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Short title: Italian ryegrass sequential treatments

Glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) control in preemergence and postemergence programs containing mixtures of residual herbicides

Taylor D. Burrell II[1], Jason A. Bond[2], Thomas W. Allen[3], Darrin M. Dodds[4] and Drew M. Gholson[5]

[1]Former Graduate Research Assistant, Mississippi State University, Delta Research and Experiment Station, Stoneville, MS, USA; [2]Extension/Research Professor, Mississippi State University, Delta Research and Experiment Station, Stoneville, MS, USA; [3]Extension/Research Professor, Mississippi State University, Delta Research and Experiment Station, Stoneville, MS, USA; [4]Professor, Plant and Soil Sciences, Mississippi State University, Starkville, MS, USA; [5]Associate Professor, Plant and Soil Sciences, Mississippi State University, Stoneville, MS

Author for correspondence: Jason A. Bond, Extension/Research Professor, Mississippi State University, Delta Research and Extension Center, P. O. Box 197, Stoneville, MS 38776.
E-mail: jbond@drec.msstate.edu

Abstract

Glyphosate-resistant (GR) Italian ryegrass is one of the most troublesome weeds of rice in Mississippi. The most effective and economical management strategy to control GR Italian ryegrass is with preemergence (PRE) followed by postemergence (POST) herbicide programs. Two separate field studies were conducted in 2021-22 and 2022-23 in Stoneville, Mississippi, to evaluate GR Italian ryegrass control with fall-applied residual herbicide mixtures (Herbicide Mixture Study) and sequential PRE followed by POST herbicide programs with multiple applications of residual herbicides (Sequential Herbicide Application Study). In the Herbicide Mixture Study, dimethenamid-P, pyroxasulfone, and *S*-metolachlor alone provided ≥ 94% control of GR Italian ryegrass 21 d after treatment (DAT). The addition of flumioxazin to dimethenamid-P, pyroxasulfone, and *S*-metolachlor did not improve control 130 DAT. In the Sequential Herbicide Application Study, treatments with fall-applied dimethenamid-P performed better than those with acetochlor, except when fall-applied acetochlor was followed by a sequential application of clethodim plus *S*-metolachlor. Fall-applied residual herbicides are a necessary component of programs for control of GR Italian ryegrass in Mississippi.

Nomenclature: acetochlor; clethodim; dimethenamid-P; flumioxazin; pyroxasulfone; *S*-metolachlor; Italian ryegrass, *Lolium perenne* ssp. *multiflorum* (Lam.)

Keywords: sequential application; fall-applied; residual herbicide mixtures

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Introduction

Weeds are one of the most limiting factors in rice (*Oryza sativa* L.) production in Mississippi (Buehring 2008). Weeds cause problems such as reductions in yield, harvest efficiency, and rice quality, and increased challenges with drying. In Mississippi, rice production typically requires a fall- or early-spring herbicide application because reducing undesirable vegetation at the time of seeding is key in establishing an adequate rice stand. To accomplish this, preplant herbicide applications (burndown) must be timely and are most often applied during February or March in Mississippi.

Herbicide resistance within Italian ryegrass populations to multiple herbicide mechanisms of action has made it a problematic weed in the midsouthern U.S. (Bond et al. 2005; Dickson et al. 2011; Heap 2025; Nandula et al. 2007; Taylor and Coats 1996). Populations of glyphosate-resistant (GR) Italian ryegrass in Mississippi were documented in 2005 (Nandula et al. 2007), and 71 of 82 counties in Mississippi contain populations of GR Italian ryegrass (Lawrence et al. 2018). Glyphosate-resistant Italian ryegrass is one of the most troublesome weeds of rice in Mississippi (Lawrence et al. 2018). The most effective and economical management strategies for GR weeds are those that incorporate soil-applied residual herbicides (Culpepper et al. 2010). Therefore, it is imperative to control GR Italian ryegrass utilizing fall-applied residual herbicides in Mississippi (Bond et al. 2022).

