Weed Technology

www.cambridge.org/wet

Research Article

Cite this article: de Sanctis JHS, Cahoon CW, Everman WJ, Gannon TW, Jennings KM, Taylor ZR (2025) Confirmation and distribution of paraquat-resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*) in North Carolina. Weed Technol. **39**(e83), 1–8. doi: 10.1017/wet.2025.10025

Received: 18 November 2024
Revised: 13 April 2025
Accepted: 8 May 2025

Associate Editor:
Authors Primary/ Alternate Email Address Provided: Josede Sanctis-jhscarparo@ncsu.edu; Charles Cahoon-charlie_cahoon@ncsu.edu; Wesley Everman-wesley_everman@ncsu.edu; Travis Gannon-travis_gannon@ncsu.edu; Katherine Jennings-katie_jennings@ncsu.edu; Zachary Taylor-zrtaylor@ncsu.edu

Nomenclature:
Clethodim; glyphosate; glufosinate; nicosulfuron; paraquat; Italian ryegrass; *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot

Keywords:
Multiple herbicide resistance; annual ryegrass

Corresponding author:
Jose H. S. de Sanctis;
Email: joseh.sanctis@gmail.com

Confirmation and distribution of paraquat-resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*) in North Carolina

Jose H.S. de Sanctis[1], Charles W. Cahoon[2], Wesley J. Everman[3], Travis W. Gannon[3], Katherine M. Jennings[4] and Zachary R. Taylor[5]

[1]Graduate Research Assistant, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [2]Associate Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [3]Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [4]Associate Professor, Department of Horticultural Science, North Carolina State University, Raleigh, NC, USA and [5]Research Specialist, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA

Abstract

Italian ryegrass is a troublesome weed species commonly found across the United States. In North Carolina, biotypes resistant to herbicides from Groups 1, 2, and 9 have been confirmed. In fall 2020, multiple growers reported unsatisfactory control of Italian ryegrass after sequential burndown applications of paraquat in the Southern Piedmont region of the state. The objectives of this study were to confirm the presence of a paraquat-resistant Italian ryegrass biotype in the state through a whole-plant dose–response bioassay and to characterize the response of Italian ryegrass accessions from the same region to commonly used burndown herbicides. Greenhouse studies were conducted at the North Carolina State University weed science laboratories to evaluate the response of three putative paraquat-resistant Italian ryegrass biotypes (B, H, SB) and four putative susceptible biotypes (S1, S2, S3, and S4) to paraquat rates ranging from 52.5 to 26,880 g ai ha^{-1} and the response of 38 accessions to clethodim (271 g ai ha^{-1}), glyphosate (1,260 g ae ha^{-1}), glufosinate (880 g ai ha^{-1}), nicosulfuron (34 g ai ha^{-1}), and paraquat (840 g ai ha^{-1}). The effective paraquat dose required to reduce biomass by 50% (GR_{50}) for the putative paraquat-resistant biotypes ranged from 570 to 1,729 g ai ha^{-1}, equivalent to 19- to 58-fold more resistant to paraquat compared to the average GR_{50} of susceptible biotypes. This study confirms the presence of paraquat-resistant Italian ryegrass in North Carolina. Results from the accessions study reveal that 29% of biotypes tested were resistant to paraquat, all of which also exhibited resistance to glyphosate and nicosulfuron. Additionally, a wide distribution of multiple herbicide–resistant biotypes was observed in the Southern Piedmont region, with 97% and 74% of accessions tested resistant to ≥1 and ≥2 sites of action, respectively.

Introduction

Italian ryegrass is a herbaceous winter annual grass species native to the Mediterranean region and introduced as a forage, turf, or cover crop in several temperate and upland tropical zones across the globe (Humphreys et al. 2010; Lacefield et al. 2003; NIES 2023; Seebens et al. 2017) because of its adaptability and fast growth rate (Ball et al. 1995). Despite its forage and turf utility, Italian ryegrass has become a problematic weed worldwide (Matzrafi et al. 2021).

In the United States, Italian ryegrass was ranked as a top 10 most troublesome weed species of wheat in 10 of the 13 southern states (Webster and Nichols 2012). This weed has prolific seed production of up to 45,000 seeds plant^{-1} (Bararpour et al. 2017) and a vigorous growth rate. For instance, Ball et al. (1995) observed that Italian ryegrass leaf production rates were greater than those of wheat (*Triticum aestivum* L. 'Stephens') and winter triticale [×*Triticosecale* Wittm. ex A. Camus (*Secale* × *Triticum*) 'Breaker']. In addition, Bararpour et al. (2017) reported that Italian ryegrass produced more tillers and more spikes per plant than did rigid (*Lolium rigidum* Gaudin), perennial (*Lolium perenne* L.), and poison (*Lolium temulentum* L.) ryegrass, which resulted in Italian ryegrass producing 3.2 to 10.4 times more seeds per plant than any of the other *Lolium* species. Previous literature has reported yield losses ranging from 3.8% to 4% in wheat for every 10 Italian ryegrass plants m^{-2} (Liebl and Worsham 1987). Bailey and Wilson (2003) reported up to 75% wheat yield loss when Italian ryegrass was left uncontrolled. Furthermore, Nandula (2014) reported up to 60% of corn yield loss from Italian ryegrass densities of 4 plants m^{-2}.

Italian ryegrass has a high outcrossing rate (Fearon et al. 1983) and high genetic variability (Karn and Jasieniuk 2017), which may increase the chances of evolution of herbicide resistance and rapid spread of weedy traits. Currently in the United States, biotypes of this weed species have evolved resistance to acetyl CoA carboxylase– (Weed Science Society of America [WSSA]

Group 1), acetolactate synthase– (WSSA Group 2), photosystem II– (PSII; WSSA Groups 5 and 6), 5-enolpyruvylshikimate-3-phosphate synthase– (WSSA Group 9), glutamine synthetase– (WSSA Group 10), very-long-chain fatty-acid- (WSSA Group 15), and PSI-inhibiting herbicides (WSSA Group 22) (Heap 2024; Liu et al. 2016).

Diclofop-methyl was introduced for Italian ryegrass control in wheat in 1980 (Khodayari et al. 1983) and was largely adopted across all wheat-growing regions (Stanger and Appleby 1989). However, its extensive use selected for diclofop-resistant biotypes in the southern United States, of which 25% exhibit cross-resistance to pinoxaden (Salas et al. 2013). The same study reported that ~80% of diclofop-resistant biotypes were also resistant to acetolactate synthase (ALS) inhibitors, exhibiting complex resistance patterns to mesosulfuron, imazamox, and pyroxsulam. Similarly, a recent study from California demonstrated a high frequency of multiple- and cross-herbicide-resistant Italian ryegrass biotypes (Brunharo and Hanson 2018). In addition, glyphosate-resistant Italian ryegrass has also been widely documented across the country (Heap 2024).

