, shoot biomass, seed number, and total seed mass compared to GS plants [17].

Differences in relative fitness between resistant and susceptible populations are usually inferred from measures of relative plant productivity or competitiveness [18]. It is important to note that the impact of resistance on plant fitness can depend upon genotype and population variation, intra- and inter-population competition, and environmental conditions such as temperature, light quality, and management practices [18-20]. Many studies have examined physiological and ecological costs associated with herbicide resistance. However, some of these studies are of limited value in addressing the growth and productivity because they compare populations from different locations that express alleles those may be associated with traits other than the resistance [21, 22]. For valid comparison, herbicide-resistant and -susceptible individuals or genotypes should share the same genetic background except for that gene or genes endowing resistance [20]. A number of techniques, including targeted mutation [23], transgenic lines [24], production of segregating populations by crossing resistant and susceptible populations [25], and vegetative propagation (cloning) [15] have been proposed to minimize the differences between genotypes. Therefore, we have used vegetative propagation technique to identify and isolate GR and GS individuals from a single field-evolved GR Italian ryegrass population.

For developing effective weed management strategies, it is essential to document the response of GR and GS Italian ryegrass phenotypes to the herbicides used for Italian ryegrass control. Because the variation in herbicide response among accessions or biotypes of an individual weed species has been reported previously [26-28]. Bond et al. [26] reported variation in control of Palmer amaranth (*Amaranthus palmeri*) accessions with registered rate of pyrithiobac (20 to 94%) but not with glyphosate or fomesafen. Differential herbicide response of common waterhemp hemp (*Amaranthus rudis* Sauer) and tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] accessions was observed for control by atrazine, glyphosate, and imazethapyr, but not for fomesafen [27]. Differential sensitivity among red rice (*Oryza sativa* L.) ecotypes was reported for thiobencarb, fenoxaprop, and sethoxydim [29].

An understanding of the basic biology of a resistant phenotype relative to a susceptible phenotype collected from a segregating population may provide beneficial information for developing effective weed management strategies to control GR Italian ryegrass. Therefore, research was conducted in the greenhouse to compare the following: level of resistance in GR and GS phenotypes following glyphosate application; early growth stage interference from GR and GS phenotypes with barley, oat, cereal rye, and wheat; effect of drought stress on GR and GS phenotypes; and response of GR and GS phenotypes to POST application of clethodim, glyphosate, imazapic, paraquat, and pinoxaden.

2. Materials and Methods

All experiments were conducted in a greenhouse maintained at 25±5 C with natural lighting supplemented for 14 h by metal halide lamps (Hubbell Lighting, Inc., Greenville, SC) delivering 400 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density. Plants were grown in round plastic pots (12 cm diam by 10 cm deep) using a commercial potting mix (Fafard 4P potting mix, Conrad Fafard Inc., Agawam, MA) and were irrigated two-three times daily with an automated irrigation system to maintain optimum soil moisture except in the drought recovery experiment. Plants were fertilized with 25 ml pot^{-1} of a 4.6 g L^{-1} fertilizer solution (Scotts Starter Fertilizer, The Scotts Company LLC, Marysville, OH). All experiments were conducted twice until specify.

2.1 Confirmation of Glyphosate Resistance in Italian Ryegrass Population

Italian ryegrass seeds were collected from plants surviving glyphosate at 840 g ae ha^{-1} in spring of 2009 prior to planting soybean in a field near New Salem, NC (35.1235 °N, 80.3789 °W). In 2010, the initial screening was performed in the greenhouse to conform the glyphosate resistance in collected seeds. A known GS population collected from Central Crops Research Station near Clayton, NC (35.6655 °N, 78.5084 °W) was included for comparison. Italian ryegrass seeds were planted, and four plants were established in each pot. Glyphosate at 0, 840, 1,680, and 3,360 g ae ha^{-1} was applied when plants had two to three tillers. The experiment was conducted three times, and treatments were replicated 4 times. Visual estimates of percent control were recorded 21 d after treatment (DAT) using a scale of 0 to 100, where 0 = no control and 100 = complete plant death or control. Plants were sectioned at the soil surface and fresh tiller biomass determined 21 DAT. Percent reduction in fresh tiller biomass was determined relative to non-treated controls for each phenotype.

In 2013, seeds of Italian ryegrass population collected from the field in 2009 was planted in the greenhouse using the procedure described previously, and vegetative clones of individual plants were propagated by tiller partition in order to obtain at least four ramets per plant. When individual ramets developed three to four tillers, half of them from each parent plant were treated with 840 g ha^{-1} glyphosate. At 21 DAT, survivor and dead clones were characterized as GR and GS Italian ryegrass phenotypes, respectively. Glyphosate-resistant and GS clones not exposed to glyphosate were grown to maturity in separate greenhouses and seeds from these plants were collected for use in all subsequent greenhouse studies. This protocol was chosen to obtain GR and GS phenotypes sharing a common genetic background [15]. An assumption was made that outcrossing between GR and GS individuals prior to field collection would ensure homogeneity of fitness affecting traits between GR and GS phenotypes.

