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Differential Response to Glyphosate in Italian Ryegrass (*Lolium multiflorum*) Populations from Mississippi

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Two Italian ryegrass populations from Mississippi, Tribbett and Fratesi, were suspected to be tolerant to glyphosate. A third population from Mississippi, Elizabeth, known to be susceptible to glyphosate, was included for comparison. Plants were treated with the isopropylamine salt of glyphosate at 0, 0.11, 0.21, 0.42, 0.84, 1.68, 3.36, and 6.72 kg ae/ha. GR_{50} (herbicide dose required to cause a 50% reduction in plant growth) values for the Tribbett, Fratesi, and Elizabeth populations were 0.66, 0.66, and 0.22 kg/ha, respectively, indicating that the Tribbett and Fratesi populations were threefold more tolerant to glyphosate compared with the Elizabeth population. These three populations were also treated with diclofop at 0, 0.13, 0.25, 0.5, 0.75, 1, and 2 kg ai/ha. Diclofop GR_{50} values for the Tribbett, Fratesi, and Elizabeth populations were 0.25, 0.28, 0.21 kg/ha, respectively, indicating similar tolerance to diclofop in the three populations. Response of all three populations to clethodim rate (0, 0.02, 0.03, 0.05, 0.06, 0.08, 0.09, and 0.13 kg ai/ha) was measured. Clethodim GR_{50} values for the Tribbett, Fratesi, and Elizabeth populations at the small growth stages were 0.016, 0.023, 0.014 kg/ha, respectively, and at the large growth stage were 0.04, 0.034, 0.02 kg/ha, respectively.

Nomenclature: Clethodim; diclofop; glyphosate; Italian ryegrass, *Lolium multiflorum* Lam. LOLMU.

Key words: Alternative control options, glyphosate resistance, herbicide resistance, resistance management, weed resistance.

Glyphosate is a nonselective, POST herbicide used extensively throughout the world during the past three decades. Because of its lack of selectivity, glyphosate use was initially limited to preplant, postdirected, and postharvest applications for weed control (Franz et al. 1997). With the introduction of glyphosate-resistant (GR) crops in the mid-1990s, glyphosate is now widely used for weed control in GR crops. Rapid adoption of GR crops by U.S. farmers (Duke et al. 2003) and glyphosate price reduction in recent years have increased glyphosate use dramatically (Nandula et al. 2005). The intensive use of glyphosate and continued adoption of GR crops without rotation to non-GR crops has increased the selection pressure to evolve resistance in certain weed populations. To date, 11 weed species have evolved resistance to glyphosate (Heap 2006; Nandula et al. 2005). They are rigid ryegrass (*Lolium rigidum* L.) in Australia (Powles et al. 1998; Pratley et al. 1999) and the United States (Simarmata et al. 2003), goosegrass [*Eleusine indica* (L.) Gaertn.] in Malaysia (Lee and Ngim 2000; Tran et al. 1999), horseweed [*Conyza canadensis* (L.) Cronq.] in the United States (Koger et al. 2004; Mueller et al. 2003; VanGessel 2001), Italian ryegrass (*Lolium multiflorum* L.) in Chile (Pérez and Kogan 2003) and the United States (Perez-Jones et al. 2005), common waterhemp (*Amaranthus rudis* Sauer) in the United States (Heap 2006; Owen and Zelaya 2005), common ragweed (*Ambrosia artemisiifolia* L.) in the United States (Sellers et al. 2005), Palmer amaranth (*Amaranthus palmeri* S. Wats) in the United States (Culpepper et al. 2006), hairy fleabane (*Conyza bonariensis* L.) in South Africa and Spain (Heap 2006), buckhorn plantain (*Plantago lanceolata* L.) in South Africa (Heap 2006), wild poinsettia (*Euphorbia heterophylla* L.) in Brazil (Heap 2006), and johnsongrass [*Sorghum halepense* (L.) Pers.] in Argentina (Heap 2006). Among the 11 species, common ragweed, common waterhemp, horseweed, johnsongrass, Palmer amaranth, rigid ryegrass, and wild poinsettia have evolved resistance to glyphosate in row crops.

