'JS501' and 'Replay' Perennial Ryegrass Glyphosate Tolerance and Rates Required for Annual Bluegrass (*Poa annua* L.) Control

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Abstract. Incorporating the use of glyphosate into a weed management program offers turfgrass managers increased flexibility and cost savings when attempting to control troublesome weeds such as annual bluegrass (ABG) (*Poa annua* L.). Field trials of glyphosate tolerant perennial ryegrass (PRG) (*Lolium perenne* L.) cultivars, JS501 and Replay, were initiated to determine glyphosate tolerance and rates required for ABG control. In the tolerance trial, glyphosate was applied on 15 Sept. 2010 and 9 Aug. 2011 at rates of 0, 0.29, 0.58, 1.16, 1.74, 2.32, and 3.48 kg·ha⁻¹ a.e. Glufosinate was also applied at 0, 1.68, and 3.37 kg·ha⁻¹ a.i. In the ABG control trial, glyphosate was applied on 17 June followed by 19 Aug. 2009 and 25 June followed by 25 Aug. 2010 at rates of 0, 0.15, 0.29, 0.44, and 0.58 kg·ha⁻¹ a.e. In the tolerance trial, linear regression analysis revealed a glyphosate application rate of 0.81 kg·ha⁻¹ a.e. was required to cause 20% leaf firing. By the end of the trial, the highest rate of glufosinate resulted in nearly complete desiccation of 'Replay' PRG. For ABG control, after four glyphosate applications over a 2-year period, a rate of 0.29 kg·ha⁻¹ a.e. or greater resulted in less than 10% ABG. Untreated plots had ≈83% ABG infestation. Discoloration was not noted for either PRG cultivar at any point over the 2-year trial period. Based on the environmental conditions of each trial, results suggest a recommended application rate should be 0.29 kg·ha⁻¹ a.e. during summer months. This rate is sufficient for ABG control and also provides protection in case spray overlap occurs during an application.

As a result of PRG's bunch-type growth, susceptibility to cold and heat stress, and slow recovery from traffic, weed invasion is often problematic where permanent stands of PRG are grown. One weed that is particularly troublesome is ABG. This is perhaps the most problematic weed species to control in fine turfgrass management as a result of prolific seed head production and genetic diversity (Cline et al., 1993; Lush, 1988). Currently, few herbicides are labeled for ABG control in permanent stands of PRG. Bispyribac-sodium has shown promise, but efficacy depends on temperature, rate, and application timing for acceptable ABG control with minimal PRG injury (McCullough and Hart, 2009). Williams et al. (2009) reported minimal PRG toxicity after mesotrione applications in a greenhouse experiment; however, this herbicide is only labeled for ABG suppression. Ethofumesate is also labeled for ABG control in PRG; however, results can be variable depending on numerous environmental factors such as temperature, light intensity, and soil moisture.

Maintaining a weed-free stand of PRG in northern climates is often a challenge as a result of weed invasion, in particular, ABG.

Although ABG is sensitive to cold, heat, disease, and drought (Beard, 1973), most biotypes of ABG are susceptible to glyphosate. Grossbard and Atkinson (1985) reported that a glyphosate rate of 0.30 kg·ha⁻¹ a.e. was sufficient for ABG removal. However, Goss et al. (2009) reported a rate of 3.30 kg·ha⁻¹ a.e. to kill 90% of ABG plants in a greenhouse experiment. Although these studies indicate ABG's genetic diversity in response to glyphosate applications, previous research indicates a wide range of diversity between ABG biotypes. Three golf courses in Minnesota, within a 5-mile radius, displayed a diverse population of ABG biotypes (Cline et al., 1993). These included prolific seed producers, minimal seed producers with dark fall color, cold-sensitive biotypes, and dark blue-green coarse biotypes. Also, ABG from the same golf course can vary because Lush (1989) noted ABG biotypes sampled on a putting green had smaller seed size, delayed flowering, and did not require chilling to break seed dormancy compared with ABG biotypes from the adjacent fairway/rough. All of these reports indicate the complexity associated when attempting to control such a diverse species.