Fall-applied residual herbicides control winter annual weeds because they target weeds prior to emergence (Hasty et al. 2004). Recommended products for fall-applied residual herbicides targeting GR Italian ryegrass in Mississippi include clomazone, pyroxasulfone, *S*-metolachlor, and trifluralin, which are common soil-applied residual herbicides for annual grass control (Bond et al. 2024).

Acetochlor, dimethenamid-P, and *S*-metolachlor are all Group 15 herbicides (Anonymous 2019; 2020a, b). These herbicides do not inhibit germination but affect susceptible weeds before emergence. Bond et al. (2014) reported flumioxazin controlled Italian ryegrass 64% 180 d after fall treatment. Therefore, the addition of flumioxazin to a fall-applied residual herbicide treatment may complement control with other herbicides recommended for GR Italian ryegrass control. However, fall-applied residual herbicides alone may be inadequate for complete control of many winter annual weed species (Bond et al. 2022). A sequential two-pass herbicide program consisting of a fall-applied residual herbicide followed by a sequential spring application may be needed to control many winter annual weed species (Bond et al. 2022; Vollmer et al. 2019).

Herbicide formulations provide the user with a convenient, safe product that will not deteriorate and allow the maximum activity of the active ingredient (Fogleman 2018). The formulation is determined by the relationship between the active and inert ingredients in the mixture. Controlled-release, or delayed-release, formulations are designed to bind the active ingredient so that it is released by an external trigger, to increase the amount of active ingredient reaching the biological target, or to extend the duration of active ingredient activity by not releasing it all at once (Naylor 2008). Microencapsulated (ME) formulations have emulsion droplets of the active ingredient encased in a tiny polymer shell, and these polymer shells often carry high concentrations of the active ingredient. Since the active ingredient is enclosed in a polymer shell, it is protected from degradation and allows the herbicide to be released slowly through molecular diffusion ultimately resulting in longer control of the target weed (Anonymous 2010).

Because Italian ryegrass is resistant to multiple herbicide mechanisms of action in Mississippi, sequential applications of herbicides are required for control (Bond et al. 2022). Acetochlor treatments utilizing the ME formulation could offer an option for a fall-applied residual herbicide targeting GR Italian ryegrass that also exhibits a rotation interval favorable for rice in the spring following application. Furthermore, after a fall-applied residual herbicide application, the addition of a residual herbicide to a sequential application when targeting GR Italian ryegrass may be beneficial. The objectives of this research were to evaluate fall-applied treatments of group 15 herbicides with and without flumioxazin for control of GR Italian ryegrass and to determine the effect of adding a residual herbicide to a sequential clethodim treatment following fall-applied residual treatment.

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Materials and Methods

Herbicide Mixture Study. A field study was conducted in 2021-22 (33.433 N 90.909 W) and 2022-23 (33.432 N 90.909 W) at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, to evaluate the control of GR Italian ryegrass with Group 15 herbicides applied with and without flumioxazin. Plot size was 2 m wide by 4.6 m in length and separated by fallow alleys. Soil was a Commerce very fine sandy loam (Silty over clay, mixed, superactive, nonacid, thermic, Fluvaquentic Endoaquepts) with a pH ranging from 4.5 to 5.5 and approximately 1.8% organic matter. The study area was infested with a naturally occurring population of GR Italian ryegrass.

The experimental design was a randomized complete block with a two-factor factorial arrangement of treatments and four replications. Factor A was flumioxazin (Valor EZ, Valent Biosciences Libertyville, IL, 870 Technology Way 60048) rates of 0 and 72 g ai ha^{-1}. Factor B was Group 15 herbicide and included no group 15 herbicide, acetochlor (Warrant, Bayer Crop Science, 2 T.W. Alexander Drive Research Triangle Park, NC 27709) at 1,266 g ai ha^{-1}, dimethenamid-P (Outlook, BASF, 100 Park Avenue, Florham Park, NJ 07932) at 841 g ai ha^{-1}, *S*-metolachlor (Dual Magnum, Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC, 27419) at 1,424 g ai ha^{-1}, and pyroxasulfone (Zidua SC, BASF, 100 Park Avenue, Florham Park, NJ 07932) at 240 g ai ha^{-1}. Fall-applied residual herbicide treatments were applied using a CO_2-pressurized backpack sprayer delivering 140 L ha^{-1} on October 26, 2021, and November 4, 2022. Rainfall was received within 3 d of application both years.

