Glyphosate Tolerance of Two Perennial Ryegrass Cultivars from Fall Applications and at Seedling Growth Stages

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Abstract. The use of glyphosate-tolerant perennial ryegrass (*Lolium perenne* L.) (PRG) cultivars JS501 and Replay provides turfgrass managers a unique option for annual bluegrass (*Poa annua* L.) (ABG) control. Both cultivars can tolerate a maximum glyphosate rate of 0.81 kg-ha⁻¹ acid equivalent (a.e.) after establishment under optimal growing temperatures (16 to 24 °C). However, tolerance to applications made immediately after germination and during low air temperatures has received limited investigation. Therefore, objectives of this research were to determine the seedling tolerance and low-temperature response after a fall season glyphosate application to both cultivars. Field trials were conducted in Idaho and Oregon. For the fall application response trial in Idaho, glyphosate was applied at 0, 0.15, 0.29, 0.58, 1.16, 1.74, 2.32, and 3.48 kg-ha⁻¹ a.e. In Oregon, glyphosate was applied at 0, 0.15, 0.29, 0.44, 0.58, 1.16, and 3.48 kg-ha⁻¹ a.e. At both sites, applications were made between late September and early October. To determine seedling tolerance, both cultivars were sprayed with glyphosate at the one-leaf stage (LS), two LS, three LS, or four LS at rates of 0, 0.15, 0.29, and 0.58 kg-ha⁻¹ a.e. Across all trials, ratings included PRG color, cover, and injury. At both trial locations, regression analysis revealed a rate of ≈0.27 kg-ha⁻¹ a.e. was required to cause 20% leaf firing in the fall application response trial. In the seedling tolerance trial, glyphosate applied at 0.58 kg-ha⁻¹ a.e. at the one LS, two LS, and three LS had color ratings 8.0 or greater; however, color ratings dropped to 4.6 when an application was made at the four LS. Based on the environmental conditions of each trial, results suggest glyphosate applications greater than 0.27 kg-ha⁻¹ a.e. as minimum air temperatures approach 0 °C should be avoided. Also, applications should be avoided at the three to four LS if the application rate is greater than 0.29 kg-ha⁻¹ a.e.

Perennial ryegrass is used as a permanent stand in temperate zone climates for overseeding dormant bermudagrass (*Cynodon dactylon* L.) in subtropical climates. A major challenge when managing permanent stands of PRG is controlling ABG during the fall season or during establishment. The introduction of two glyphosate-tolerant perennial ryegrass cultivars provides turfgrass managers a new tool in weed control, specifically ABG (Samudio et al., 2011). However, the glyph-

osate tolerance of these cultivars at the seedling stage and after a late-season, cool-temperature application has received limited investigation.

Although PRG is considered a fast-germinating cool-season species, ABG has greater seedling vigor (Grime and Hunt, 1975). When establishing a newly seeded PRG area, a glyphosate application during the PRG seedling phase would be beneficial in controlling newly emerging ABG. There have been several reports detailing the seedling tolerance of various turfgrass species to numerous herbicides and plant growth regulators (PGRs). Kaminski et al. (2004) reported on the effects of siduron, bensulide, ethofumesate, prodi-amine, paclobutrazol, and chlorsulfuron on young seedlings of creeping bentgrass (CBG) (*Agrostis stolonifera* L.). In general, a mature stand of CBG is tolerant to the previous products listed. However, the authors noted

seedling tolerance varied depending on whether certain herbicides were applied 2, 4, or 7 weeks after emergence. Dernoeden et al. (2008) investigated CBG and PRG seedling tolerance to bispyribac-sodium and ethofumesate when both species were between the two to five LS. Applications made during early seedling emergence (two LS) resulted in more injury to both species than applications when plants were fully established.

Regarding a glyphosate application during the seedling stage of glyphosate-resistant and -tolerant cultivars, Dant et al. (2005) applied glyphosate over Roundup Ready® CBG seedlings (Scotts Company, Marysville, OH) within 4 weeks of seeding and did not note any injury to the seedlings. ‘Aurora Gold’ hard fescue (*Festuca longifolia* Thuill.) seedling response to glyphosate has received limited investigation because applications were made the next year after seeding (Hart et al., 2005). Similarly, the glyphosate tolerance of mature stands of ‘JS501’ and ‘Replay’ PRG has been established (Baldwin et al., 2012), yet the seedling tolerance has received limited investigation. This is an important management aspect of these PRG cultivars to minimize weed competition in a newly seeded area or to develop renovation programs that allow for minimal weed pressure.