Few commercially available cultivars possess a sufficient amount of glyphosate tolerance to control ABG. Hart et al. (2005) reported that 'Aurora Gold' hard fescue (*Festuca longifolia* Thuill.) can tolerate a single glyphosate application of 0.6 to 0.8 kg·ha⁻¹ a.e., which would be sufficient for ABG removal. To date, there

have been no reports of a glyphosate-tolerant PRG cultivar in the literature. 'JS501' and 'Replay', glyphosate-tolerant PRG cultivars, were part of a population improvement program that included selection, paired crosses, and polycrosses, which originated from a greenhouse cross in 1992 of 'APM' PRG, which was pollinated by 'Birdie II' PRG. The glyphosate tolerance of the cultivars is associated with double mutations of the 5-enolpyruvylshikimate-3-phosphate synthase gene (EPSPS) and its encoded EPSPS protein (Samudio et al., 2011). The glyphosate tolerance is naturally occurring, which has been documented in other *Lolium* species (Lorraine-Colwell et al., 2001, 2003).

The introduction of a glyphosate-tolerant PRG cultivar in fine turfgrass management would provide a unique tool in weed control for cool-season turfgrass managers. To preserve the glyphosate tolerance trait, rates used in 'JS501' and 'Replay' seedstock production fields are annually sprayed at 1.16 kg·ha⁻¹ a.e. with minimal PRG injury. However, determining glyphosate rates adequate for ABG control and testing the tolerance in a fine turfgrass environment are needed to refine application rates. Therefore, research objectives were to determine the glyphosate tolerance of 'Replay' PRG and to determine glyphosate application rates required for effective ABG control in a stand of 'JS501' and 'Replay' PRG.

Materials and Methods

Two field trials were conducted from 2009 to 2011 at the Jacklin Seed research farm in Post Falls, ID. For both trials, site preparation and management were identical. Before seeding, the area was sprayed with glyphosate at 1.74 kg·ha⁻¹ a.e., scalped to 0.38 cm (Greens King 500 Series; Jacobsen, Charlotte, NC), and verticut in two directions with 2.5-cm blade spacing set to a depth of 0.2 cm (Graden GS04; Graden Company, Victoria, Australia). After all debris was removed, the area was seeded at 390 kg·ha⁻¹ on a Garrison gravelly silt loam (fine-loamy, mixed, superactive, calcareous, hyperthermic, Typic, Torrifluent). Plots were fertilized at 48 kg·ha⁻¹ nitrogen (N) using 15N–6.6P–12.5K the day of seeding and again 2 weeks after seeding. Once all plots were established, grass was mowed at a height of 1.27 cm three times weekly without clipping removal. Irrigation was provided as needed to prevent wilt stress and no additional herbicide, fungicide, or insecticide applications were necessary.

Glyphosate tolerance of 'Replay' perennial ryegrass. 'Replay' was seeded on 6 May 2010 at a rate of 390 kg·ha⁻¹. Glyphosate (Departure; Syngenta Crop Protection Inc., Greensboro, NC) rates were 0, 0.29, 0.58, 1.16, 1.74, 2.32, and 3.48 kg·ha⁻¹ a.e. Glufosinate (Finale; Bayer Environmental Science, Research Triangle Park, NC) was also applied at 0, 1.68, and 3.37 kg·ha⁻¹ a.i. Individual plot size was 1.8 m × 2.4 m. Applications occurred on 15 Sept. 2010 and 9 Aug. 2011. A different section of the PRG block that was seeded on

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6 May 2010 was sprayed in 2010 and 2011. Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, 100% = complete desiccation. Leaf firing was rated 8, 16, 24, and 31 d after application (DAA).