2.2 Experiment 1: Glyphosate Dose Response

Eight to ten Italian ryegrass seeds were planted in each pot. Seedlings of both the GR and GS phenotypes were thinned to one plant per pot at 8 d after planting (DAP). Glyphosate at 0, 50, 100, 500, 1000, and 5000 g ha^{-1} was applied when plants were 5 to 8 cm tall with 2 to 3 tillers. Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with 11002 AIXR nozzles (TeeJet Technologies, Wheaton, IL) calibrated to deliver 140 L ha^{-1} of spray solution at 207 kPa. The experimental design was a randomized complete block with eight replications and blocking against plant size.

At 21 DAT, above ground plant material harvested and dried in an oven at 55 C for 3 d to determine dry biomass. Percent reduction in tiller dry biomass was converted relative to the non-treated control for each Italian ryegrass phenotype. Regression analysis were performed using PROC NLIN in SAS (Version 9.3, SAS Institute, Cary, NC) to describe the best fits of the experimental data to appropriate functions [30]. Plots were generated using Sigma-Plot (SigmaPlot 12.0, Systat Software Inc., San Jose, CA).

A three-parameter sigmoidal log-logistic model was fit to describe glyphosate rate effect on percent reduction of tiller dry biomass:

$$Y = a \,/\, (1 + e^{-[(x - X0)/b]}) \qquad (1)$$

In this model, Y is percent reduction of tiller dry biomass, x is glyphosate rate, *a* is the difference of the upper and lower response limits (asymptotes), X_0 is the glyphosate rate giving 50% response, and *b* is the slope of the curve around X_0. Root mean square error (RMSE) and modelling efficiency coefficient (EF) were used to determine the goodness of fit for the model [31].

2.3 Experiment 2: Interference of GR and GS Italian ryegrass Phenotypes with Selected Crops

Six seeds of barley (cultivar unknown), oat cultivar “Gerard 229” (NC Foundation Seed Producers, Inc., Zebulon, NC), cereal rye cultivar “Wrens Abruzzi” (Crop Production Services, Loveland, CO), or wheat cultivar “DG Shirley” [32] and 8 to10 Italian ryegrass seeds were planted per pot. Seedlings were thinned to one crop and one Italian ryegrass plant per pot 10 DAP. Pots containing a single crop or a single Italian ryegrass plant were used as controls. The experimental design was a randomized complete block with treatments replicated 10 times.

Plant height of Italian ryegrass and crop was determined at 5-d intervals from 20 to 45 DAP. Plant height was measured from the soil surface to the point of newest leaf blade or spike of upper most tiller for all crops and Italian ryegrass plants. At 45 DAP, Italian ryegrass and crop plants were severed at the soil surface, and tillers were counted and oven-dried at 55 C for 3 d to measure tiller dry biomass. Percent reduction in height, tiller count, and dry biomass was calculated with respect to the controls of either crop or Italian ryegrass without interference. All data were subjected to analysis of variance (ANOVA) in SAS using PROC MIXED considering the factorial arrangement of four levels of crop (barley, oat, cereal ryegrass, and wheat) and two levels of Italian ryegrass phenotypes (GR and GS). Means of significant main effects and interactions were separated using Fisher’s Protected LSD test at significance level of 0.05.

2.4 Experiment 3: Recovery of GR and GS Italian Ryegrass Phenotypes from Drought Stress

The experiment was conducted using a factorial arrangement of plant type (wheat alone, GR Italian ryegrass alone, GS Italian ryegrass alone, wheat plus GR Italian ryegrass, and wheat plus GS Italian ryegrass) and drought stress durations 0, 4, 6, 8, 10, and 12 d. The experimental design was a randomized complete block with treatments replicated eight times. Each pot contained a similar biomass (175 to 180 g) of potting mix. Six crop seeds and 8 to10 Italian ryegrass seeds were planted in each pot and seedlings were thinned to one crop and one Italian ryegrass plant per pot 10 DAP. Pots contained a single wheat or Italian ryegrass plant where species were grown individually. Before the initiation of drought stress treatments, pots were watered to the field capacity on a daily basis.

Beginning 20 DAP, water was withheld in order to induce drought stress for 0, 4, 6, 8, 10 or 12 d. Soil was brought back to full saturation after completion of the stress period and was thereafter maintained at adequate moisture to ensure optimum growth for the remainder of the experiment. Plant height, tiller count, and biomass were recorded 38 DAP, or 6 d after recovery from the last drought treatment. Tillers of each Italian ryegrass or wheat plant were harvested separately and oven-dried at 55 C for 3 d. Percent reduction in height, tiller count, and tiller dry biomass was calculated with respect to the control for each plant type. Data were subjected to ANOVA in SAS using PROC MIXED appropriate for the factorial arrangement of plant type × duration of drought stress. For percent reduction in wheat height, tiller count, and tiller dry biomass, the plant type had three levels including wheat grown alone, wheat grown with GR Italian ryegrass, and wheat grown with GS Italian ryegrass. For percent reduction in Italian ryegrass height, tiller count, and tiller dry biomass, the plant type had four levels including GR Italian ryegrass grown alone, GS Italian ryegrass grown alone, wheat grown with GR Italian ryegrass, and wheat grown with GS Italian ryegrass. Percent reduction in wheat height, tiller count, and dry biomass were regressed against duration of stress using PROC REG in SAS to fit quadratic models [33, 34]:

$$y = a + bx + cx^2 \tag{2}$$

where y = percent reduction in height, tiller count, or tiller dry biomass, a, b, and c are constants, and x = duration of drought stress in d.