The first evidence of evolved resistance to glyphosate in a weed species was reported by Powles et al. (1998). A rigid ryegrass population from an orchard in Australia receiving two or three glyphosate applications per year for 15 yr exhibited a 7- to 11-fold resistance compared with a susceptible population. In a second report on GR rigid ryegrass from Australia, a ryegrass biotype selected from a population exposed to glyphosate applications for 15 yr was nearly 10-fold more resistant compared with a susceptible biotype (Pratley et al. 1999). Italian ryegrass populations from a fruit orchard in Chile exposed to three glyphosate applications annually for 8 to 10 yr evolved two- to fourfold resistance compared with a susceptible population (Pérez and Kogan 2003). An Italian ryegrass population selected from an orchard in Oregon where glyphosate effectively controlled weeds during the previous 15 yr exhibited a fivefold tolerance to glyphosate compared with a susceptible population (Perez-Jones et al. 2005). This population was previously reported to survive glyphosate at 1.68 kg ae/ha (Perez-Jones et al. 2004).

Italian ryegrass is an erect winter annual with a biennial-like growth habit. It grows vigorously in winter and early spring and is highly competitive. Recently, unacceptable control of Italian ryegrass with glyphosate has been observed in selected populations in Mississippi. Glyphosate-tolerant Italian ryegrass populations could jeopardize preplant burndown options in reduced-tillage crop production systems. The

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objectives of this study were to determine if the Italian ryegrass populations were tolerant to glyphosate, and if so, the level of tolerance. Field observations indicated tolerance of the Italian ryegrass populations to diclofop. Hence the Italian ryegrass populations were also examined for tolerance to diclofop as well as clethodim, another herbicide with a similar mode of action to diclofop. An additional objective was to evaluate nonglyphosate herbicide treatments for Italian ryegrass control.

Materials and Methods

Seed Selection, Storage, Germination, Planting, Growth, and Treatment Conditions. Three Italian ryegrass populations, Tribbett and Fratesi, suspected to be tolerant to glyphosate, and Elizabeth, a glyphosate-susceptible population with no known prior exposure to glyphosate, were selected for comparisons. The Fratesi population was collected from a field where GR soybean was grown from 2002 to 2005. This field was treated with glyphosate or glyphosate + (2,4-dichlorophenoxy)acetic acid as a preplant burndown treatment followed by glyphosate for in-season weed control from 2002 to 2005. The Tribbett population was collected from a field planted to GR cotton in 2001 to 2003 and planted to GR soybean in 2004 and 2005. Reduced control of Italian ryegrass has been observed since 2002 at the Tribbett population site. The Elizabeth population was collected from an abandoned home garden site with no history of herbicide applications. All three sites are located near Stoneville (33°22′30″N, 90°52′30″W), Washington County, MS. Italian ryegrass seed was collected in summer of 2005, air-dried in the greenhouse (28/20 C day/night) for 48 h, and stored in a freezer at 0 C until further use. Italian ryegrass seeds were planted at 1-cm depth in 50-cm by 20-cm by 6-cm plastic trays containing a commercial potting mix.[1] Plants were grown in a growth chamber maintained at 12/6 C day/night temperatures with a 13-h photoperiod. Fluorescent and incandescent lamps were utilized to produce a photosynthetic photon flux density of 600 $\mu mol/m^2\ s^{-1}$. Two weeks after emergence, Italian ryegrass plants were transplanted into 6-cm by 6-cm by 6-cm pots containing the soil mix mentioned before and placed in a greenhouse under natural sunlight conditions. Temperatures during day and night were 20 ± 4 and 15 ± 2 C, respectively. Day length averaged 12 h through the duration of the experiments. Plants were fertilized once with a nutrient solution[2] containing 200 mg/L each of N, P_2O_5, and K_2O at 1 wk after transplanting and subirrigated as needed. Herbicide treatments were applied to Italian ryegrass plants at selected growth stages with a moving-nozzle sprayer equipped with 8002E[3] nozzles delivering 140 L/ha at 280 kPa. Italian ryegrass injury was estimated on a scale of 0 (no injury) to 100% (plant death) at 4 wk after treatment. Treated plants were also evaluated for survival on the basis of live/dead growing point and tillers. Percentage survival of Italian ryegrass populations are reported to estimate the potential of the plant to reaching maturity and produce seed.