Another important consideration in developing weed control programs is determining the time of year to make herbicide applications while minimizing injury on the desirable species. Although no reports on seasonal application timing of glyphosate-tolerant turfgrasses are known, timing of application when using other herbicides has been reported. Research has noted increased toxicity on CBG and PRG after amicarbazone applications when applied in the fall compared with the spring (McCullough et al., 2010; Perry, 2011). Also, fall applications of bispyribac-sodium and sulfosulfuron caused greater injury to Kentucky bluegrass (*Poa pratensis* L.) compared with spring applications (Hart and McCullough, 2007).

Herbicide success is highly dependent on species maturity and application timing; therefore, the objectives of this research were to determine tolerance of ‘JS501’ and ‘Replay’ PRG to glyphosate during the seedling stage and after a late-season fall application.

Materials and Methods

Fall application response. Field trials in Idaho and Oregon determined the effect of a late-season, cool-temperature glyphosate application on the glyphosate tolerance of either ‘JS501’ or ‘Replay’ PRG. The Idaho trial was conducted at the Jacklin Seed research farm in Post Falls, ID (lat. 47°44’46” N, long. 116°52’56” W). Before seeding in both years, the areas were 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 a 2.5-cm blade spacing set to a depth of 0.2 cm (Graden GS04; Graden Company, Victoria, Australia). After all debris was removed, ‘Replay’ was broadcast-seeded on

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7 May 2010 and 16 May 2011 at 390 kg·ha⁻¹ on a Garrison gravelly silt loam (fine-loamy, mixed, superactive, calcareous, hyperthermic, Typic, Torrifluvent). Glyphosate (Depature, potassium salt form; Syngenta Crop Protection Inc., Greensboro, NC) was applied on 30 Sept. 2010 and 14 Oct. 2011 at 0, 0.15, 0.29, 0.58, 1.16, 1.74, 2.32, and 3.48 kg·ha⁻¹ a.e. Glyphosate treatments were applied using a CO₂-pressurized backpack sprayer calibrated to deliver 326 L·ha⁻¹ with TeeJet XR 8004 nozzles (TeeJet Technologies, Wheaton, IL). Individual plot size was 1.2 m × 1.8 m with greater than 90% coverage at the time of the initial application. 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. An additional fertilizer application using the same rate and product was applied in mid-September of both years. Irrigation was not applied after fertilizer applications. Once established, plots were mowed three times weekly at 1.27 cm during the growing season without clipping removal. Irrigation was provided as needed to prevent wilt stress and no additional herbicide, fungicide, or insecticide applications were necessary. Percent PRG leaf firing (i.e., injury) was rated 7, 14, 21, and 28 d after application (DAA) on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, 100% = brown, dead turfgrass.

The Oregon trial was conducted at the Oregon State University Lewis Brown Research Farm located in Corvallis, OR (lat. 44°33'52" N, long. 123°15'44" W). Before seeding, the area was sprayed with glyphosate at 1.74 kg·ha⁻¹ a.e., scalped to 1.27 cm using a Hayter Rotary mower (Hayter, Hertfordshire, England), then flail-mowed using a tractor-mounted flail mower. Separate plots of 'JS501' and 'Replay' were then seeded on 30 July 2010 at 490 kg·ha⁻¹ on a Chehalis silty clay loam (Cumulic Ultic Haploxerolls). Although the Oregon site was not replicated in time, several studies in the literature report data conducted during one growing season when replicated at another site (Brosnan et al., 2011, 2012, 2013; Flessner et al., 2013; Lewis et al., 2010; McCullough et al., 2011). Individual plot size was 1.5 m × 1.5 m with greater than 90% coverage at the time of the initial application. Plots were fertilized at 96 kg·ha⁻¹ N using 15N-3.9P-14.9K the day of seeding. Once all plots were established, mowing height was 5.1 cm two times weekly during the growing season with clippings returned. Glyphosate (same formulation as previously described) was applied on 6 Oct. 2010 at 0, 0.15, 0.29, 0.44, 0.58, 1.16, and 3.48 kg·ha⁻¹ a.e. All glyphosate treatments were applied using a CO₂-pressurized backpack sprayer calibrated to deliver 407 L·ha⁻¹ with TeeJet 80015 nozzles. Unless rainfall occurred, plots were irrigated every other day at 80% of evapotranspiration based on a Rain Bird Weather Station (CR10X; Campbell Scientific, Logan, UT). No additional herbicide, fungicide, or insecticide applications were necessary. Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury,

Table 1. Percent injury of 'Replay' perennial ryegrass after various glyphosate application rates on 30 Sept. 2010 and 14 Oct. 2011 in Post Falls, ID.