2.5 Experiment 4: Efficacy of Herbicides on GR and GS Italian Ryegrass Phenotypes

Eight seeds of GR or GS Italian ryegrass phenotypes were sown per pot. Seedlings similar in height and number of leaves were thinned to one per pot 10 DAP. Herbicides (Table 1) were applied when plants were 5 to 8 cm tall with 2 to 3 tillers. Herbicide application rates reflect the 0.75X of label rate because of high sensitivity of plants in the greenhouse. A nonionic surfactant (Induce® nonionic surfactant, Helena Chemical Company, Collierville, TN) at 0.25% (v/v) was included with imazapic, and paraquat whereas crop oil concentrate (Agri-Dex® spray adjuvant, Helena Chemical Company, Collierville, TN) at 0.5% (v/v) was included with clethodim, and pinoxaden. No adjuvant was included with glyphosate. A non-treated control was included for comparison. Herbicides were applied using a spray chamber equipped with a single 8002 EVS nozzle (TeeJet Technologies, Wheaton, IL) delivering 140 L ha^{-1} at 207 kPa. Following herbicide application, plants were returned to the greenhouse where irrigation was withheld for 24 h. Treatments were replicated six times in a randomized complete block design with blocking against plant size. At 21 DAT, visual estimates of Italian ryegrass control were made as previously described and tiller dry biomass was determined. Percent reduction in tiller dry biomass was calculated relative to the non-treated control for each Italian ryegrass phenotype. Effect of herbicides,

Table 1 Herbicides used in greenhouse experiment 4.

Herbicide			
Common name	Trade name	Application rates[a]	Manufacturer
Clethodim	Select®	118 g ai ha^{-1}	Valent U.S.A. Corporation, CA; valent.com
Glyphosate	Roundup Weathermax®	710 g ae ha^{-1}	Monsanto Company, St. Louis, MO; monsanto.com
Imazapic	Cadre®	39 g ai ha^{-1}	BASF Corp., Research Triangle Park, NC; basf.com
Paraquat	Parazone®	315 g ae ha^{-1}	ADAMA, Raleigh, NC; adama.com
Pinoxaden	Axial®	42 g ai ha^{-1}	Syngenta Crop Protection LLC; Greensboro, NC; syngentacropprotection-us.com

[a] The use rate of each herbicide represents the 0.75X of manufacturer's recommended field use rate.

Italian ryegrass phenotypes, and their two-way interaction were determined using ANOVA in SAS with PROC MIXED. Means of significant main effects and interactions were separated using the Fisher's Protected LSD test at the significance level of 0.05.

3. Results and Discussion

The interactions for experiment run × treatment were not significant in any experiment; therefore, data were pooled over experiment runs.

3.1 Confirmation of Glyphosate Resistance in Italian Ryegrass Population

Visual control and percent reduction in tiller fresh biomass were 37% and 40%, respectively, when glyphosate was applied at 840 g ha^{-1} to suspected GR Italian ryegrass plants compared with 99% reduction in both parameters for the known GS Italian ryegrass population (Table 2). A difference in visual control and percent reduction in tiller fresh biomass was also noted between populations when glyphosate was applied at 1,680 g ha^{-1} but not when applied at 3,360 g ha^{-1}. These results demonstrated the conformation of GR in the Italian ryegrass population collected from field during 2009.

3.2 Experiment 1: Glyphosate Dose Response

An interaction of phenotype × glyphosate rate was noted for Italian ryegrass tiller dry biomass reduction ($P < 0.0001$). The GR_{50} (glyphosate rate providing 50% reduction in tiller dry biomass) was 3.2-fold greater for the GR phenotype (770 g ha^{-1}) than the GS phenotype (239 g ha^{-1}) (Fig. 1 and Table 3). Varying levels of glyphosate resistance among Italian ryegrass phenotypes have been reported previously, depending

Table 2 Percent visual control and tiller fresh biomass reduction of Italian ryegrass progeny of plants surviving glyphosate 840 g ha^{-1} in the field and a known GS population in the greenhouse during 2010.

	Visual control		Tiller fresh biomass reduction	
Glyphosate	Known GS population	Suspected GR progeny from the field	Known GS population	Suspected GR progeny from the field
g ae ha^{-1}	%			
840	99 a	37 c	99 a	40 c
1,680	100 a	84 b	100 a	76 b
3,360	100 a	99 a	100 a	97 a

[a]Means within a column followed by the same letter are not different according to Fisher's Protected LSD ($\alpha = 0.05$). Data are pooled over three runs of the experiment.

Table 3 Parameter estimates and the goodness of fit (RMSE and EF)[a] of the three-parameter log-logistic model[b] fitted to Italia ryegrass phenotypes for percent tiller dry biomass after glyphosate application (with 95% confidence interval in parenthesis) (experiment 1).