Annual bluegrass control. 'JS501' and 'Replay' were seeded on 4 Sept. 2008 at a rate of 390 kg·ha⁻¹. Glyphosate rates of 0, 0.15, 0.29, 0.44, and 0.58 kg·ha⁻¹ a.e. were applied on 17 June followed by 19 Aug. 2009 and 25 June followed by 25 Aug. 2010. The same plots were sprayed in 2009 and 2010. Individual plot size was 1.2 m × 2.4 m. The ABG was a result of a natural infestation. For both trials, all herbicide treatments were applied using a CO₂ backpack sprayer calibrated to deliver 326 L·ha⁻¹ set to 245 kPa with TeeJet XR 8004 nozzles (Tee Jet Technologies, Wheaton, IL). Data collected included PRG and ABG color and percent ABG. Species color was rated on a scale from 1 to 9, where 1 = brown turfgrass, 9 = dark green turfgrass, 7 = commercially acceptable color. Color was recorded 1 month after the initial and sequential glyphosate application. Percent ABG was rated on a scale of 0% to 100%, where 0% = no ABG encroachment, 100% = complete ABG encroachment. This parameter was rated before the initial glyphosate application each year and also recorded 1 month after the initial and sequential glyphosate applications.

Data analysis. In both trials, treatments were arranged in a randomized complete block design with three replications. In the ABG control trial, 'JS501' and 'Replay' were planted in separate blocks; therefore, data for each cultivar are presented separately. Treatment effects were evaluated using analysis of variance with means separation based on Fisher's least significant difference test at alpha = 0.05 (Statistica; Stat-Soft, Tulsa, OK). In the tolerance trial, regression analysis was used to predict at what rate glyphosate caused 20% injury. In the tolerance trial, there was no significant year-by-treatment interaction. In the ABG control trial, there was a significant year-by-treatment interaction on one rating date. However, this interaction occurred as a result of a numerical scale change between years. Therefore, data for both trials were pooled over the 2-year study period.

Results and Discussion

Tolerance trial. At 8 DAA, a glyphosate rate 1.16 kg·ha⁻¹ a.e. or less had less than 20% leaf firing (Table 1). However, 16 DAA, rates greater than or equal to the 1.16 kg·ha⁻¹ a.e. rate had injury greater than the minimally acceptable leaf firing rating of 20%. This rate continued to show unacceptable leaf firing ratings through the duration of the trial. On all rating dates, minimal injury (less than 10%) occurred at rates 0.58 kg·ha⁻¹ a.e. or less. Regression analysis revealed that a rate of 0.81 kg·ha⁻¹ a.e. was required to cause 20% leaf firing injury (Fig. 1). Similar results were noted on a hard fescue cultivar, Aurora

Table 1. Percent leaf firing of 'Replay' perennial ryegrass after various glyphosate and glufosinate application rates on 15 Sept. 2010 and 9 Aug. 2011 in Post Falls, ID.

Treatment	Rate	Percent leaf firing ²			
		8 DAA ¹	16 DAA	24 DAA	31 DAA
Glyphosate (kg·ha ⁻¹ a.e.)	0	0	0	0	0
	0.29	0	1	0	0
	0.58	0	5	2	0
	1.16	15	31	26	21
	1.74	23	61	62	62
	2.32	30	66	66	77
	3.48	53	86	87	94
Glufosinate (kg·ha ⁻¹ a.i.)	0	0	0	0	0
	1.68	90	90	80	70
	3.37	98	100	98	99
LSD _{0.05}		7.3	11.9	13.7	10
Analysis of variance					
Herbicide rate (HR)		**	**	**	**
Year (Y)		NS*	*	NS	NS
HR*Y		NS	NS	NS	NS

¹Percent leaf firing based on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, 100% = complete desiccation.

²DAA = days after application.

*NS, nonsignificant.

*Significant at $P \leq 0.05$;

**significant at $P \leq 0.01$.

LSD = least significant difference.