Rate (kg·ha ⁻¹ a.e.)	Replay percent injury ^z			
	7 DAA	14 DAA	21 DAA	28 DAA
0	0	0	0	0
0.15	0	0	8	9
0.29	1	3	21	22
0.58	7	28	46	61
1.16	25	35	60	78
1.74	45	70	79	81
2.32	59	72	84	87
3.48	64	80	98	99
LSD _{0.05}	7.3	6.8	6.7	7.2

^zPercent injury based on a scale of 0% to 100%, where 0% = no injury and 100% = brown, dead turfgrass. DAA = days after application; LSD = least significant difference.

Table 2. Percent injury of 'JS501' and 'Replay' in Corvallis, OR, after various glyphosate application rates on 5 Oct. 2010.^z

Rate (kg·ha ⁻¹ a.e.)	JS501 percent injury ^y				
	7 DAT	14 DAT	21 DAT	28 DAT	36 DAT
0	0	0	0	0	0
0.15	0	0	2	2	3
0.29	0	2	8	20	25
0.45	0	4	27	47	60
0.58	1	17	45	72	82
1.16	2	77	88	93	98
2.32	12	99	100	100	100
LSD _{0.05}	5.1	6.0	3.6	9.2	9.9

Rate (kg·ha ⁻¹ a.e.)	Replay percent injury ^y				
	7 DAT	14 DAT	21 DAT	28 DAT	36 DAT
0	0	0	0	0	0
0.15	0	0	2	2	2
0.29	0	2	6	23	25
0.45	0	3	20	57	60
0.58	1	30	48	92	97
1.16	1	85	90	99	100
2.32	4	99	100	100	100
LSD _{0.05}	1.9	5.3	4.7	10.8	12.9

^zAll plots were seeded on 30 July 2010.

^yPercent injury based on a scale of 0% to 100%, where 0% = no injury and 100% = brown, dead turfgrass. DAT = days after treatment; LSD = least significant difference.

100% = brown, dead turfgrass, 7, 14, 21, 28, and 36 DAA.

Seedling tolerance. An additional trial at the Jacklin Seed research farm in Post Falls, ID, determined the seedling tolerance of 'JS501' and 'Replay' PRG. Both cultivars were seeded on 7 May 2010 and 5 Aug. 2011 at a rate of 390 kg·ha⁻¹. Method of seeding, management practices, plot size, and sprayer equipment were identical to the Idaho trial previously described. Glyphosate (same formulation as previously described) rates were 0, 0.15, 0.29, and 0.58 kg·ha⁻¹ a.e. and applied at the one LS, two LS, three LS, and four LS. Four separate areas in each plot were examined to ensure the majority (greater than 75%) of the plot area was at the intended leaf stage before an application. Application dates in 2010 were 17 May, 24 May, 1 June, and 8 June. Application dates in 2011 were 15 Aug., 23 Aug., 1 Sept., and 12 Sept. Percent PRG cover was rated on a scale of 0% to 100%, where 0% = bare ground and 100% = complete green living PRG cover 16, 32, 48, and 60 d after seeding (DAS). Perennial ryegrass color was rated 32, 48, and 60 DAS on a scale from 1 to 9, where 1 = brown turfgrass, 9 = dark green turfgrass, 7 =

commercially acceptable color. Before glyphosate applications, all plots received a rating of 9. Therefore, plots were rated solely based on injury from the glyphosate application treatments.

Data analysis. In both trials, treatments were arranged in a randomized complete block design with three replications. At the Oregon location, 'JS501' and 'Replay' were planted in separate plots; 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 $\alpha = 0.05$ with SAS (Version 9.3; SAS Institute Inc., Cary, NC). In the fall application response trials, data were analyzed using nonlinear regression analysis with the NLIN procedure of SAS to indicate the rate at which glyphosate would cause a mean predicted leaf firing response of 20%. The following log-logistic dose-response function was used:

$$y = C + \frac{D - C}{1 + e^{[b(\log(x) - \log(I_{50}))]}}$$

where C is the lower limit, D is the upper limit, b equals the slope, and I₅₀ equals dose

giving 50% response (Seefeldt et al., 1995). For both trials in Idaho, analysis with linear models indicated no year $\times$ treatment interaction for any parameter measured; therefore, data collected over the 2-year study period for the seedling tolerance trial were pooled and the low-temperature response trial was pooled for the nonlinear regression analysis.