Phenotype	A	X_0	b	RSME	EF	GR^c_{50} g ai ha^{-1}
	Percent tiller dry biomass					
GR Italian ryegrass	97.8 (97-98)	2.9 (2.8-2.9)	0.11 (0.08-0.11)	0.02	0.99	770
GS Italian ryegrass	100.7 (81-120)	2.4 (2.1-2.6)	0.28 (0.12-0.45)	2.74	0.99	239

[a] Abbreviations: RMSE, root mean square error; EF, modelling efficiency coefficient.

[b] $Y = a / (1 + e^{-[(x-X_0)/b]})$, where a is the difference of the upper and lower response limits (asymptotes), X_0 is the glyphosate rate giving 50% response, and b is the slope of the curve around X_0.

[c] GR_{50} glyphosate rate g ai ha^{-1} (back transformed) required for 50% reduction in tiller dry biomass.

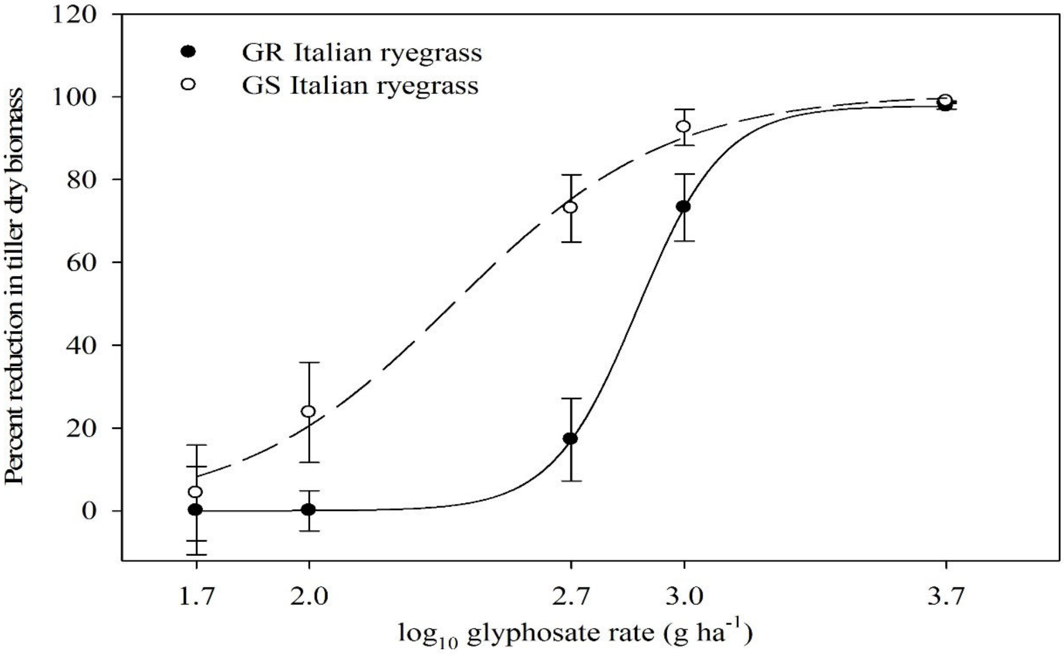


Fig. 1 Percent tiller dry biomass reduction of GR and GS Italian ryegrass phenotypes 21 d after glyphosate application (experiment 1). Data are pooled over two experiment runs and points are means ± SE.

upon location and measured response variables. In California, GR Italian ryegrass populations were between 2- and 15-fold resistant to glyphosate based upon the amount of glyphosate needed to reduce shoot biomass by 50% [5]. Perez-Jones et al. [9] reported a 5-fold level of glyphosate resistance in an Italian ryegrass population in Oregon based on shoot fresh biomass reduction. Salas et al. [7] reported a 7- to 13-fold level of glyphosate resistance in Arkansas based on visual injury.

3.3 Experiment 2: Interference of GR and GS Italian Ryegrass Phenotypes with Crops

Height, tiller count, and tiller dry biomass of the two Italian ryegrass phenotypes grown in the absence of a crop were similar. Heights, tiller count, and tiller dry biomass of GR and GS phenotypes at 45 DAP in the absence of a crop were 50.6 cm, 33.2 plant^{-1}, and 4.9 g plant^{-1} and 46.2 cm, 35.7 plant^{-1}, and 4.8 g plant^{-1}, respectively (data not presented). In the absence of Italian ryegrass, heights of barley, oat, cereal rye, and wheat were 41.9, 66.6, 35.5, and 33.7 cm, respectively, tiller counts were 30.4, 10.4, 50.1, and 41.8 plant^{-1}, respectively, and dry tiller biomass values were 8.0, 4.4, 6.3, and 6.0 g plant^{-1}, respectively (data not presented).

3.3.1 Effect of Italian Ryegrass on Crops

The interaction of crop × phenotype ($P = 0.1001$ to 0.6550) and the main effect of phenotype ($P = 0.1743$ to 0.9539) were not significant for percent reduction in crop height, tiller count, or tiller dry biomass. Averaged over crops, no differences in crop height, tiller count, and tiller dry biomass reduction were noted between Italian ryegrass phenotypes (Table 4). This indicates that both Italian ryegrass phenotypes were similarly competitive against crops.