Fig. 1. Percent leaf firing 16 d after application of 'Replay' perennial ryegrass (PRG) after various glyphosate application rates on 15 Sept. 2010 and 9 Aug. 2011 in Post Falls, ID. ²Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, 100% = complete desiccation.

Gold, because injury exceeded 20% when glyphosate rates approached 0.80 kg·ha⁻¹ a.e. (Hart et al., 2005).

Meanwhile, 16 DAA, glufosinate applied at 3.37 kg·ha⁻¹ a.i. completely desiccated 'Replay' PRG (Table 1). By the end of the trial, the highest rate of glyphosate and glufosinate resulted in greater than 90% injury. This indicates that an alternative chemistry, glufosinate, can effectively remove 'Replay' PRG within label rates. Future trials should include other chemistries to ensure that this PRG cultivar can be removed using other modes of action through various herbicides.

Annual bluegrass trial. Data for both cultivars are presented separately because they were planted in two blocks; however, data are nearly identical. Therefore, species color and percent ABG data will only be discussed for 'JS501' (Table 2).

'JS501' did not exhibit any discoloration regardless of the glyphosate application rate throughout the trial (Table 2). This is in agreement with results from the tolerance trial. At the 0.15 kg·ha⁻¹ a.e. rate, ABG had color greater than 7.0 on all rating dates. However, once rates exceeded 0.15 kg·ha⁻¹ a.e., ABG was completely desiccated.

Before initial glyphosate applications, percent ABG in plots ranged from 28% to 40% (Table 3). One month after the initial application, untreated plots were comprised of more than 80% ABG; however, areas treated with 0.29 kg·ha⁻¹ a.e. had 21% ABG infestation. Plots treated with rates greater than 0.29 kg·ha⁻¹ a.e. had less than 10% ABG. One month after the second application, plots treated with glyphosate 0.29 kg·ha⁻¹ a.e. or greater had less than 10% ABG, whereas the

Table 2. Color of 'JS501' and 'Replay' perennial ryegrass and *Poa annua* after four different glyphosate application rates on 17 June and 19 Aug. 2009 and 25 June and 25 Aug. 2010 in Post Falls, ID.

Treatment	Rate (kg·ha ⁻¹ a.e.)	Color ^z			
		1 MAIA ^y		1 MASA ^x	
		JS501	<i>Poa annua</i>	JS501	<i>Poa annua</i>
Untreated	0.00	9.0	9.0	9.0	9.0
Glyphosate	0.15	9.0	7.7	9.0	7.3
	0.29	9.0	1.0	9.0	1.0
	0.44	9.0	1.0	9.0	1.0
	0.58	9.0	1.0	9.0	1.0
Analysis of variance					
Glyphosate rate (GR)		NS ^w	**	NS	**
Year (Y)		NS	NS	NS	NS
GR*Y		NS	NS	NS	NS

Treatment	Rate (kg·ha ⁻¹ a.e.)	Color ^z			
		1 MAIA ^y		1 MASA ^x	
		Replay	<i>Poa annua</i>	Replay	<i>Poa annua</i>
Untreated	0.00	9.0	9.0	9.0	9.0
Glyphosate	0.15	9.0	8.0	9.0	8.0
	0.29	9.0	1.0	9.0	1.0
	0.44	9.0	1.0	9.0	1.0
	0.58	9.0	1.0	9.0	1.0
Analysis of variance					
Glyphosate rate (GR)		NS	**	NS	**
Year (Y)		NS	NS	NS	NS
GR*Y		NS	NS	NS	NS

^zColor based on a scale of 1 to 9, where 1 = brown, dead turfgrass and 9 = dark green, healthy turfgrass.

^yOne month after initial application.

^xOne month after second application.

^wNS = nonsignificant.