Data collection included visible estimate of GR Italian ryegrass control on a scale of 0 to 100% where 0 indicated no control and 100 indicated complete plant death (Frans et al. 1986) at 21, 100, and 130 d after treatment (DAT) each year. Italian ryegrass density from two randomly selected 1-m^2 quadrats in each plot was recorded 49 and 91 DAT. Aboveground weight of GR Italian ryegrass was collected by harvesting plants from two randomly selected 1-m^2 quadrats in each plot 130 DAT. Hand-harvested samples were removed by cutting at the crown and placed into separate paper bags. Bags containing plant material were placed in forced draft ovens at 35 C for seven days, and weights were recorded.

Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., 100 SAS Campus Drive Cary, USA) with year and replication (nested within year) set as random effect parameters (Blouin et al. 2011). Type III statistics were applied to test

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the fixed effects of flumioxazin rate and group 15 herbicide for GR Italian ryegrass control, density, and dry weight. Estimates of least-squared means were utilized for mean separation ($p \leq 0.05$).

Sequential Herbicide Application Study. A field study was conducted in 2021-22 (33.4328 N 90.9089 W) and 2022-23 (33.4329 N 90.9090 W) at the Mississippi State University Delta Research and Extension Center in Stoneville, MS, to evaluate control of GR Italian ryegrass with sequential applications of PRE and POST herbicides applied in fall and winter. Plot dimensions, site information, treatment application, and weed establishment method were identical to the Herbicide Mixture Study.

The experimental design was a randomized complete block with a two-factor factorial arrangement of treatments and four replications. Factor A was fall treatment and included no fall treatment, dimethenamid-P at 842 g ha^{-1}, and acetochlor at 1,267 g ha^{-1}. Fall treatments were applied on October 26, 2021, and November 4, 2022. Factor B was sequential herbicide treatment and included no sequential treatment, clethodim at 140 g ai ha^{-1}(Select 2 EC, Valent Biosciences Libertyville, IL, 870 Technology Way 60048), clethodim at 140 g ha^{-1} plus *S*-metolachlor at 1,424 g ha^{-1}, and clethodim at 140 g ha^{-1} plus flumioxazin at 72 g ha^{-1}. Sequential herbicide treatments were applied January 18, 2021, and February 14, 2022. All sequential treatments included a nonionic surfactant (Activator 90, Loveland Products, Greeley, NC) at 0.5% v/v and ammonium sulfate (Class Act NG, Winfield Solutions, St. Paul, MN) at 2.5% v/v. Target GR Italian ryegrass population was 20 to 28 cm at the time of sequential application each year.

Data collection included visible estimates of GR Italian ryegrass control on the previously described scale at monthly intervals following fall treatment and 21 and 35 d after sequential herbicide treatment. Italian ryegrass was collected from two randomly selected 1-m^2 quadrats in each plot utilizing the same method outlined in the Herbicide Mixture Study to record aboveground dry weight 28 d after sequential herbicide treatment. Data analyses were similar to those in the Herbicide Mixture Study.

Results and Discussion

Herbicide Mixture Study. Interactions of flumioxazin rate and group 15 herbicide were detected for GR Italian ryegrass control 21, 100, and 130 DAT ($p \leq 0.0001$). Dimethenamid-P, pyroxasulfone, and *S*-metolachlor alone provided ≥ 94% GR Italian ryegrass control 21 DAT, which was greater than with acetochlor (Table 1). Dimethenamid-P and *S*-metolachlor controlled more GR Italian ryegrass 100 DAT with and without flumioxazin compared with acetochlor (Table 1). Glyphosate-resistant Italian ryegrass control 130 DAT with dimethenamid-P was 26 and 40% greater than with pyroxasulfone and acetochlor, respectively, but it was similar to that with *S*-metolachlor (Table 1). The addition of flumioxazin to dimethenamid-P, pyroxasulfone, and *S*-metolachlor did not improve control 130 DAT. Flumioxazin and acetochlor alone controlled GR Italian ryegrass ≤ 53% 130 DAT. When flumioxazin was included, *S*-metolachlor and pyroxasulfone provided 82 and 73% control, respectively.