The magnitude of Italian ryegrass interference varied among crops. Averaged over Italian ryegrass phenotypes, crop height reduction due to Italian ryegrass interference was similar for all crops across evaluation timings (Table 4). At 45 DAP, Italian ryegrass reduced barley, oat, cereal rye, and wheat height 7, 5, 5, and 4%, respectively. Tiller count for barley and cereal rye was reduced 19% compared with 25 to 28% reduction for oat and wheat. Dry biomass of tillers for barley, oat, and cereal rye was reduced similarly (14 to 20%) and less than the 28% reduction in wheat tiller dry biomass. These differences in crop response were expected due to the crops' inherent variation in growth and ability to compete with weeds [13, 35, 36, 37].

3.3.2 Effect of Crops on Italian Ryegrass

The interaction of phenotype × crop was not significant for height, tiller count, or tiller dry biomass reduction, indicating Italian ryegrass phenotypes responded similarly to crop interference. However, the main effect of phenotype was significant only for Italian ryegrass height reduction (P = 0.0005 to 0.0224). The GR phenotype experienced a greater height reduction compared to GS phenotype 25 through 45 DAP (Table 5), suggesting a disadvantage in competition with crops for glyphosate resistance in Italian ryegrass. However, the main effect of phenotype was not significant for Italian ryegrass tiller count (P = 0.9622) or dry biomass reduction (P = 0.2469), and similar level of reduction in both variables was reported for both GR and GS phenotypes from crop interference. The main effect of crop was not significant for reduction of Italian ryegrass height at 25

Table 4 Percent reduction in crop height, tiller count, and tiller dry biomass with respect to the controls of crops without interference as influenced by Italian ryegrass phenotype and crop (experiment 2)[a].

	Height							Tiller dry
Main effect	20 DAP[b]	25 DAP	30 DAP	35 DAP	40 DAP	45 DAP	Tiller count	biomass
	%							
Phenotype (P)[b]	11 a	10 a	8 a	6 a	5 a	5 a	24 a	22 a
GR Italian ryegrass	11 a	10 a	8 a	6 a	5 a	5 a	24 a	22 a
GS Italian ryegrass	12 a	8 a	8 a	7 a	6 a	6 a	21 a	18 a
Crop (C)								
Barley	9 a	8 a	5 a	6 a	5 a	7 a	19 b	18 b
Oat	10 a	7 a	9 a	8 a	7 a	5 a	28 a	14 b
Cereal rye	17 a	9 a	7 a	4 a	6 a	5 a	19 b	20 b
Wheat	11 a	11 a	9 a	7 a	3 a	4 a	25 a	28 a
P × C (P value)	0.6550	0.1056	0.1735	0.1001	0.5425	0.1008	0.6481	0.6857

[a] Data pooled over two experiment runs. Means within columns for main effects (phenotype or crop) followed by the same letter are not different according to Fisher's Protected LSD (α = 0.05).

[b] Abbreviations: DAP, days after planting; GR, glyphosate-resistant; GS, glyphosate-susceptible.

Table 5 Percent reduction in Italian ryegrass height, tiller count, and tiller dry biomass with respect to the controls of Italian ryegrass without interference as influenced by Italian ryegrass phenotype and crop (experiment 2)[a].

	Height							Tiller dry
Main effect	20 DAP[b]	25 DAP	30 DAP	35 DAP	40 DAP	45 DAP	Tiller count	biomass
	%							
Phenotype (P)[b]								
GR Italian ryegrass	18 a	20 a	23 a	21 a	21 a	17 a	41 a	56 a
GS Italian ryegrass	17 a	12 b	14 b	14 b	11 b	9 b	41 a	51 a
Crop (C)								
Barley	16 a	17 a	23 a	24 a	22 a	15 a	45 a	57 a
Oat	20 a	19 a	19 a	17 a	16 a	14 a	34 a	47 a
Cereal rye	21 a	16 a	17 a	15 a	14 a	12 a	44 a	55 a
Wheat	12 a	12 a	15 a	15 a	13 a	11 a	42 a	55 a
P × C (P value)	0.8532	0.3589	0.3477	0.6583	0.7036	0.7320	0.5820	0.6923

[a] Data pooled over two experiment runs. Means within columns for main effects (phenotype or crop) followed by the same letter are not different according to Fisher's Protected LSD (α = 0.05).

[b] Abbreviations: DAP, days after planting; GR, glyphosate-resistant; GS, glyphosate-susceptible.

through 45 DAP (P = 0.0745 to 0.6225), tiller count (P = 0.1486), and tiller dry biomass (P = 0.2975). Barley, oat, cereal rye, and wheat interference caused a similar reduction in both GR and GS Italian ryegrass height, tiller count, and tiller dry biomass. The potential impact of greater height reduction for GR phenotype compared with GS phenotype after 45 d or a field setting could be informative in determining a possible fitness penalty associated with GR. This is one of the limitations of our work.