Results and Discussion

Fall application response. In the Idaho trial location, glyphosate rates greater than 0.58 kg·ha⁻¹ a.e. resulted in more than 20% injury by 7 d after treatment (DAT) (Table 1). By 21 and 28 DAT, only the 0.15 kg·ha⁻¹ a.e. rate had less than 20% injury. An application at the highest rate (3.48 kg·ha⁻¹ a.e.) resulted in nearly complete kill at 99% injury 28 DAT.

In the Oregon trial location, data for both cultivars are presented separately because they were planted in two blocks; however, data are nearly identical; therefore, injury data will only be discussed for 'JS501' (Table 2). Similar to the trial conducted in Idaho, by 28 DAT, an application of 0.15 kg·ha⁻¹ a.e. was the only treatment that resulted in less than 20% injury. Meanwhile, complete removal of 'JS501' was achieved by the end of the trial at the 2.32 kg·ha⁻¹ a.e. rate. These data indicate the cultivars can be removed using high rates of glyphosate. An application with an alternative chemistry (glufosinate) within label rates will eradicate these glyphosate-tolerant cultivars as well (Baldwin et al., 2012).

Regression analysis revealed a rate of 0.25 kg·ha⁻¹ a.e. was required to cause 20% leaf firing in the Idaho trial location 28 DAA (Fig. 1), whereas a 0.27 and 0.29 kg·ha⁻¹ a.e. rate caused 20% leaf firing for 'JS501' and 'Replay' in the Oregon trial location 36 DAA, respectively (Figs. 2 and 3). These tolerance levels are lower than previously reported by Baldwin et al. (2012) who noted a glyphosate application of 0.81 kg·ha⁻¹ a.e. was required to cause 20% leaf firing. The previous trial was conducted in the summer months, whereas the ryegrass plants were actively growing. In the current trial, applications were conducted in the fall as air/soil temperatures cooled, daylength decreased, and plant growth slowed. Other herbicides targeted for ABG control also have similar seasonal timing limitations. For example, trials in New Jersey and Indiana noted increased injury to various cool-season turfgrasses after amicarbazone applications in the fall compared with the spring (McCullough et al., 2010). Another herbicide targeting ABG, bispyribac-sodium, was more injurious to CBG when applications were made at 10 °C than 20 and 30 °C in a growth chamber study (McCullough and Hart, 2006).

In this trial, it is difficult to conclusively state that air temperature was the predominant factor that caused increased susceptibility because applications were limited to the fall season. However, 2010 and 2011 minimum air

Fig. 1. Percent leaf firing 28 d after treatment of 'Replay' perennial ryegrass (PRG) after various glyphosate application rates on 30 Sept. 2010 and 14 Oct. 2011 in Post Falls, ID. Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, and 100% = complete desiccation. Black dots indicate mean responses at the selected glyphosate application rates and the curve displays the log-logistic dose-response from the nonlinear regression equation shown within the plot.

Fig. 2. Percent leaf firing 36 d after treatment of 'JS501' perennial ryegrass (PRG) after various glyphosate application rates on 5 Oct. 2010 in Corvallis, OR. Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, and 100% = complete desiccation. Black dots indicate mean responses at the selected glyphosate application rates and the curve displays the log-logistic dose-response from the nonlinear regression equation shown within the plot.

temperatures in the Idaho trial location the day of application were 9 and 5 °C, respectively (Table 3). Meanwhile, 12 DAT, minimum air temperatures were near or below the freezing mark with similar temperatures noted throughout the remainder of the study period. Although air temperatures were not as

cold in the Oregon trial location, similar trends were noted. Although only fall applications were assessed in this trial, injury from spring applications (April) at similar rates is limited to leaf tip burn with complete recovery noted within 14 DAT (C. M. Baldwin, unpublished data). Although it is probable

Fig. 3. Percent leaf firing 36 d after treatment of 'Replay' perennial ryegrass (PRG) after various glyphosate application rates on 5 Oct. 2010 in Corvallis, OR. Percent PRG leaf firing was rated on a scale of 0% to 100%, where 0% = no injury, 20% = acceptable level of injury, and 100% = complete desiccation. Black dots indicate mean responses at the selected glyphosate application rates and the curve displays the log-logistic dose-response from the nonlinear regression equation shown within the plot.

that air temperatures would have been similar in the spring, a warming trend would have likely been observed instead of a cooling trend (Table 3). Therefore, fall is likely the time of year when a glyphosate application should be avoided. Presumably, this is the result of herbicide translocation toward the stem and crown of the plant, which would result in increased susceptibility compared with applications in the spring and summer seasons. Future growth chamber studies with ¹⁴C glyphosate to determine the translocation in the plant with different temperature regimes and daylengths would clarify the previous statement.