In North Carolina, Italian ryegrass has been problematic in wheat and other small grain crops since the late 1970s (Liebl and Worsham 1987). The first recorded case of herbicide-resistant Italian ryegrass in the state was a biotype resistant to diclofop-methyl and sethoxydim reported in 1990. Since then, biotypes resistant to ALS inhibitors (WSSA Group 2) and glyphosate (WSSA Group 9) have been reported across the state (Heap 2024). While investigating the susceptibility of Italian ryegrass to diclofop, pinoxaden, mesosulfuron, and pyroxsulam across North Carolina, Jones et al. (2021) observed widespread distribution of herbicide-resistant biotypes, with 100%, 5%, 11%, and 19% of biotypes tested resistant to the aforementioned herbicides, respectively. In addition, the same study reported that the four biotypes resistant to all the herbicides tested were collected in the Southern Piedmont region of North Carolina. However, with limited options for postemergence Italian ryegrass control in wheat, growers have continued to rely on WSSA Groups 1 and 2 herbicides (Carleo and Everman 2020), increasing selection pressure. Moreover, Italian ryegrass biotypes resistant to glyphosate, which was typically used for burndown applications, have quickly spread across North Carolina (C. W. Cahoon and W. J. Everman, personal communication, 2022). Consequently, growers have shifted to paraquat for preplant burndown of multiple herbicide–resistant Italian ryegrass. However, owing to overreliance on paraquat and lack of alternative postemergence options, multiple reports of suspected paraquat-resistant Italian ryegrass emerged from the Southern Piedmont region of North Carolina during fall 2020. Therefore the objectives of this study were to confirm the presence of a putative paraquat-resistant Italian ryegrass biotype in the Southern Piedmont region and to investigate the distribution of multiple herbicide–resistant biotypes within that region.

Materials and Methods

Plant Material

In October 2020, multiple farmers reported ineffective control of ≤10-cm-tall Italian ryegrass with paraquat. Preliminary assessments at the reported locations were consistent with observation by farmers as Italian ryegrass plants were found to survive paraquat at rates up to 3,362 g ai ha^{-1} or 4X the standard rate (data not shown). Surviving plants from three locations (named B, H, and SB) were collected, placed in separate greenhouses to avoid cross-pollination, and grown for seeds. When seeds reached maturity, seedheads were harvested, kept at room temperature (20 C) for 10 d to reduce moisture while maintaining viability, manually threshed, and stored at −10 C. Four putative paraquat-susceptible biotypes (named S1, S2, S3, and S4) were obtained from an herbicide-resistant Italian ryegrass distribution study conducted previously in North Carolina (Jones et al. 2021).

Whole-Plant Dose–Response Bioassay

Progeny from each putative paraquat-resistant biotype were then seeded in 21 × 28 cm flats and transplanted at the coleoptile stage into plastic pots (a single plant per pot; 12 cm diameter by 10 cm deep) containing potting soil mix (Fafard® 4P Mix, SunGro, Agawam, MA, USA) and approximately 1 g of Osmocote® Flower Food Granules (14-14-14; Scotts Company, Marysville, OH, USA) for optimal growth. Greenhouse temperature was maintained at 25/15 C diurnal fluctuation, irrigation was applied overhead to maintain field capacity, and supplemental artificial lighting was provided (600 to 1,000 µmol m^{-2} s^{-1} photosynthetic photon flux density) for a 14-h photoperiod. Each experimental unit consisted of a single plant, and plants were treated once they reached 10 cm in height.

The study was conducted as a randomized complete block design with five replications and two experimental runs. Seven biotypes (three putative resistant and four putative susceptible) were tested, and treatments consisted of ten paraquat (Gramoxone® SL 3.0, Syngenta Crop Protection LLC, Greensboro, NC, USA) rates plus a nontreated control. Paraquat rates were 0.0625X, 0.125X, 0.5X, 1X, 2X, 4X, 8X, 16X, and 32X of the label recommended rate (840 g ai ha^{-1}); crop oil concentrate at 1% v/v was included with all treatments. Herbicide treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1} with TeeJet® 11002AIXR nozzles (TeeJet® Technologies, Wheaton, IL, USA).

At 28 d after application, aboveground biomass was collected by clipping Italian ryegrass plants at the soil surface; plants were individually packaged in paper bags, dried at 65 C for 10 d, and weighted. Biomass reduction was calculated as

$$\mathrm{BR} = 100 \times \left(1 - \frac{T}{C}\right)$$

where BR is the biomass reduction relative to the nontreated plants, T is the treated plant weight, and C is the average weight of nontreated plants.

Statistical analysis was performed in R (R Core Team 2019) utilizing the base packages plus the DRC package (Ritz et al. 2015). The four-parameter log-logistic model was used to describe the relationship between Italian ryegrass biomass reduction and paraquat rates in g ai ha^{-1} (De Sanctis et al. 2021; Knezevic et al. 2007):

$$Y = C + \frac{d - c}{\{1 + \exp[b(\log x - \log e)]\}}$$

where Y is the response variable (Italian ryegrass biomass reduction), c is the lower limit, d is the upper limit, x is the paraquat rate in 840 g ai ha^{-1}, e is the GR_{50} (paraquat rate where 50% response between lower and upper limit occurs; inflection point), and b is the slope of the line at the inflection point.

https://doi.org/10.1017/wet.2025.10025 Published online by Cambridge University Press

Table 1. Herbicide products and application rates for greenhouse experiments conducted at the North Carolina State University Weed Science Laboratories.[a]

Common name	WSSA group number	Trade name	Rate	Manufacturer
			g ai/ae ha^{-1}	
Clethodim[b,c]	1	Select Max®	272	Valent (San Ramon, CA)
Nicosulfuron[b,c]	2	Accent® Q	63	Du Pont (Wilmington, DE)
Glyphosate	9	Roundup PowerMAX® 3	1,260	Bayer Crop Science (St. Louis, MO)
Glufosinate[c]	10	Liberty®	880	BASF (Research Triangle Park, NC)
Paraquat[d]	22	Gramoxone® SL 3.0	840	Syngenta Crop Protection (Greensboro, NC)

[a]Abbreviation: WSSA, Weed Science Society of America.
[b]Nonionic surfactant at 0.25% (v/v) as included as instructed by label directions.
[c]Ammonium sulfate at 3% (v/v) as included as instructed by label directions.
[d]Crop oil concentrate at 1% (v/v) as included as instructed by label directions.