3.4 Experiment 3: Recovery of GR and GS Italian Ryegrass Phenotypes from Drought Stress

When grown alone under no drought stress, height (21.2 and 22.5 cm, respectively), tiller count (28.3 and 28.8 plant^{-1}, respectively), and tiller dry biomass (1.3 and 1.2 g plant^{-1}, respectively) of GR and GS Italian ryegrass phenotypes were similar at 38 DAP. Wheat had only minor effects on the height of Italian ryegrass phenotypes (1 to 17% reduction) but reduced tiller count and dry biomass by 43 to 45% and 53 to 61%, respectively under no drought stress (data not presented). When grown alone under no drought stress, height, tiller count, and tiller dry biomass of wheat were 26.4 cm, 17.6 plant^{-1}, and 2.0 g plant^{-1} at 38 DAP. The two Italian ryegrass phenotypes similarly reduced wheat height (1 to 3%), tiller count (1 to 5%), and tiller dry biomass (15%) under no drought stress (data not presented).

3.4.1 Effect of Drought Stress on Italian Ryegrass Interference with Wheat

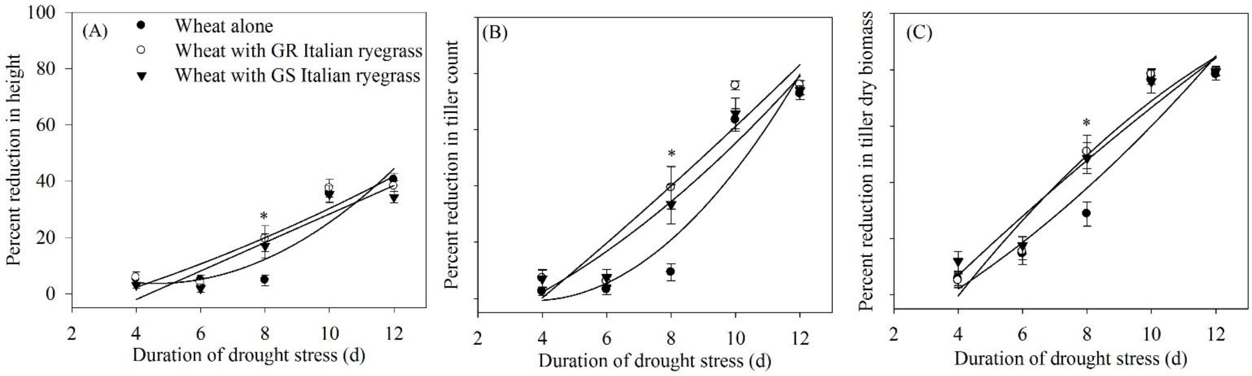
Interactions of duration of drought stress and plant type were significant for wheat height (P = 0.0082), tiller count (P = 0.0003), and tiller dry biomass (P = 0.0009) reduction. The interaction occurred because wheat height, tiller count, and tiller dry biomass reductions were similar with and without Italian ryegrass for drought stress durations of 4, 6, 10, and 12 d whereas drought stress for 8 d had less impact on wheat grown alone compared with wheat grown with Italian ryegrass (Fig. 2). Wheat height, tiller count, and tiller dry biomass reductions increased as duration of drought increased, with a greater impact on tiller count and tiller dry biomass than height. Averaged over plant type, drought stress for 12 d reduced wheat height, tiller count, and tiller dry biomass 38, 74, and 79%, respectively.

3.4.2 Effect of Drought Stress on Wheat Interference with Italian Ryegrass

The interaction of duration of drought stress and plant type was significant for Italian ryegrass height (P < .0001), tiller count (P < .0001), and tiller dry biomass (P = 0.0004) reduction. Italian ryegrass height, tiller count, and tiller dry biomass reductions increased as duration of drought increased, with a greater impact on tiller dry biomass and tiller count than height (Fig. 3). The interaction occurred because height and tiller dry biomass reduction in Italian ryegrass phenotypes was similar when grown with and without wheat for 4 and 12 d of drought stress whereas a greater reduction in both variables was noted with 6 to 10 d of drought stress when Italian ryegrass phenotypes were grown with wheat compared to being grown alone. However, tiller count reduction of Italian ryegrass phenotypes was similar when grown either alone or with wheat with drought stress durations of 4, 6, and 12 d. Tiller count reduction of Italian ryegrass phenotypes was greater when grown with wheat as compared to grown alone with drought stress durations of 8 and 10 d. Due to competition for resources (space, light, water, and nutrients), greater reduction in height, tiller count, and tiller dry biomass of plants was expected when growing both wheat and Italian ryegrass together than each growing alone [34].

3.5 Experiment 4: Efficacy of Herbicides for GR and GS Italian Ryegrass Phenotypes

The interaction of phenotype × herbicide for Italian ryegrass control and tiller dry biomass reduction was significant (P < 0.0001) (Table 6). The two phenotypes responded similarly to all herbicides except glyphosate. Glyphosate controlled the GS phenotype 67% and reduced tiller dry biomass 74% (Table 6). In contrast, glyphosate controlled the GR phenotype only 19% and reduced tiller dry biomass only 35%. Clethodim, paraquat, and pinoxaden controlled both phenotypes ≥ 99% and reduced tiller dry biomass ≥ 98% (Table 6). Similarly, Kuk et al. [38] reported 100% control of Italian ryegrass with pinoxaden at 60 g ha^{-1} and clethodim at 140 g ha^{-1} when applied POST in the greenhouse. Nandula et al. [39] reported almost complete control (≥ 98%) of Italian ryegrass by POST-applied clethodim at 140 g ha^{-1} and paraquat at 700 g ha^{-1}. Jordan et al. [40] reported Italian ryegrass control of no more than 60% 4 wk after treatment (WAT) with paraquat at 530 g ha^{-1} applied at four-leaf or jointing stages in field. They also reported that same rate of paraquat controlled Italian ryegrass 87% when applied 10 to 20 cm tall (tillering) plants. Imazapic at 39 g ha^{-1} controlled Italian ryegrass at least 74% and reduced tiller dry biomass at least 79%, with no differences among phenotypes. Clemmer et al. [41]