Interactions of flumioxazin rate and group 15 herbicide were detected for GR Italian ryegrass density at 49 ($p \leq 0.0001$) and 91 DAT ($p=0.0139$). As weed densities increase, the effectiveness of a fall-applied residual herbicide often decreases (Avent et al. 2023). With no flumioxazin, plots treated with acetochlor contained at least 63 plants m^{-2} more than those receiving dimethenamid-P, pyroxasulfone, and *S*-metolachlor 49 DAT (Table 2). Similar to Avent et al. (2023), pyroxasulfone provided better GR Italian ryegrass control than acetochlor. Dimethenamid-P, pyroxasulfone, and *S*-metolachlor reduced GR Italian ryegrass density to < 7 plants m^{-2} 91 DAT with and without flumioxazin (Table 2).

The main effect of flumioxazin rate ($p=0.3962$) and the interaction of flumioxazin rate and group 15 herbicide ($p=0.7742$) were not significant for aboveground dry weight; however, a group 15 herbicide main effect was significant ($p \leq 0.0001$). Aboveground dry weight of GR Italian ryegrass following dimethenamid-P and *S*-metolachlor was ≤ 33 g m^{-2} (Table 3). Acetochlor and pyroxasulfone treatments resulted in ≥ 184 g m^{-2} dry weight. Bond et al. (2014) reported that treatments containing clomazone, pyroxasulfone, and *S*-metolachlor provide adequate control of GR Italian ryegrass when applied in the fall; however, control decreased approximately 140 to 180 DAT.

Sequential Application Study. A main effect of fall treatment ($p \leq 0.0001$) was detected for GR Italian ryegrass control 70 d after fall herbicide treatment. Dimethenamid-P controlled GR Italian ryegrass 96% 70 d after fall treatment, and this was greater than control with acetochlor (Table 4). Fall-applied dimethenamid-P can be an effective weed management strategy for controlling winter annual weed species (Vollmer et al. 2019). Because no fall-applied treatment provides complete control, a sequential herbicide application is necessary to approach complete control (Bond et al. 2022).

An interaction of fall and sequential herbicide treatments was detected for GR Italian ryegrass control 21 ($p \leq 0.0001$) and 35 ($p \leq 0.0001$) d after sequential treatment. All treatments containing dimethenamid-P as a fall treatment controlled Italian ryegrass ≥ 96% 21 d after sequential herbicide treatment (Table 5). Fall-applied acetochlor followed by a sequential application of clethodim plus *S*-metolachlor provided comparable control to treatments where dimethenamid-P was applied in the fall. When no fall herbicide treatment was utilized, the addition of flumioxazin to clethodim increased control ≥ 14%. When acetochlor was applied in the fall, mixtures of *S*-metolachlor plus clethodim as a sequential treatment provided comparable control to a sequential flumioxazin plus clethodim treatment (Table 5).

A fall treatment of dimethenamid-P controlled GR Italian ryegrass ≥ 94% regardless of sequential treatment 35 d after sequential treatment (Table 5). Acetochlor followed by any sequential treatment controlled GR Italian ryegrass better than acetochlor alone 35 d after sequential herbicide treatment. However, no sequential treatment provided ≥ 74% control in the absence of fall treatment. Italian ryegrass plants that have survived an October or November herbicide application have fewer and smaller leaves compared with those that survived a September herbicide application. Therefore, POST herbicide coverage is much greater following a fall application in October or November compared with September application or no application (Bond et al. 2014).

No interaction of fall and sequential herbicide treatments was detected for GR Italian ryegrass aboveground dry weight ($p = 0.2753$) 28 d after sequential treatment. However, main effects of fall herbicide ($p=0.0002$) and sequential herbicide ($p=0.0083$) treatments were significant. Glyphosate-resistant Italian ryegrass dry weight following acetochlor was 161 g m^{-2}, while that following dimethenamid-P was <4 g m^{-2} (Table 6). Pooled over fall herbicide treatment, clethodim and clethodim plus flumioxazin reduced GR Italian ryegrass aboveground dry weight more than clethodim plus *S*-metolachlor (Table 7).