Root mean square error (RMSE) and modeling efficiency (ME) were calculated to evaluate goodness of fit for Italian ryegrass biomass reduction (Barnes et al. 2018; De Sanctis et al. 2021):

$$\mathrm{RMSE} = \left[\frac{1}{n}\sum\nolimits_{i=1}^{n}(Pi - Oi)^2\right]^{\frac{1}{2}}$$

where Pi and Oi are the predicted and observed values, respectively, and n is the total number of comparisons. The smaller the RMSE, the closer the model-predicted values are to the observed values. The ME was calculated using the following equation (De Sanctis et al. 2021; Mayer and Butler 1993):

$$\mathrm{ME} = 1 - \left[\frac{\sum_{i=1}^{n}(Oi - Pi)^2}{\sum_{i=1}^{n}(Oi - \bar{O}i)^2}\right]$$

where $\bar{O}i$ is the mean observed value and all other parameters are the same as in Equation 3. An ME value closer to 1.00 means more accurate prediction.

Herbicide Screening of Whole-Plant Dose–Response Biotypes

Biotypes utilized in the dose–response bioassay study were also screened against five commonly used POST herbicides from different sites of action (SOAs). Growing conditions in the greenhouse, plant size, and application parameters were the same as aforementioned for the dose–response bioassay study.

The study was conducted as a randomized complete block design with five replications and two experimental runs; each individual plant was considered an experimental unit. Treatments consisted of five herbicides (clethodim, nicosulfuron, glyphosate, glufosinate, and paraquat) each belonging to a different SOA plus a nontreated control. Rates, WSSA group numbers, and adjuvants are described in Table 1.

Data collected consisted of weekly visible estimation of control (VEC) until 28 DAT. Statistical analysis was conducted in R (R Core Team 2019) utilizing the base packages plus the LME4 package. VEC data were subjected to analysis of variance (ANOVA) to test for significance of fixed and random effects and means. Experimental run and replications were treated as random effects and herbicide treatment and biotypes as fixed. Fisher's least significant difference was used to separate means at $\alpha = 0.05$.

Italian Ryegrass Accessions Study

Over the course of fall and winter 2020–2021, additional reports of ineffective paraquat control of Italian ryegrass in the same general area of initial putative resistant biotypes were received (Figure 1). Owing to the historical issues with herbicide-resistant Italian ryegrass in the Southern Piedmont region of North Carolina, a local assessment of Italian ryegrass response to POST herbicides from different SOAs was deemed necessary. Italian ryegrass seeds were collected in June 2021 when plants reached maturity. A total of 38 seed samples were randomly collected from fields naturally infested with Italian ryegrass in Stanly, Union, Anson, Cabarrus, and Rowan counties. Samples were kept at room temperature (20 C) for 10 d to reduce moisture while maintaining viability and then manually threshed, cleaned, and stored at −10 C.

Treatments, experimental design, growing conditions in the greenhouse, plant sizes, and application parameters were the same as described in the herbicide screening of whole-plant dose–response biotypes. Data collected consisted of weekly VEC, and at 28 DAT, plant mortality was assessed visibly as dead (no green tissue; assessed value of 1) or alive (green tissue with evidence of regrowth; assessed value of 0). Accessions with ≤50% mortality were classified as resistant to the specific herbicide (Faleco et al. 2022). Statistical analysis was conducted in R (R Core Team 2019) utilizing the base packages plus the LME4 package. Italian ryegrass VEC data were subjected to ANOVA to test for significance of fixed and random effects and means, where experimental run and replications were treated as random effects and herbicide treatment and biotypes as fixed. Fisher's least significant difference was used to separate means at $\alpha = 0.05$.

Results and Discussion

Whole-Plant Dose–Response Bioassay

Confirming susceptibility of the putative susceptible biotypes, paraquat GR_{50} and GR_{90} (paraquat rate required to reduce biomass by 90%) values ranged from 15 to 37 and from 120 to 128 g ai ha^{-1}, respectively. The GR_{50} values of the putative paraquat-resistant Italian ryegrass biotypes were 899.9 (H), 570.4 (B), and 1,729.5 (SB) g ai ha^{-1}. The GR_{90} for H and B was 6,670.8 and 1,7609.0 g ai ha^{-1}, respectively; however, this value was not observed for the SB biotype (Table 2; Figure 2). The calculated resistance ratio based on the GR_{50} was 30-, 19-, and 58-fold for the H, B, and SB biotypes when compared to the averaged values of susceptible biotypes, respectively. Therefore, owing to the high levels of differential susceptibility observed, paraquat-resistant Italian ryegrass was confirmed in North Carolina. In the United States, paraquat-resistant Italian ryegrass was first observed in a California orchard (Brunharo and Hanson 2018) with a GR50 of 1,089 g ai ha^{-1} and a resistance ratio of 19-fold. Recently, another paraquat-resistant Italian ryegrass population was confirmed in Louisiana sugarcane (*Saccharum officinarum* L.) (Coco 2022), where paraquat is the only labeled postemergence herbicide. For this biotype, the GR50

https://doi.org/10.1017/wet.2025.10025 Published online by Cambridge University Press

Table 2. Estimates of the model parameters, paraquat dose required to reduce the aboveground biomass of Italian ryegrass biotypes by 90% (GR_{90}), resistance ratio, and model goodness of fit at 28 d after paraquat treatment in a whole-plant dose–response bioassay conducted in a greenhouse at the North Carolina State University Weed Science Laboratories.[a]

	Parameter estimate[b]						Model goodness of fit	
Population	Lower limit (±*SE*)	Upper limit (±*SE*)	Slope (±*SE*)	GR_{50}[c] (±*SE*)	GR_{90} (±*SE*)	Resistance ratio (GR_{50}(R)/GR_{50}(S))[c,d]	RMSE	ME
					g ai ha^{-1}			
S1	−0.1 (1.5)	94.1 (0.6)	−2.5 (0,4)	37.0 (2.7)	128.2 (19.2)		4.7	0.97
S2	0.0 (1.4)	95.5 (0.6)	−2.1 (0.3)	33.2 (2.7)	120.1 (13.9)		4.4	0.98
S3	−0.1 (1.4)	96.7 (0.6)	−1.3 (0.2)	15.2 (4.4)	121.8 (18.3)		4.2	0.98
S4	−0.6 (1.9)	95.6 (0.8)	−2.2 (0.4)	34.6 (4.0)	124.5 (19.8)		5.9	0.96
H	9.5 (5.0)	95.0 (4.9)	−1.4 (0.4)	899.9 (168.7)	6,670.8 (3,881.6)	30	13.5	0.86
B	2.8 (5.0)	90.7 (4.2)	−1.4 (0.3)	570.4 (99.3)	1,7609.0 (14,090.0)	19	18.0	0.79
SB	10.8 (2.5)	74.6 (3.5)	−5.3 (4.3)	1,729.5 (130.7)	—	58	18.1	0.72