Glyphosate Dose–Response Experiment. Plants at the 10- to 15-cm-tall (three to six leaves, two to three tillers) stage were treated with glyphosate (0, 0.11, 0.21, 0.42, 0.84, 1.68, 3.36, 6.72 kg ae/ha) using an isopropylamine (IPA) formulation.[4] The IPA formulation does not contain surfactant, so adjuvant overload would be avoided in the spray mix at higher rates of glyphosate. The 0.84 kg/ha rate corresponds to the 1× registered rate for preplant burndown applications. A nonionic surfactant (NIS) at 0.25% v/v was added to all treatments.

Diclofop Dose–Response Experiment. Field observations and anecdotal reports have indicated less-than-satisfactory control with diclofop of certain Italian ryegrass populations. Response of the three Italian ryegrass populations to diclofop rate at two growth stages, small (8- to 10-cm tall, two to three leaves, two to three tillers) and large (15- to 20-cm tall, four to five leaves, 10 to 12 tillers), was evaluated. Preliminary evaluations indicated no difference between the levels of Italian ryegrass control at the two growth stages by diclofop. Hence, dose–response experiments are reported with only the small growth stage. Diclofop was applied at 0, 0.13, 0.25, 0.5, 0.75, 1, and 2 kg ai/ha.

Clethodim Dose Response. In addition to diclofop, clethodim was evaluated. Mode of action of clethodim is similar to diclofop, i.e., inhibition of the enzyme acetyl coenzyme A carboxylase. Italian ryegrass plants from the three populations were treated with clethodim at 0, 0.02, 0.03, 0.05, 0.06, 0.08, 0.09, and 0.13 kg ai/ha at both small and large growth stages described previously. A crop oil concentrate (COC) at 1.7% v/v was added to all the clethodim treatments.

Alternative Herbicide Options Experiment. Alternative, non-glyphosate herbicides were also evaluated for efficacy on the three Italian ryegrass populations. Diclofop at 0.56 and 1.12 kg ai/ha, mesosulfuron at 0.015 kg ai/ha, clethodim at 0.11 and 0.14 kg ai/ha, nicosulfuron + rimsulfuron at 0.027 + 0.013 kg ai/ha, glufosinate at 0.47 and 0.58 kg ai/ha, and paraquat at 0.7 and 0.98 kg ai/ha were applied to Italian ryegrass plants at the two growth stages as described in diclofop dose–response experiment. Glyphosate at 0.84 and 1.68 kg ae/ha was also included for comparison. A NIS at 0.25% v/v with glyphosate, ammonium sulfate at 3.4 kg/ha with mesosulfuron, methylated soybean oil at 1.3% v/v with nicosulfuron + rimsulfuron, and COC at 1.7% v/v with clethodim was included.

Statistical Analysis. All experiments were conducted using a completely randomized design and experiments were repeated once. There were six replications per treatment in the glyphosate dose–response experiment and four replications per treatment in all other experiments. All data were analyzed by ANOVA. No significant experiment effect was observed in all experiments; therefore, data from experiments were pooled. Nonlinear regression analysis determined the effect of glyphosate, diclofop, and clethodim rate on control of each Italian ryegrass population. A sigmoidal log-logistic model (Seber and Wild 1989) was used to relate percentage control (Y) to herbicide rate (x).

$$Y = \frac{a}{1 + e^{-(x - X_0)/b}} \qquad [1]$$

In this equation, a is the difference of the upper and lower response limits (asymptotes), X_0 is the herbicide rate that

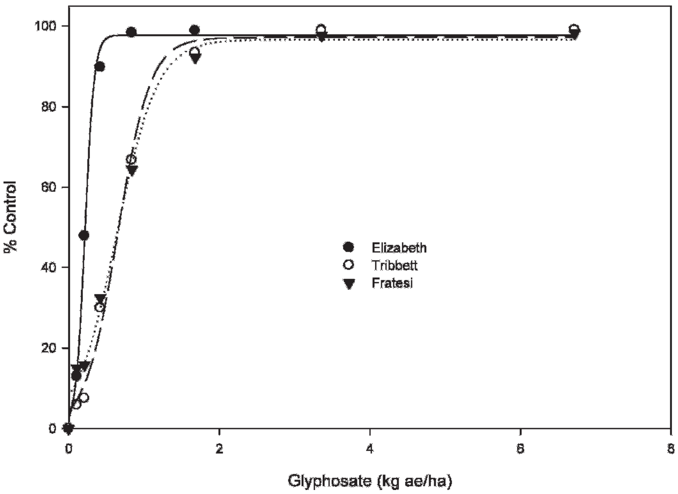

Figure 1. Response of two glyphosate-tolerant Italian ryegrass populations (Tribbett and Fratesi) and one susceptible population (Elizabeth) at the 10- to 15-cm-tall (three to six leaves, two to three tillers) growth stage to glyphosate 4 wk after treatment. Mean values are plotted. Log-logistic dose–response curves:

Elizabeth $Y = \frac{98}{1 + e^{-(x - 0.06)/0.27}}$ $R^2 = 0.81, I_{50} = 0.22$ kg ae/ha

Tribbett $Y = \frac{97}{1 + e^{-(x - 0.65)/0.22}}$ $R^2 = 0.86, I_{50} = 0.66$ kg ae/ha

Fratesi $Y = \frac{97}{1 + e^{-(x - 0.64)/0.27}}$ $R^2 = 0.81, I_{50} = 0.66$ kg ae/ha

results in a 50% reduction in biomass (GR_{50}), and b is the slope of the curve around X_0. The regression parameters from the response curves of the Italian ryegrass populations were computed[5] and compared for significant differences with a sum-of-squares reduction test. Means from the alternative herbicide treatments study were separated using Fisher's Protected LSD test at P = 0.05. Survival data from glyphosate and diclofop dose–response experiments were analyzed by Mantel–Haenszel chi-square test for significance of slope assuming a linear association between herbicide dose and survival. Survival data from clethodim dose–response experiments were analyzed by regression analysis using Fisher's Exact test assuming normal distribution.

Results and Discussion

Glyphosate Dose Response. Response of the Italian ryegrass populations to glyphosate rate is shown in Figure 1. The Tribbett, Fratesi, and Elizabeth populations had GR_{50} values of 0.66, 0.66, and 0.22 kg/ha, respectively. Both Tribbett and Fratesi populations were threefold more tolerant to glyphosate compared with the Elizabeth population. The level of glyphosate tolerance we report is lower than the fivefold tolerance level observed in Italian ryegrass in Chile (Perez and Kogan 2003) and Oregon (Perez-Jones et al. 2005), and in rigid ryegrass in Australia (Powles et al. 1998; Pratley et al. 1999), which was 7- to 11-fold. It should be noted, however, that plants from another known susceptible population appeared to be more susceptible to glyphosate than the Elizabeth population, which could have increased the level of tolerance. This population was not included in our analysis because of prior exposure to herbicides. Italian ryegrass plants from the Elizabeth population did not survive when treated with glyphosate at 0.84 kg/ha or higher (Table 1). However, the Tribbett and Fratesi populations survived glyphosate rates up to 0.84 and 1.68 kg/ha, respectively, and plants that survived were not left to go to seed.

This report of reduced control of Italian ryegrass in a field crop situation is the first of its kind. All of the previously documented cases of GR Italian ryegrass were selections from orchards. It is believed that glyphosate-tolerant Italian ryegrass is an emerging problem in the midsouthern United States and there is potential for development of tolerance to glyphosate to levels higher than reported here.

Table 1. Survival of three Italian ryegrass populations from Mississippi on the basis of ratings taken 4 wk after treatment with glyphosate, diclofop, and clethodim.

		Population		
Herbicide[a]	Rate	Elizabeth	Tribbett	Fratesi
	kg/ha[b]		% survived	
Glyphosate	0.11	100	100	100
	0.21	100	100	100
	0.42	100	100	100
	0.84	0	100	50
	1.68	0	0	50
	3.36	0	0	0
	6.72	0	0	0
Significance			*[c]	
Diclofop	0.13	50	100	100
	0.25	100	100	100
	0.5	100	50	50
	0.75	100	100	50
	1.0	100	100	50
	2.0	100	50	50
Significance			*[c]	
Clethodim (small)	0.02	63	90	63
	0.03	50	63	50
	0.05	38	40	13
	0.06	13	63	50
	0.08	38	13	25
	0.09	38	25	13
	0.13	25	38	13
Significance			*[d]	
Clethodim (large)	0.02	100	100	100
	0.03	100	100	100
	0.05	100	100	75
	0.06	100	90	63
	0.08	100	50	75
	0.09	100	63	50
	0.13	90	50	13
Significance			*[d]	

[a] Italian ryegrass plant size treated with glyphosate was 10- to 15-cm tall (three to six leaves, two to three tillers), with diclofop and clethodim (small) was 8- to 10-cm tall (two to three leaves, two to three tillers), and with clethodim (large) was 15 to 20-cm tall (4 to 5 leaves, 10 to 12 tillers).