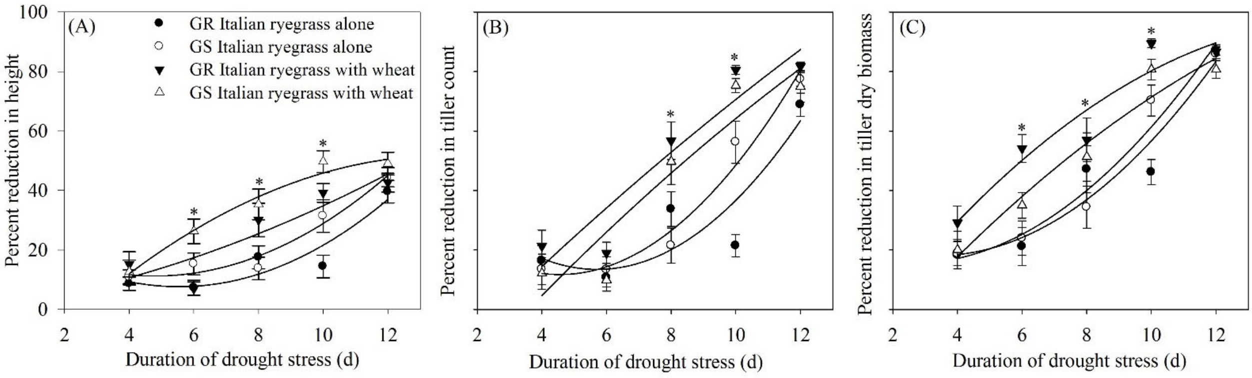


wheat alone $Y = 19 - 6.9x + 0.7x^2$, $R^2 = 0.86$
wheat with GR $Y = -10 + 2.8x + 0.13x^2$, $R^2 = 0.89$
wheat with GS $Y = -16 + 3.8x + 0.06x^2$, $R^2 = 0.87$

wheat alone $Y = 15 - 8.6x + 1.16x^2$, $R^2 = 0.89$
wheat with GR $Y = -36 + 8.7x + 0.10x^2$, $R^2 = 0.89$
wheat with GS $Y = -17 + 3.4x + 0.38x^2$, $R^2 = 0.93$

wheat alone $Y = -22 + 4.8x + 0.35x^2$, $R^2 = 0.91$
wheat with GR $Y = -65 + 18.1x - 0.46x^2$, $R^2 = 0.94$
wheat with GS $Y = -38 + 11.9x - 0.14x^2$, $R^2 = 0.93$

Fig. 2 Percent reduction in wheat (A) plant height, (B) tiller count, and (C) tiller dry biomass as affected by plant type and duration of drought stress (experiment 3). Data pooled over two experiment runs and points are means ± SE. Asterisk (*) indicates significant difference between plant type for each duration of drought stress based on Fisher's Protected LSD (α = 0.05).



GR alone $Y = 29 - 7.8x + 0.70x^2$, $R^2 = 0.86$
GS alone $Y = 26 - 6.2x + 0.65x^2$, $R^2 = 0.95$
GR with wheat $Y = 1 + 1.7x + 0.16x^2$, $R^2 = 0.81$
GS with wheat $Y = -27 + 11.4x - 0.41x^2$, $R^2 = 0.97$

GR alone $Y = 54 - 14.4x + 1.2x^2$, $R^2 = 0.79$
GS alone $Y = 37 - 11.2x + 1.2x^2$, $R^2 = 0.97$
GR with wheat $Y = -27 + 11x - 0.12x^2$, $R^2 = 0.88$
GS with wheat $Y = -42 + 12x - 0.18x^2$, $R^2 = 0.88$

GR alone $Y = 28 - 5.8x + 0.87x^2$, $R^2 = 0.92$
GS alone $Y = 20 - 4.2x + 0.82x^2$, $R^2 = 0.96$
GR with wheat $Y = -22 + 14.9x - 0.4x^2$, $R^2 = 0.91$
GS with wheat $Y = -30 + 13.2x - 0.3x^2$, $R^2 = 0.95$

Fig. 3 Percent reduction in Italian ryegrass (A) height, (B) tiller count, and (C) tiller dry biomass as affected by plant type and duration of drought stress (experiment 3). Data pooled over two experiment runs and points are means ± SE. Asterisk (*) indicates significant difference between plant type for each duration of drought stress based on Fisher's Protected LSD (α = 0.05).