[a]Abbreviations: GR_{90}, effective paraquat rate that reduces Italian ryegrass biomass by 90%; ME, modeling efficiency; R, resistant; RMSE, root mean square error; S, susceptible; SE, standard error.
[b]Four-parameter log-logistic model: $Y = c + (d - c)/\{1 + \exp[b(\log x - \log e)]\}$, where *Y* is the response variable (biomass reduction), *c* is the lower limit, *d* is the upper limit, *x* is the paraquat rate expressed in g ai ha^{-1}, *e* is the GR_{50} (paraquat rate where 50% response between lower and upper limit occurs; inflection point), and *b* is the slope of the line at the inflection point.
[c]Resistance ratio was determined by dividing the predicted value of the putative resistant (R) accession by the predicted value of the average of the susceptible (S) accessions.
[d]All values are significant based on 95% confidence intervals.

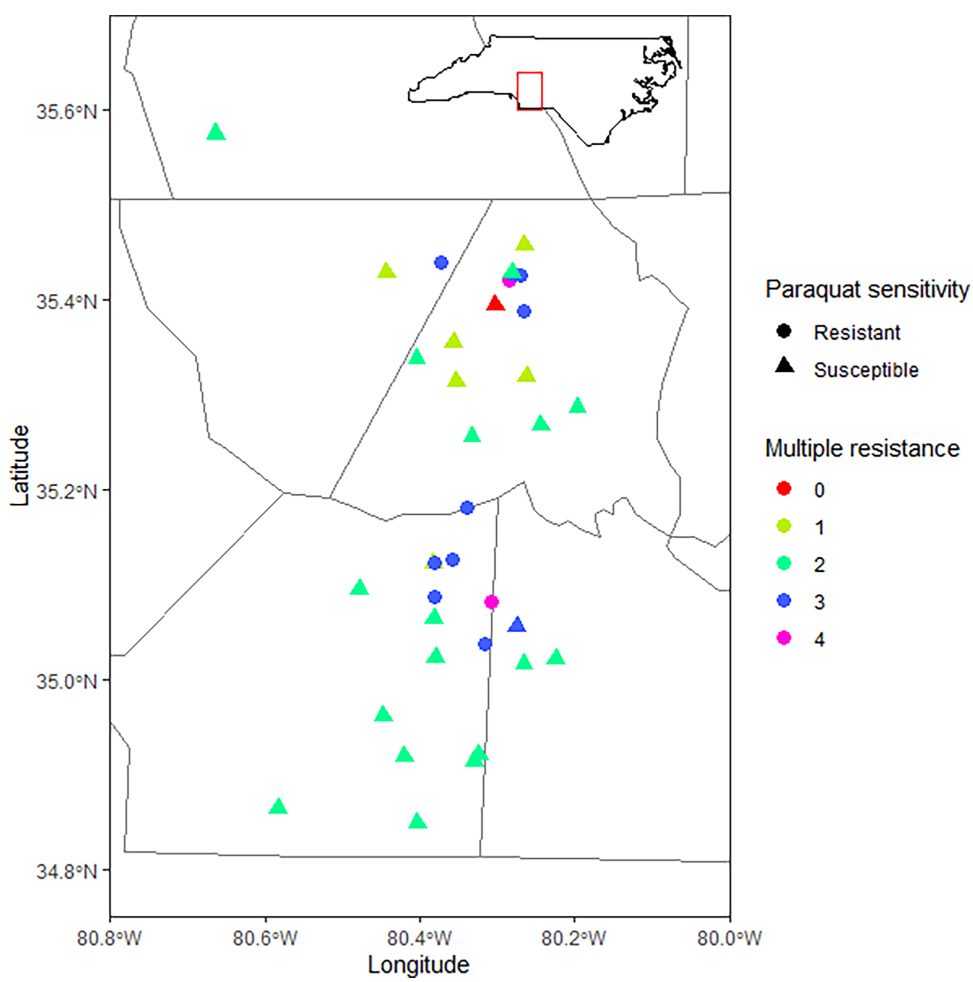

Figure 1. Geographic distribution of Italian ryegrass accessions collected from counties within the Southern Piedmont region of North Carolina and the resistance profile of those accessions. Data from the 38 accessions evaluated for clethodim, glufosinate, glyphosate, nicosulfuron, and paraquat applied postemergence are presented. Multiple resistance indicates the number of herbicides from different sites of action that the specific populations exhibited mortality ≤50%, and paraquat sensitivity indicates whether the population exhibited mortality ≤50% to paraquat.

https://doi.org/10.1017/wet.2025.10025 Published online by Cambridge University Press

Table 3. Italian ryegrass visible estimations of control at 28 d after herbicide application on paraquat-resistant and -susceptible biotypes tested in a whole-plant, dose–response assay conducted in a greenhouse at the North Carolina State University Weed Science Laboratories.[a,b,c]

	Herbicide				
Population	Clethodim[d,e]	Glufosinate[d]	Glyphosate	Nicosulfuron[d,e]	Paraquat[f]
	%				
S1	100	53	100 a	100 a	100 a
S2	100	64	100 a	87 ab	100 a
S3	100	60	100 a	61 c	100 a
S4	100	52	98 a	91 a	100 a
H	91	64	36 b	17 d	29 c
B	100	69	30 b	31 d	51 b
SB	100	64	36 b	70 bc	13 c
	NS	NS	***	***	***

[a]Means presented within the same column and with no common letter(s) are significantly different according to Fisher's protected LSD.
[b]Abbreviation: NS, nonsignificant at $\alpha = 0.05$
[c]Herbicide treatments: clethodim (272 g ai ha^{-1}), glufosinate (880 g ai ha^{-1}), glyphosate (1,260 g ae ha^{-1}), nicosulfuron (34.4 g ai ha^{-1}), and paraquat (840 g ai ha^{-1}).
[d]Nonionic surfactant at 0.25% (v/v) as included as instructed by label directions.
[e]Ammonium sulfate at 3% (v/v) as included as instructed by label directions.
[f]Crop oil concentrate at 1% (v/v) as included as instructed by label directions.
*$P \leq 0.05$. **$P \leq 0.01$. ***$P \leq 0.001$.