*Significant at $P \leq 0.05$;

**significant at $P \leq 0.01$.

Table 3. Percent *Poa annua* contamination in 'JS501' and 'Replay' perennial ryegrass plots after four different glyphosate application rates on 17 June and 19 Aug. 2009 and 25 June and 25 Aug. 2010 in Post Falls, ID.

Treatment	Rate (kg·ha ⁻¹ a.e.)	'JS501' perennial ryegrass			
		21 May 2009 ^z	3 June 2010 ^y	1 MAIA ^x	1 MASA ^w
		<i>Poa annua</i> (%)			
Untreated	0.00	40	81 a	84 a	83 a
Glyphosate	0.15	28	60 b	43 b	27 b
	0.29	30	25 c	21 c	5 c
	0.44	33	15 c	8 c	0 c
	0.58	38	20 c	3 c	0 c
Analysis of variance					
Glyphosate rate (GR)		NS ^y	**	**	**
Year (Y)		—	—	*	*
GR*Y		—	—	NS	NS

Treatment	Rate (kg·ha ⁻¹ a.e.)	'Replay' perennial ryegrass			
		21 May 2009	3 June 2010	1 MAIA	1 MASA
		<i>Poa annua</i> (%)			
Untreated	0.00	42	77 a	80 a	82 a
Glyphosate	0.15	37	48 b	59 b	28 b
	0.29	42	20 c	17 c	3 c
	0.44	38	17 c	7 c	0 c
	0.58	47	18 c	7 c	0 c
Analysis of variance					
Glyphosate rate (GR)		NS	**	**	**
Year (Y)		—	—	**	NS
GR*Y		—	—	**	NS

^zInitial estimate of *Poa annua* infestation before initial glyphosate application in 2009.

^yInitial estimate of *Poa annua* infestation before initial glyphosate application in 2010.

^xOne month after initial application.

^wOne month after second application.

^vNS = nonsignificant.

* Significant at $P \leq 0.05$;

** significant at $P \leq 0.01$.

0.15 kg·ha⁻¹ a.e. application rate had 27% ABG encroachment. These data indicate that a repeat application is necessary for season-long ABG control.

Percent ABG and ABG color data are similar to previous research reported in the literature. Grossbard and Atkinson (1985) reported a glyphosate rate of 0.30 kg·ha⁻¹ a.e.

was sufficient for removal of ABG. Similarly, in a greenhouse experiment, Brosnan et al. (2012) reported susceptible ABG biotypes had a F_v/F_m value of 0.000 at rates greater than 0.21 kg·ha⁻¹ a.e., indicating the plants ceased photosynthesis. Also, Binkholder et al. (2011) noted a glyphosate rate of 0.09 kg·ha⁻¹ a.e. was required to reduce ABG aboveground biomass by 50%. However, Goss et al. (2005, 2009) reported higher rates of glyphosate were required to remove ABG used in a greenhouse trial. Data from this research and previous research indicate the genetic diversity of ABG in response to glyphosate.

This research represents the first report of glyphosate-tolerant PRG cultivars with sufficient levels of tolerance to eradicate most ABG biotypes. However, the level of ABG control is likely to vary as a result of the number of diverse biotypes that exist, maturity of the ABG plant, perennial vs. annual ABG biotype, application timing, glyphosate formulation, and diverse microenvironments where turfgrasses are planted. Based on the environmental conditions of each trial, results suggest a recommended application rate should be 0.29 kg·ha⁻¹ a.e. This rate is sufficient for ABG control and also provides protection in case spray overlap occurs during an application. Also, a repeat application, 8 weeks after an initial application, is necessary for season-long ABG control. Although this trial focused solely on using a single chemistry, glyphosate, future trials should focus on incorporating other pre- and post-emergent herbicides for broad-spectrum weed control. This type of future research will help minimize selection pressure for glyphosate-resistant ABG biotypes, which have been observed in Missouri and Tennessee (Binkholder et al., 2011; Brosnan et al., 2012).

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