Table 6 Percent visual control and reduction in tiller dry biomass of Italian ryegrass as influenced by phenotypes and POST rates 21 d after treatment (experiment 4).[a]

Herbicide	Rates[a]	Control		Tiller dry biomass	
		GR Italian ryegrass	GS Italian ryegrass	GR Italian ryegrass	GS Italian ryegrass
		%		% reduction of non-treated	
Glyphosate	710 g ae ha^{-1}	19*	67	35*	74
Paraquat	315 g ae ha^{-1}	100	100	99	99
Clethodim	118 g ai ha^{-1}	99	99	99	99
Pinoxaden	42 g ai ha^{-1}	99	99	98	98
Imazapic	39 g ai ha^{-1}	74	84	79	87

[a] Asterisk (*) indicates significance at Fisher's Protected LSD ($\alpha = 0.05$) within phenotypes for each herbicide. Data pooled over two experiment runs.

[b] Abbreviations: DAP, days after planting; GR, glyphosate-resistant; GS, glyphosate-susceptible.

[c] The use rate of each herbicide represents the 0.75X of manufacturer's recommended field use rate.

reported 97 to 100% control of Italian ryegrass at 10 WAT with POST applied imazapic at 70 g ha^{-1}. The similar response of both GR and GS Italian ryegrass phenotypes to clethodim, imazapic, paraquat, and pinoxaden indicate that the presence of glyphosate resistance in Italian ryegrass does not influence their susceptibility to these herbicides. Clethodim, imazapic, paraquat, and pinoxaden can use to effective control both GR and GS Italian ryegrass.

Overall, these results show that GR and GS Italian ryegrass phenotypes collected from a segregating population most likely will not respond differently under drought stress or control with herbicides currently used in several economically important agronomic crops. However, with early season crop interference, tiller count and biomass of Italian ryegrass did not differ between phenotypes, but height reduction was greater for the GR phenotype compared with the GS phenotype regardless of crop. A greater reduction in height for the GR phenotype suggests resistance may impart a disadvantage when the GR phenotype is in competition with crops. Additional research is needed to determine if the greater height reduction for GR phenotype compared with GS phenotype would translate into difference in Italian ryegrass seed production.

Similar research by Chaudhari et al. [33] reported no significant differences for plant height and shoot biomass of GR and GS Palmer amaranth phenotypes obtained from a single population when subjected to drought stress, grown with corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.), or treated with atrazine, dicamba, fomesafen, glufosinate, paraquat, tembotrione, thifensulfuron, or 2,4-D. Glyphosate-resistant and –susceptible giant ragweed (*Ambrosia trifida*) from Indiana grown in field were found to have similar height, leaf area, and shoot biomass accumulation [42]. Pedersen et al. [15] also observed no significant differences in biomass accumulation and competitiveness of GR and GS rigid ryegrass phenotypes collected from a single population, but they reported that in the absence of crop competition or at low wheat densities, GS phenotype exhibited a potential fitness advantage and produced more seeds than GR plants. However, this difference in seed production between these phenotypes was not evident with increase on the intensity of competition or at higher crop densities. In contrast, Yanniccari et al. [17] demonstrated greater reduction in height, leaf blade area, shoot biomass, seed number, and total seed mass of GR perennial ryegrass compared to GS phenotypes, suggesting that the resistant biotype was less fit and its frequency would decrease over time in the absence of glyphosate.

The presented results should be interpreted carefully because these studies were conducted using small pots in controlled environment where limited ecological interactions occurred. It has been well studied that pot size can influence the plant growth, and growth reduction in smaller pots compared to larger pots may reported mainly due to a decrease in number of physiological processes including nutrient efficiency, photosynthesis, and/or transpiration [43, 44]. A number of factors including growth stage, environmental stress, intensity and nature of competitive interactions, genetic background of resistance trait, physiological mechanism of resistance, and weed management practices has been reported to play an important role to influence the magnitude of a fitness penalty [6, 15, 19, 45, 46]. Future research should conduct to address the growth and productivity of GR Italian ryegrass, relative to the GS phenotypes, under competitive and across diverse environmental gradients.

4. Conclusions

Results from these experiments do not describe presence or absence of a fitness penalty for glyphosate resistance in Italian ryegrass because fecundity was not determined. Thus, it would be difficult to determine the impact of weed management practices on frequency of GR Italian ryegrass population over time. The comparison of seed production among the phenotypes (not measured in this study) would have been more useful, although plant biomass and seed production are often highly correlated [47]. Therefore, additional work is necessary to determine whether differences in late-season vegetative growth and fecundity exist between GR and GS Italian ryegrass phenotypes before predictions can be made regarding the likelihood of persistence and prevalence of the GR phenotype in the absence of glyphosate selection pressure. However, no other research is published in the peer-reviewed literature addressing this topic for Italian ryegrass. These results indicate that in the absence of glyphosate selection pressure, resistance to glyphosate does not influence the basic biology and management of GR and GS Italian ryegrass phenotypes collected from a segregating population. Further, the present research findings can be useful in the development of resistant evolution simulation models to identify potential management strategies to prevent and/or contain the spread of glyphosate resistance. These models would lead to more accurate predictions towards the impact of different weed management strategies on control of GR and GS Italian ryegrass populations.

Conflicts of Interest

None of the authors has a conflict of interests in terms of the products mentioned in the paper.

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