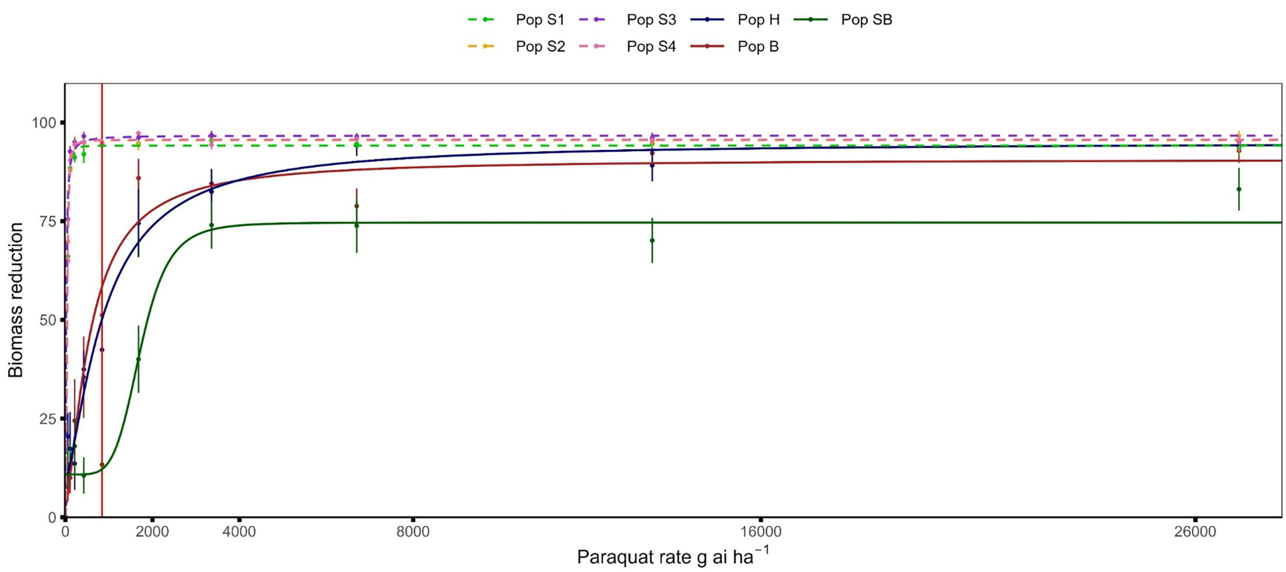

Figure 2. Dose–response curves of three putative paraquat-resistant biotypes (B, H, SB; solid lines) and four putative susceptible biotypes (S1, S2, S4, S4; dashed lines) collected from North Carolina. Graph represents the effect of aboveground biomass reduction of Italian ryegrass biotypes harvested at 28 d after herbicide treatment in the whole-plant dose–response bioassays conducted in the greenhouse at the North Carolina State University Weed Science Laboratories. The red vertical line represents the standard paraquat rate (840 g ai ha^{-1}).

was 643 g ai ha^{-1} with a resistance ratio of 15-fold. In both instances, biotypes were found in perennial cropping systems, where the paraquat-resistant biotypes from North Carolina would be the first case reported in an annual crop setting. The precise mechanism of resistance is yet to be determined. Restricted paraquat translocation primarily caused by vacuolar sequestration has been observed in paraquat-resistant biotypes from California (Brunharo and Hanson 2017). Enhanced reactive oxygen species detoxification has also been hypothesized as a potential mechanism of paraquat resistance (Hart and Di Tomaso 1994). To this date, no binding-site mutations or enhanced metabolisms have been observed as mechanisms of paraquat resistance in plants (Hawkes 2014).

Herbicide Screening of Whole-Plant Dose–Response Biotypes

Italian ryegrass biotypes resistant to enolpyruvyl shikimate phosphate synthase–, ALS-, and acetyl CoA carboxylase (ACCase)-inhibiting herbicides have already been confirmed in North Carolina (Chandi et al. 2011; Heap 2024; Nandula et al. 2020). Therefore an herbicide screening was established to investigate the resistance profile of biotypes used in the dose–response study. Clethodim was the most effective herbicide, with ≥91% control for all biotypes (Table 3). Moreover, glyphosate performed poorly on all paraquat-resistant biotypes, with ≤36% control when compared to ≥98% control for the susceptible biotypes. Biotypes B and H had the lowest nicosulfuron control, with 31% and 17%, respectively. Furthermore, no differences among biotypes were observed in response to glufosinate, and control ranged from 52% to 69%. California researchers reported variable Italian ryegrass control by glufosinate, ranging from 58% to 83% (Moretti 2021). As expected, paraquat controlled susceptible biotypes 100% and resistant biotypes ≤51%.

https://doi.org/10.1017/wet.2025.10025 Published online by Cambridge University Press