[b] Glyphosate in kg ae/ha; diclofop and clethodim in kg ai/ha.

[c] * indicates that Italian ryegrass survival is associated with applied herbicide dose in case of glyphosate and diclofop according to Mantel–Haenszel chi-square test at 5% level of significance.

[d] * indicates that Italian ryegrass survival is affected by clethodim dose and slopes of response of the three populations as well as between susceptible (Elizabeth) and tolerant (averaged across Tribett and Fratesi) populations are different according to Fisher's Exact test at 5% level of significance.

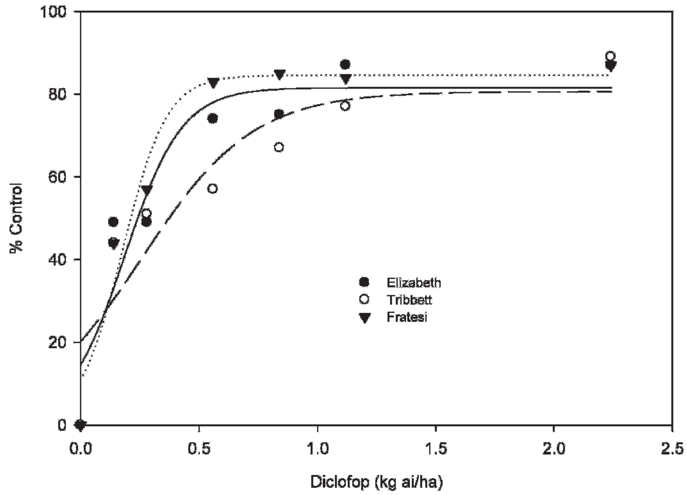

Figure 2. Response of two glyphosate-tolerant Italian ryegrass populations (Tribbett and Fratesi) and one susceptible population (Elizabeth) at the 8- to 10-cm-tall (two to three leaves, two to three tillers) growth stage to diclofop 4 wk after treatment. Mean values are plotted. Log-logistic dose–response curves:

Elizabeth $Y = \frac{82}{1 + e^{-(x - 0.19)/0.12}}$ $R^2 = 0.89, I_{50} = 0.25$ kg ae/ha

Tribbett $Y = \frac{81}{1 + e^{-(x - 0.26)/0.24}}$ $R^2 = 0.83, I_{50} = 0.28$ kg ae/ha

Fratesi $Y = \frac{85}{1 + e^{-(x - 0.18)/0.10}}$ $R^2 = 0.96, I_{50} = 0.21$ kg ae/ha

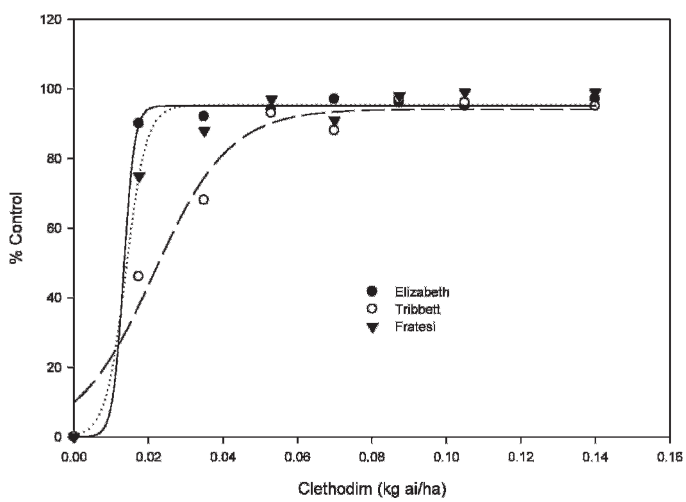

Figure 3. Response of two glyphosate-tolerant Italian ryegrass populations (Tribbett and Fratesi) and one susceptible population (Elizabeth) at the 8- to 10-cm-tall (two to three leaves, two to three tillers) growth stage to clethodim 4 wk after treatment. Mean values are plotted. Log-logistic dose–response curves:

Elizabeth $Y = \frac{95}{1 + e^{-(x - 0.01)/0.002}}$ $R^2 = 0.99, I_{50} = 0.016$ kg ae/ha

Tribbett $Y = \frac{94}{1 + e^{-(x - 0.02)/0.010}}$ $R^2 = 0.97, I_{50} = 0.023$ kg ae/ha

Fratesi $Y = \frac{95}{1 + e^{-(x - 0.01)/0.003}}$ $R^2 = 0.99, I_{50} = 0.014$ kg ae/ha

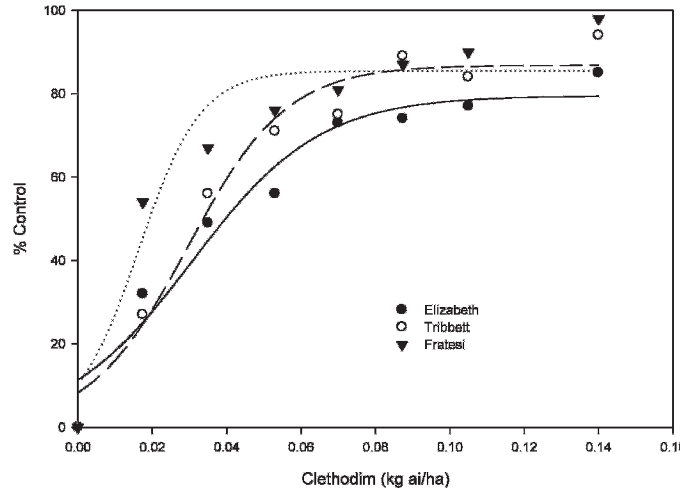

Figure 4. Response of two suspected glyphosate-tolerant Italian ryegrass populations (Tribbett and Fratesi) and one susceptible population (Elizabeth) at the 15- to 20-cm-tall (4 to 5 leaves, 10 to 12 tillers) growth stage to clethodim 4 wk after treatment. Mean values are plotted. Log-logistic dose–response curves:

Elizabeth $Y = \frac{80}{1 + e^{-(x - 0.03)/0.02}}$ $R^2 = 0.95, I_{50} = 0.04$ kg ae/ha

Tribbett $Y = \frac{87}{1 + e^{-(x - 0.03)/0.01}}$ $R^2 = 0.97, I_{50} = 0.034$ kg ae/ha

Fratesi $Y = \frac{85}{1 + e^{-(x - 0.02)/0.01}}$ $R^2 = 0.92, I_{50} = 0.020$ kg ae/ha

Diclofop Dose Response. Response of the Italian ryegrass populations to diclofop rate is shown in Figure 2. The Elizabeth, Tribbett, and Fratesi populations had GR_{50} values of 0.25, 0.28, 0.21 kg/ha, respectively. All three populations were somewhat similar in their tolerance to diclofop, with the most tolerant being Tribbett, followed by Elizabeth and Fratesi in decreasing order. Increased tolerance of Italian ryegrass to diclofop is troubling because of the possibility of cross-resistance to clethodim. At least 50% of Italian ryegrass plants survived diclofop treatment regardless of rate or population (Table 1).

Clethodim Dose Response. Response of the Italian ryegrass populations to clethodim rate is shown in Figures 3 and 4. The Elizabeth, Tribbett, and Fratesi populations had GR_{50} values of 0.016, 0.023, 0.014 kg/ha, respectively, at the small growth stage and 0.04, 0.034, 0.02 kg/ha, respectively, at the large growth stage. The above results indicate that as plant size increased, a higher rate of clethodim was required to cause a 50% reduction in Italian ryegrass plant growth. Comparison of GR_{50} values of clethodim for the three populations indicates that the Fratesi population was the most susceptible to clethodim irrespective of the plant size, whereas Tribbett was least susceptible at the small growth stage and Elizabeth was least susceptible at the large growth stage. Thus, it is evident that neither Tribbett nor Fratesi is clearly tolerant to clethodim. However, a percentage of plants from all three populations survived regardless of clethodim rate or plant growth stage (Table 1).

Alternative Herbicide Options. Glyphosate controlled the Elizabeth population 91 to 99% regardless of plant size

Table 2. Control of three Italian ryegrass populations 4 wk after treatment with several POST herbicide treatments.