Practical Implications

The effectiveness of fall-applied herbicide treatments to manage GR Italian ryegrass populations has previously been documented (Bond et al. 2014, 2022), but populations of GR Italian ryegrass continue to increase. Furthermore, fall tillage may season-long control of GR Italian ryegrass by terminating initial emergence in the fall (Bond et al. 2022); however, fall tillage in years when rainfall totals are greater than normal may contribute to increased soil erosion. Dimethenamid-P applied alone as a fall treatment was effective targeting GR Italian ryegrass (Tables 1, 2, 3). At present, POST herbicides for GR Italian ryegrass control in Mississippi are limited to clethodim and paraquat (Bond et al. 2022; 2024). When comparing sequential herbicide applications targeting GR Italian ryegrass, clethodim plus flumioxazin provided the greatest control 21 d after sequential treatment in the absence of fall-applied residual herbicides (Table 5). Treatments with fall-applied dimethenamid-P performed better than those with acetochlor except when fall-applied acetochlor was followed by a sequential application of clethodim plus *S*-metolachlor. Poor control with acetochlor might be related to the formulation of the product utilized in the current work since it was an ME formulation while dimethenamid-P is an EC. The addition of a residual herbicide to a clethodim application did not influence control with clethodim 35 d after sequential treatment in plots receiving a fall treatment (Table 5). However, the addition of *S*-metolachlor reduced control with clethodim when no fall treatment was applied. This research indicates that fall-applied residual herbicides such as dimethenamid-P should be utilized to control GR Italian ryegrass in Mississippi. In the absence of a fall-applied residual herbicide, *S*-metolachlor can reduce efficacy of clethodim targeting GR Italian ryegrass. Also, little benefit was realized from adding a residual herbicide to a sequential clethodim application targeting GR Italian ryegrass. As Bond et al. (2014) reported that clomazone, pyroxasulfone, *S*-metoloachlor, and trifluralin were effective residual herbicide treatments targeting GR Italian ryegrass, our current research indicates that fall-applied dimethenamid-P can also provide effective GR Italian ryegrass control.

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Competing Interest

The authors declare they have no competing interests.

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Table 1. Glyphosate-resistant Italian ryegrass control 21, 100, and 130 d after treatment (DAT) in a study evaluating fall-applied residual herbicide mixtures for control of GR Italian ryegrass from 2021-22 to 2022-23 at Stoneville, MS.[a]

Flumioxazin rate	Group 15 herbicide	Rate	21 DAT	100 DAT	130 DAT
g ai ha^{-1}		g ai ha^{-1}	%		
0	No group 15 herbicide	0	0	0	0
	acetochlor	1266	79 c	68 d	48 d
	dimethenamid-p	841	95 a	93 a	88 ab
	pyroxasulfone	240	94 a	80 c	63 cd
	S-metolachlor	1424	95 a	90 ab	74 abc
72	No group 15 herbicide	0	87 b	79 c	53 d
	acetochlor	1266	91 ab	84 bc	53 d
	dimethenamid-P	841	96 a	91 ab	89 a
	pyroxasulfone	240	94 a	86 abc	73 bc
	S-metolachlor	1424	96 a	90 ab	82 ab

[a] Data were pooled across two years. Means followed by the same letter within a column are not different at $p \leq 0.05$.

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Table 2. Glyphosate-resistant Italian ryegrass density 49 and 91 d after treatment (DAT) in a study evaluating fall-applied residual herbicide mixtures for control of GR Italian ryegrass from 2021-22 to 2022-23 at Stoneville, MS[a].

Flumioxazin rate	Group 15 herbicide	Rate	49 DAT	91 DAT
g ai ha^{-1}		g ai ha^{-1}	—— No. m^{-2} ——	
0	No group 15 herbicide	0	214 a	214 a
	acetochlor	1266	70 b	84 b
	dimethenamid-p	841	0 b	0 c
	pyroxasulfone	240	7 b	7 c
	S-metolachlor	1424	2 b	2 c
72	No group 15 herbicide	0	72 b	85 b
	acetochlor	1266	18 b	33 bc
	dimethenamid-p	841	0 c	4 c
	pyroxasulfone	240	3 b	4 c
	S-metolachlor	1424	0 b	2 c

[a] Data were pooled across two years. Means followed by the same letter within a column are not different at $p \leq 0.05$.