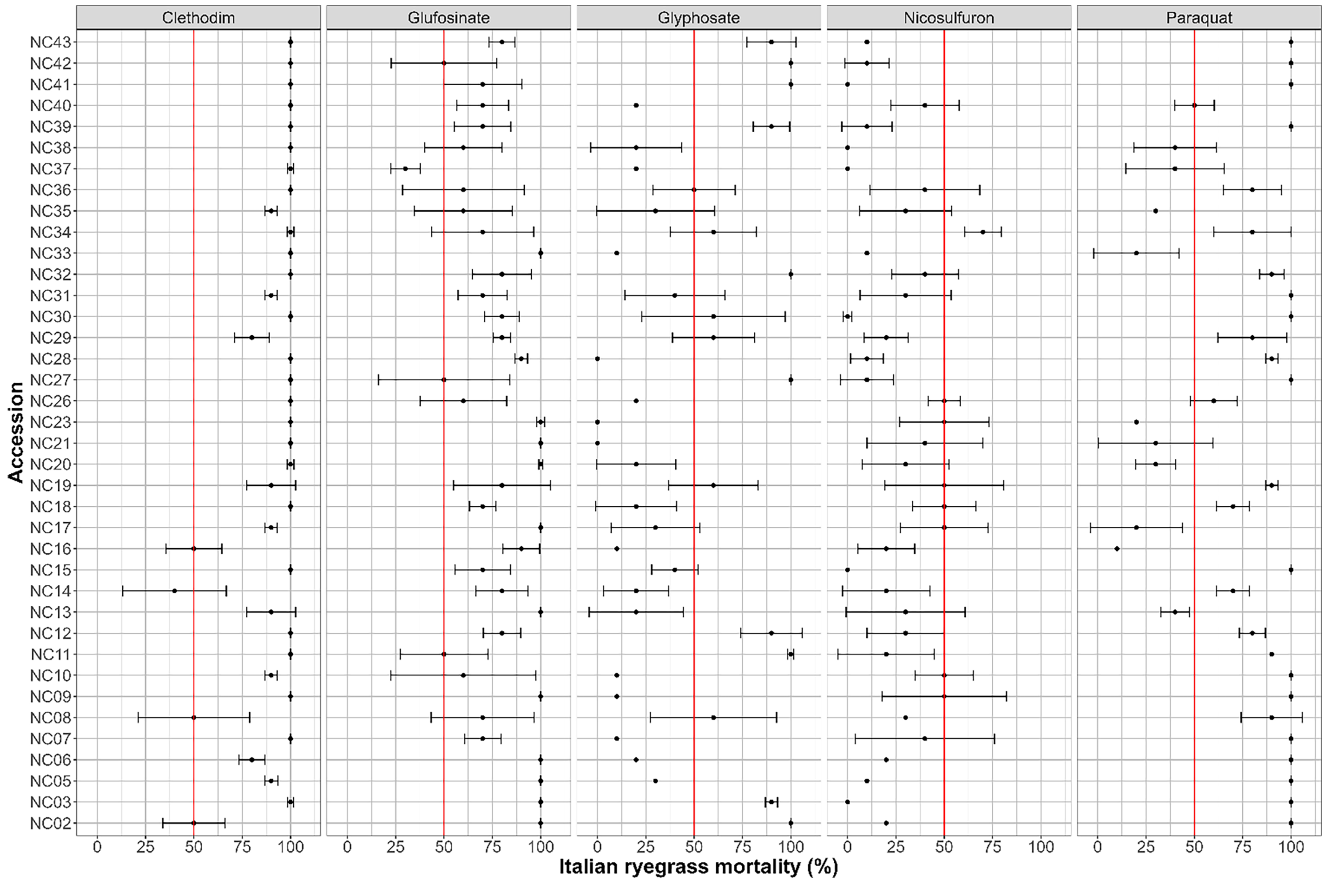

Figure 3. Italian ryegrass plant mortality (±*SE*) in response to commonly used spring burndown herbicides. Accessions with mortality ≤50% (represented by the red line) were classified as resistant. Data from the 38 accessions evaluated for all herbicides applied postemergence are presented. This study was conducted under greenhouse conditions at the North Carolina State University Weed Science Laboratories.

Italian Ryegrass Accessions Study

It is important to note that the goal of the accessions study was to identify multiple-resistant biotypes in the area. Therefore plant samples were collected only from agronomic fields where Italian ryegrass seedheads were visible, which might indicate that these plants survived preplant burndown and postemergence herbicides.

Among the herbicides tested, nicosulfuron and glyphosate resulted in the lowest mortality rates; 97% and 60% of the 38 accessions exhibited ≤50% mortality, respectively, and thus were classified as resistant (Figure 3). Furthermore, 29%, 13%, and 10% of accessions were resistant to paraquat, glufosinate, and clethodim, respectively. Although variable Italian ryegrass response to glufosinate was expected, as this herbicide is generally more effective on broadleaf weeds compared to grasses (Takano and Dayan 2020), glufosinate-resistant Italian ryegrass has been confirmed in North Carolina (Molin et al. 2017), and our results indicate that such resistance is present in different locations across the Southern Piedmont region.

A similar trend was observed for VEC and mortality pooled over accessions (Table 4), where glyphosate and nicosulfuron were the least effective treatments, with both resulting in 73% VEC and 45% and 26% mortality, respectively. In addition, clethodim was again the most effective, with 98% VEC and 92% mortality. Bobadilla et al. (2021), while investigating the frequency and distribution of herbicide-resistant Italian ryegrass accessions from western Oregon, reported that 88% of accessions tested were resistant to at least one herbicide, while 84%, 43%, 11%, and 8% of biotypes were resistant to mesosulfuron-methyl, glyphosate, clethodim, and paraquat, respectively.

Only one accession (NC34) exhibited susceptibility to all herbicides, whereas 97% and 74% of accessions tested resistant to ≥1 and ≥2 SOAs, respectively (Figure 3; treatments applied separately). Among the 11 accessions resistant to paraquat, all also exhibited resistance to nicosulfuron and glyphosate. Two accessions, NC16 and NC37, were classified as resistant to four herbicides. NC16 was resistant to clethodim, glyphosate, nicosulfuron, and paraquat, while NC37 was resistant to glufosinate, glyphosate, nicosulfuron, and paraquat. The existence of four-way-resistant Italian ryegrass biotypes is concerning and poses a great challenge to farmers who may not have a viable postemergence herbicide option to control this biotype. These results indicate that the majority of Italian ryegrass within the Southern Piedmont region of North Carolina has resistance to at least one herbicide; such findings reflect the consequences of excessive reliance on a single approach to weed management. Reports of Italian ryegrass populations resistant to multiple herbicides are abundant in the literature. In California, Brunharo and Hanson (2018) reported a population resistant to clethodim, fluazifop-P-butyl, pyroxsulam, glyphosate, and paraquat (WSSA Groups 1, 1, 2, 9, and 22, respectively).

Table 4. Italian ryegrass visible estimations of control and mortality at 28 d after herbicide application on 38 accessions collected from the Southern Piedmont region of North Carolina.[a,b]

Herbicide	Rate	Visible estimations of control	Mortality
	g ai/ae ha^{-1}	%	
Clethodim[c,d]	272	98 a	92 a
Glufosinate[d]	63	93 b	77 b
Glyphosat[e]	1,260	73 c	45 c
Nicosulfuron[c,d]	880	73 c	26 d
Paraquat[e]	840	92 b	73 b

[a]The study was conducted in a greenhouse at the North Carolina State University Weed Science Laboratories.
[b]Means presented within the same column and with no common letter(s) are significantly different according to Fisher's protected LSD.
[c]Nonionic surfactant at 0.25% (v/v) was included as instructed by label directions.
[d]Ammonium sulfate at 3% (v/v) was included as instructed by label directions.
[e]Crop oil concentrate at 1% (v/v) was included as instructed by label directions.