Seedling tolerance. No significant PRG cover differences were noted at 16 or 32 DAS (Table 4). There was a significant rate and timing main effect and a rate by timing interaction at 48 and 60 DAS. On both of these rating dates, glyphosate applied at 0.58 kg·ha⁻¹ a.e. resulted in the lowest PRG cover. For the main effect timing means, glyphosate applied at the four LS resulted in the lowest PRG cover.

At 60 DAS, all timing and glyphosate rates applied at 0.15 and 0.29 kg·ha⁻¹ a.e. had greater than 85% PRG cover (Table 5). However, applying glyphosate at 0.29 kg·ha⁻¹

Table 3. Maximum and minimum air temperature (°C) and precipitation (mm) during the study period of 30 Sept. to 28 Oct. 2010 and 14 Oct. to 11 Nov. 2011 in Post Falls, ID and 6 Oct. to 2 Nov. 2010 in Corvallis, OR.

2010, Post Falls, ID					2011, Post Falls, ID					2010, Corvallis, OR				
Month	Temp. (°C)		Rainfall (mm)	Month	Temp. (°C)		Rainfall (mm)	Month	Temp. (°C)		Rainfall (mm)	Month	Temp. (°C)	
	Maximum	Minimum			Maximum	Minimum			Maximum	Minimum			Maximum	Minimum
September	23	15.6	2.8	October	7	12.7	7.8	September	29	25.6	11.7	October	29	25.6
	24	17.7	8.9		8	15.0	1.7		30	30.0	8.9		30	30.0
	25	25.5	6.7		9	12.7	2.8		1	18.9	12.7		1	18.9
	26	21.7	5.5		10	11.7	6.7		2	22.8	15.6		2	22.8
	27	25.5	10.0		11	15.0	8.9		3	17.8	10.6		3	17.8
	28	26.7	11.7		12	12.7	6.7		4	16.7	5.0		4	16.7
	29	23.9	10.0		13	12.7	0.6		5	21.6	3.8		5	21.6
	30 ^a	23.9	8.9		14 ^a	10.0	5.0		6 ^a	26.7	5.0		6 ^a	26.7
	1	25.6	13.9		15	12.8	1.7		7	17.8	8.9		7	17.8
	2	26.7	7.8		16	12.8	2.8		8	23.8	11.7		8	23.8
October	3	17.8	11.7	November	17	12.8	2.8	October	9	18.8	13.9	November	9	18.8
	4	12.8	10.6		18	13.9	1.7		10	17.8	13.9		10	17.8
	5	17.8	5.6		19	15.0	2.8		11	17.8	3.8		11	17.8
	6	20.0	6.7		20	13.9	6.7		12	20.0	1.7		12	20.0
	7	12.8	10.0		21	13.9	2.8		13	22.8	1.7		13	22.8
	8	12.8	10.0		22	13.9	6.7		14	22.8	2.8		14	22.8
	9	17.8	6.7		23	13.9	5.0		15	16.7	5.6		15	16.7
	10	16.7	11.7		24	10.6	-1.1		16	17.8	2.8		16	17.8
	11	12.8	3.9		25	8.9	-4.4		17	17.8	0.6		17	17.8
	12	15.0	0.0		26	7.8	-3.3		18	18.8	0.0		18	18.8
	13	16.7	2.8		27	8.9	-3.3		19	22.8	1.7		19	22.8
	14	20.6	2.8		28	10.0	-4.4		20	22.8	1.7		20	22.8
	15	12.8	6.7		29	10.6	0.6		21	17.8	7.2		21	17.8
	16	12.8	0.0		30	8.9	-2.2		22	17.8	10.6		22	17.8
	17	13.9	-3.3		31	10.0	1.7		23	16.7	10.0		23	16.7
	18	13.9	-2.2		1	7.8	-3.3		24	15.0	8.9		24	15.0
	19	15.6	-2.2		2	10.6	-6.1		25	12.8	7.8		25	12.8
	20	16.7	1.7		3	6.7	-2.2		26	12.8	5.6		26	12.8
	21	18.9	1.7		4	3.9	0.6		27	8.9	1.7		27	8.9
	22	13.9	1.7		5	2.8	-4.4		28	11.7	7.8		28	11.7
	23	8.9	3.9		6	3.9	-7.2		29	16.7	8.9		29	16.7
	24	8.9	3.9		7	2.8	-7.2		30	12.8	7.8		30	12.8
	25	5.6	3.9		8	6.7	-1.1		31	13.9	5.6		31	13.9
	26	6.7	2.8		9	8.9	-2.2		November	1	20.0	12.8	1	20.0
	27	7.8	2.8		10	10.0	-3.3			2	18.8	13.9	2	18.8
	28	5.6	1.7		11	8.9	-5.0			3	20.0	6.7	3	20.0