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Table 3. Main effect of fall-applied residual herbicides on aboveground dry weight 130 d after fall treatment in a study evaluating GR Italian ryegrass control with fall-applied residual herbicides in Stoneville, MS, from 2021-22 to 2022-23.

Group 15 herbicide	Rate	Aboveground dry weight
	g ai ha^{-1}	g
No group 15 herbicide	0	162 a
acetochlor	1266	204 a
dimethenamid-p	841	5 b
pyroxasulfone	240	184 a
S-metolachlor	1424	33 b

[a] Data were pooled across two flumioxazin rates and two years. Means followed by the same letter within a column are not different at $p \leq 0.05$.

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Table 4. Glyphosate-resistant Italian ryegrass control 70 d after fall treatment in a study evaluating control of glyphosate-resistant Italian ryegrass with sequential applications of residual herbicides from 2021-22 to 2022-23 at Stoneville, MS[a].

Fall herbicide treatment	Rate	Control
	g ai ha^{-1}	%
No fall herbicide	0	0 c
acetochlor	1,266	80 b
dimethenamid-P	841	96 a

[a] Data were pooled across four sequential herbicide treatments and two years. Means followed by the same letter are not different at $p \leq 0.05$.

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Table 5. Glyphosate-resistant Italian ryegrass control 21 and 35 d after sequential treatment in a study evaluating control of GR Italian ryegrass from 2021-22 to 2022-23 at Stoneville, MS.[a]

Fall herbicide[b]	Sequential herbicide[b]	21 d after sequential	35 d after sequential
		%	
No fall herbicide	No sequential herbicide	0	0
	clethodim	57 de	68 d
	clethodim plus flumioxazin	70 cd	74 cd
	clethodim plus *S*-metolachlor	55 e	49 e
acetochlor	No sequential herbicide	54 e	50 e
	clethodim	74 c	69 cd
	clethodim plus flumioxazin	82 bc	78 cd
	clethodim plus *S*-metolachlor	84 ab	81 bc
dimethenamid-P	No sequential herbicide	96 ab	94 ab
	clethodim	98 a	97 a
	clethodim plus flumioxazin	96 ab	96 a
	clethodim plus *S*-metolachlor	97 a	95 a

[a] Data were pooled across two years. Means followed by the same letter within a column are not different at $p \leq 0.05$.

[b]Acetochlor was applied at 1,266 g ai ha^{-1}, clethodim was applied at 140 g ai ha^{-1} dimethenamid-P was applied at 841 g ai ha^{-1}, flumioxazin was applied at 72 a ai ha^{-1}, and *S*-metolachlor was applied at 1,424 g ai ha^{-1}.

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Table 6. Main effect of fall residual herbicide treatment on aboveground dry weight 28 d after sequential treatment in a study evaluating glyphosate-resistant Italian ryegrass control with sequential treatments of residual herbicides in Stoneville, MS, from 2021-22 to 2022-23.[a]

Fall herbicide treatment	Rate	Aboveground dry weight
	g ai ha^{-1}	g
No fall herbicide	0	141 a
Acetochlor	1,266	161 a
dimethenamid-P	841	4 b

[a] Data were pooled across four sequential herbicide treatments and two years. Means followed by the same letter are not different at $p \leq 0.05$.

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Table 7. Main effect of sequential herbicides on aboveground dry weight 28 d after sequential treatment in a study evaluating glyphosate-resistant Italian ryegrass control with sequential treatments of residual herbicides in Stoneville, MS, from 2021-22 to 2022-23.

Sequential herbicide	Rate	Aboveground dry weight
	g ai ha^{-1}	g
No sequential herbicide	0	160 a
clethodim	140	46 b
clethodim plus flumioxazin	140 + 72	42 b
clethodim plus *S*-metolachlor	140 + 1,424	160 a

[a] Data were pooled across four sequential herbicide treatments and two years. Means followed by the same letter are not different at $p \leq 0.05$

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