Italian ryegrass biotypes from Washington and Idaho were reported to be resistant to herbicides from WSSA Groups 1, 2, and 15 (Rauch et al. 2010); in the same study, the authors reported that 12% and 25% of accessions were cross-resistant to all ACCase- and ALS-inhibiting herbicides tested, respectively (Group 1: diclofop, clodinafop, quizalofop, tralkoxydim, sethoxydim, pinoxaden, and clethodim; Group 2: triasulfuron, mesosulfuron, flucarbazone, and imazamox). Bobadilla et al. (2021) reported that 75% of Italian ryegrass populations screened were resistant to multiple herbicides, and 20% were resistant to herbicides from WSSA Groups 1, 2, 9, and 15. In a statewide survey of North Carolina, Jones et al. (2021) reported Italian ryegrass biotypes resistant to multiple ACCase- and ALS-inhibiting herbicides; in addition, the authors reported that all 155 populations evaluated were resistant to diclofop-methyl. Therefore it is likely that the majority of the 38 accessions in this study also exhibit diclofop resistance.

Practical Implications

Results from the whole-plant dose–response bioassay confirmed high levels of paraquat resistance in all three biotypes tested, with resistance ratios of 19- to 58-fold. The confirmation of paraquat-resistant Italian ryegrass further complicates management of a weed that North Carolina farmers rank as one of the most troublesome in the state. More worrisome, the widespread distribution of multiple herbicide–resistant biotypes in the Southern Piedmont region leaves farmers with limited postemergence herbicide options to effectively manage this weed. Biotypes resistant to glyphosate and paraquat pose a challenge to preplant burndown operations, and growers may have to rely on alternative methods to manage Italian ryegrass during the fall and winter months, such as fall-applied residual herbicides, cover crops, and/or tillage. Furthermore, in small grain systems, virtually no postemergence herbicide options are available to effectively manage four-way-resistant Italian ryegrass. Preemergence herbicides, especially those from WSSA Group 15, remain an effective and reliable option, but selection pressure on this valuable SOA will only increase as postemergence options are lost.

To successfully manage multiple-resistant Italian ryegrass, a multifaceted approach that does not rely solely on herbicides is needed. Best management practices for mitigating the evolution and/or spread of herbicide-resistant weed biotypes include using multiple effective herbicide SOAs, preventing or reducing weed soil seedbank replenishment, and incorporating cultural and mechanical practices (Norsworthy et al. 2012). Fall-applied residual herbicides have been studied for fallow management of herbicide-resistant Italian ryegrass (Bond et al. 2014); however, the activity of residual herbicides is reduced over time, and additional preplant control tactics might be necessary. Cover crops can effectively suppress troublesome winter annual weeds during the fallow months (Hayden et al. 2012; Pittman et al. 2019) if adequate biomass is produced, with studies estimating at least 5,000 kg ha^{-1} for satisfactory weed suppression (Nichols et al. 2020). Cechin et al. (2022) reported that 3 yr of continuous use of fall and winter cover crops resulted in up to 96% Italian ryegrass control and up to 99% reduction in Italian ryegrass soil seedbank when compared to nontreated check. De Sanctis et al. (2025), while investigating fall-applied residual herbicides combined with cover crops, observed up to 85% control and 96% seed reduction in Italian ryegrass by the following growing season when these two tools were effectively integrated. However, the authors also observed that the efficacy of this strategy was reduced when a significant cover crop injury occurred as a result of the preemergence herbicide selection. Furthermore, windrow burning is a technique widely implemented in Australia to reduce seedbank replenishment of herbicide-resistant rigid ryegrass (Walsh and Newman 2007). In the United States, Lyon et al. (2016) reported that this tactic reduced Italian ryegrass seed viability by 99%. Other harvest weed seed control tactics include impact mill seed destruction, chaff lining, and chaff removal (Beam et al. 2019; Walsh et al. 2017). Although several alternative and effective weed control tactics exist, further research is needed to determine how to best integrate chemical and nonchemical tactics to better control multiple herbicide–resistant Italian ryegrass.

Funding. Funding for this work was provided in part by Cotton Incorporated, the North Carolina Cotton Producers Association, the Corn Growers Association of North Carolina, and Syngenta Crop Protection.

Competing interests. The authors declare no conflicts of interest.

References

Bailey WA, Wilson HP (2003) Control of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*) with postemergence herbicides. Weed Technol 17:534–542

Ball DA, Klepper B, Rydrych DJ (1995) Comparative above-ground development rates for several annual grass weeds and cereal grains. Weed Sci 43: 410–416

Bararpour MT, Norsworthy JK, Burgos NR, Korres NE, Gbur EE (2017) Identification and biological characteristics of ryegrass (*Lolium* spp.) accessions in Arkansas. Weed Sci 65:350–360

Barnes ER, Jhala AJ, Knezevic SZ, Sikkema PH, Lindquist JL (2018) Common ragweed (*Ambrosia artemisiifolia* L.) interference with soybean in Nebraska. Agron J 110:646–653

Beam SC, Mirsky S, Cahoon C, Haak D, Flessner M (2019) Harvest weed seed control of Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], common ragweed (*Ambrosia artemisiifolia* L.), and Palmer amaranth (*Amaranthus palmeri* S. Watson). Weed Technol 33:627–632

Bobadilla LK, Hulting AG, Berry PA, Moretti ML, Mallory-Smith C (2021) Frequency, distribution, and ploidy diversity of herbicide-resistant Italian ryegrass (*Lolium perenne* spp. *multiflorum*) populations of western Oregon. Weed Sci 69:177–185

Bond JA, Eubank TW, Bond RC, Golden BR, Edwards HM (2014) Glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) control with fall-applied residual herbicides. Weed Technol 28:361–370

Brunharo CACG, Hanson BD (2017) Vacuolar sequestration of paraquat is involved in the resistance mechanism in *Lolium perenne* L. spp. *multiflorum*. Front Plant Sci 8:1485

Brunharo CACG, Hanson BD (2018) Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: characterization, mechanism of resistance, and chemical management. Weed Sci 66:696–701

Carleo J, Everman W (2020) Post-emergence control of Italian ryegrass. https://smallgrains.ces.ncsu.edu/2020/12/post-emergence-control-of-italian-ryegrass/. Accessed: June 24, 2023

Cechin J, Schmitz MF, Torchelsen JS, Durigon MR, Agostinetto D, Vargas L (2022) Winter cover crops reduce the soil seed bank and infestations of Italian ryegrass in no-tillage system. Crop Sci 62:479–488