Treatment[b]	Rate	Growth stage[a]					
		Small			Large		
		Elizabeth	Tribett	Fratesi	Elizabeth	Tribett	Fratesi
	kg/ha[c]	% control					
Glyphosate + NIS	0.84	98	60	69	94	40	60
Glyphosate + NIS	1.62	99	77	91	91	63	73
Diclofop	0.56	67	48	71	46	47	43
Diclofop	1.12	93	87	94	64	55	46
Mesosulfuron + MSO + AMS	0.02	97	96	98	77	82	85
Clethodim + COC	0.11	99	99	99	95	94	85
Clethodim + COC	0.14	99	99	99	99	98	98
Nicosulfuron + rimsulfuron MSO + AMS	0.04	99	96	99	90	88	94
Glufosinate	0.47	81	66	79	69	70	86
Glufosinate	0.58	82	82	92	89	74	86
Paraquat + NIS	0.70	99	99	99	98	99	99
Paraquat + NIS	0.98	99	99	99	99	99	99
LSD (0.05)							
Within growth stage & population		10	18	9	10	15	13
Within growth stage, across populations			7			7	
Across growth stages and populations				5			

[a] Italian ryegrass growth stages: small, 8 to 10-cm tall (two to three leaves, two to three tillers); large, 15 to 20-cm tall (4 to 5 leaves, 10 to 12 tillers).

[b] Abbreviations: AMS, ammonium sulfate added at 3.4 kg/ha; COC, crop oil concentrate added at 1.7% v/v; MSO, methylated soybean oil added at 1.3%; NIS, nonionic surfactant added at 0.25% v/v.

[c] Glyphosate in kg ae/ha; all other herbicides in kg ai/ha.

(Table 2). Control of Tribbett and Fratesi plants with glyphosate was higher in small plants (60 to 91%) than in large plants (40 to 73%). Diclofop at 0.56 kg/ha was ineffective on Italian ryegrass in this study regardless of population or plant size (43 to 71% control). Effective control (87 to 94%) of small Italian ryegrass plants was obtained with diclofop at 1.12 kg/ha. However, it was not effective on larger-sized plants of all three populations, providing only 46 to 64% control. Mesosulfuron provided excellent control of small plants (≥ 96%) of all three populations, but only 77 to 85% control of larger plants. Glufosinate at 0.47 kg/ha controlled small Italian ryegrass plants 66 to 81%, and larger plants 69 to 86%. Only the Elizabeth population was less susceptible to glufosinate when plants were larger. Similar results were observed when glufosinate rate was 0.58 kg/ha. While uniform foliar coverage is critical for consistent control of weeds from POST herbicide treatments, prevailing weather conditions had a greater impact on performance of glufosinate. Italian ryegrass, being a winter annual, thrives in cool conditions. Temperatures of 20 C or less in these studies may have limited the effectiveness of glufosinate. The days immediately after glufosinate application were cloudy and cool. All three populations were susceptible (≥ 88% control) to clethodim, nicosulfuron + rimsulfuron, and paraquat, regardless of herbicide rate or growth, except larger-sized Fratesi plants treated with clethodim at 0.11 kg/ha. However, it should be noted that this level of Italian ryegrass control with paraquat is uncommon in the field. None of the Italian ryegrass plants, regardless of population or growth stage, survived when treated with clethodim at 0.14 kg/ha or paraquat at 0.98 kg/ha (data not shown).

In summary, we characterized two Italian ryegrass populations that are threefold more tolerant to glyphosate compared with a susceptible population. These populations were also tolerant to diclofop, but not clethodim. Alternative chemical control options exist for managing these populations. Effective management of Italian ryegrass populations, however, is determined by timing, economics, and prevailing weather conditions. This is the first report of glyphosate-tolerant Italian ryegrass populations from GR cotton and GR soybean crop situations.

Sources of Materials

[1] Jiffy Products of America Inc. Batavia, IL 60510.

[2] Peters Fertilizer Products, W. R. Grace and Co., Fogelsville, PA 18051.

[3] Spraying Systems Co., Wheaton, IL 60189.

[4] Rodeo®, Monsanto Company, St. Louis, MO 63167.

[5] Sigma Plot®, version 9.0, Systat Software Inc. Point Richmond, CA 94804.

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