Chandi A, York AC, Jordan DL, Beam JB (2011) Resistance to acetolactate synthase and acetyl co-a carboxylase inhibitors in North Carolina Italian ryegrass (*Lolium perenne*). Weed Technol 25:659–666

Coco A (2022) Paraquat-resistant Italian ryegrass (*Lolium multiflorum*) confirmed in Louisiana. MAg thesis, Louisiana State University and Agricultural and Mechanical College. 39 p

De Sanctis JHS, Barnes ER, Knezevic SZ, Kumar V, Jhala AJ (2021) Residual herbicides affect critical time of Palmer amaranth removal in soybean. Agron J 113:1920–1933

De Sanctis JHS, Cahoon CW, Everman WJ, Gannon TW, Jennings KM, Taylor ZR, Dean BA, Forehand JC, Lee JH (2025) Cover crops and fall residual herbicides for managing Italian ryegrass. Weed Technol 37:e27

Faleco FA, Oliveira MC, Arneson NJ, Renz M, Stoltenberg DE, Werle R (2022) Multiple herbicide resistance in waterhemp (*Amaranthus tuberculatus*) accessions from Wisconsin. Weed Technol 36:597–608

Fearon CH, Hayward MD, Lawrence MJ (1983) Self-incompatibility in ryegrass: V. Genetic control, linkage and seed-set in diploid *Lolium multiflorum* Lam. Heredity 50:35–45

Hart JJ, Di Tomaso JM (1994) Sequestration and oxygen radical detoxification as mechanisms of paraquat resistance. Weed Sci 42:277–284

Hawkes TR (2014) Mechanisms of resistance to paraquat in plants. Pest Manag Sci 70:1316–1323

Hayden ZD, Brainard DC, Henshaw B, Ngouajio M (2012) Winter annual weed suppression in rye–vetch cover crop mixtures. Weed Technol 26:818–825

Heap I (2024) International Herbicide-Resistant Weed Database. https://www.weedscience.org/. Accessed: June 22, 2024

Humphreys M, Feuerstein U, Vandewalle M, Baert J (2010) Ryegrasses. Pages 211–260 *in* Boller B, Posselt0 UK, Veronesi F, eds. Fodder Crops and Amenity Grasses. New York: Springer

Jones EAL, Taylor ZR, Everman WJ (2021) Distribution and control of herbicide-resistant Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] in winter wheat (*Triticum aestivum* L.) in North Carolina. Front Agron 2:601917

Karn E, Jasieniuk M (2017) Genetic diversity and structure of *Lolium perenne* ssp. *multiflorum* in California vineyards and orchards indicate potential for spread of herbicide resistance via gene flow. Evol Appl 10:616–629

Khodayari K, Frans RE, Collins FC (1983) Diclofop—a selective herbicide for Italian ryegrass (*Lolium multiflorum*) control in winter wheat (*Triticum aestivum*). Weed Sci 31:436–438

Knezevic SZ, Streibig JC, Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol 21:840–848

Lacefield G, Collins M, Henning J, Phillips T, Rasnake M, Spitaleri R, Grigson D, Turner K (2003) Annual ryegrass. http://www2.ca.uky.edu/agcomm/pubs/agr/agr179/agr179.pdf. Accessed: June 22, 2025

Liebl R, Worsham AD (1987) Interference of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*). Weed Sci 35:819–823

Liu M, Hulting AG, Mallory-Smith C (2016) Characterization of multiple herbicide–resistant Italian ryegrass (*Lolium perenne ssp. multiflorum*) populations from winter wheat fields in Oregon. Weed Sci 64:331–338

Lyon DJ, Huggins DR, Spring JF (2016) Windrow burning eliminates Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) seed viability. Weed Technol 30:279–283

Matzrafi M, Preston C, Brunharo CA (2021) Review: evolutionary drivers of agricultural adaptation in *Lolium* spp. Pest Manag Sci 77:2209–2218

Mayer DG, Butler DG (1993) Statistical validation. Ecol Modell 68:21–32

Molin WT, Nandula VK, Wright AA (2017) Italian ryegrass from Iredell County, North Carolina is resistant to glufosinate, ACCase- and ALS-inhibiting herbicides. Page 112 *in* Proceedings of the 57th Annual Meeting of the Weed Science Society of America. Westminster, CO: Weed Science Society of America

Moretti ML (2021) POST control of Italian ryegrass in hazelnut orchards. Weed Technol 35:638–643

Nandula VK (2014) Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) and corn (*Zea mays*) competition. Am J Plant Sci 5:3914–3924

Nandula VK, Giacomini DA, Lawrence BH, Molin WT, Bond JA (2020) Resistance to clethodim in Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) from Mississippi and North Carolina. Pest Manag Sci 76:1378–1385

Nichols V, Martinez-Feria R, Weisberger D, Carlson S, Basso B, Basche A (2020) Cover crops and weed suppression in the U.S. Midwest: a meta-analysis and modeling study. Agric Environ Lett 5:e20022

[NIES] National Institute for Environmental Studies (2023) Italian ryegrass/invasive species of Japan. https://www.nies.go.jp/biodiversity/invasive/DB/detail/80990e.html. Accessed: June 10, 2023

Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60:31–62

Pittman KB, Barney JN, Flessner ML (2019) Horseweed (*Conyza canadensis*) suppression from cover crop mixtures and fall-applied residual herbicides. Weed Technol 33:303–311

R Core Team (2019) R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/. Accessed: June 9, 2024

Rauch TA, Thill DC, Gersdorf SA, Price WJ (2010) Widespread occurrence of herbicide-resistant Italian ryegrass (*Lolium multiflorum*) in northern Idaho and eastern Washington. Weed Technol 24:281–288

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose–response analysis using R. PLOS One 10:e0146021

Salas RA, Burgos NR, Mauromoustakos A, Lassiter RB, Scott RC, Alcober EA (2013) Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. *multiflorum* in the United States. J Crop Weed 9:168–183

Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, Van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. Nat Commun 8:14435

Stanger CE, Appleby AP (1989) Italian ryegrass (*Lolium multiflorum*) accessions tolerant to diclofop. Weed Sci 37:350–352

Takano H, Dayan F (2020) Glufosinate-ammonium: a review of the current state of knowledge. Pest Manag Sci 76:3911–3925

Walsh MJ, Aves C, Powles S (2017) Harvest weed seed control systems are similarly effective on rigid ryegrass. Weed Technol 31:178–183

Walsh MJ, Newman P (2007) Burning narrow windrows for weed seed destruction. Field Crop Res 104:24–30

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. Weed Sci 60:145–157