^aIndicates the date of the initial application.

Table 4. Percent cover of 'JS501' and 'Replay' perennial ryegrass after various glyphosate application rates and application timing following seeding on 7 May 2010 and 5 Aug. 2011 in Post Falls, ID.

Main factors	Timing	Percent cover ^z			
		16 DAS	32 DAS	48 DAS	60 DAS
Cultivar	JS501	49	77	90	94
	Replay	49	77	89	95
Rate (kg·ha ⁻¹ a.e.)	0	51	81	95	98
	0.15	50	78	94	99
	0.29	50	77	88	96
	0.58	47	74	80	83
Timing	1 LS	47	81	95	100
	2 LS	51	78	94	100
	3 LS	48	74	86	94
	4 LS	50	76	82	84
ANOVA					
Cultivar (C)		NS ^y	NS	NS	NS
Rate (R)		NS	NS	**	**
Timing (T)		NS	NS	**	**
C*R		NS	NS	NS	NS
C*T		NS	NS	NS	NS
R*T		NS	NS	**	**
C*R*T		NS	NS	NS	NS

^zPercent cover based on a scale of 0 to 100, where 0 = bare ground and 100 = complete green living perennial ryegrass cover.

^yNS = nonsignificant; *significant at 0.05 *P* level; **significant at 0.01 *P* level.

DAS = days after seeding; ANOVA = analysis of variance; LS = leaf stage.

Table 5. Percent cover of 'JS501' and 'Replay' perennial ryegrass after various glyphosate application rates and application timing when seeded on 7 May 2010 and 5 Aug. 2011 in Post Falls, ID.

Rate (kg·ha ⁻¹ a.e.)	Timing	Percent cover ^z	
		48 DAS	60 DAS
0	1 LS	96	100
	2 LS	98	100
	3 LS	94	98
	4 LS	88	98
0.15	1 LS	99	100
	2 LS	96	100
	3 LS	92	98
	4 LS	91	100
0.29	1 LS	92	100
	2 LS	98	100
	3 LS	82	98
	4 LS	78	87
0.58	1 LS	92	100
	2 LS	85	100
	3 LS	77	84
	4 LS	67	49
LSD _{0.05}		15.2	9.7

^zPercent cover based on a scale of 0 to 100, where 0 = bare ground and 100 = complete green living perennial ryegrass cover.

DAS = days after seeding; LS = leaf stage; LSD = least significant difference.

a.e. at the four LS had a 11% cover reduction compared with when applied at the three LS. Regardless, both cultivars appear sensitive to glyphosate when applied at the four LS because PRG cover was only 49% when treated at 0.58 kg·ha⁻¹ a.e. Both cultivars appear to tolerate glyphosate at this rate early in the seedling stage because percent cover was greater than 80% at the one, two, and three LS treatment stage.

No significant PRG color differences were noted at 32 DAS (Table 6). However, there was a rate and timing main effect and a rate × timing interaction at 48 and 60 DAS. For main effect rate means, on both of these rating dates, glyphosate applied at 0.58 kg·ha⁻¹ a.e. was the only treatment that produced a color rating below 8.0. For the main

effect timing means, applying glyphosate at the four LS resulted in the lowest color ratings.

At 48 DAS, ryegrass treated with glyphosate at 0.15 kg·ha⁻¹ a.e. resulted in color ratings of 9.0, regardless of application timing (Table 7). Applying glyphosate at 0.29 kg·ha⁻¹ a.e. at the four LS had a ≈2.5 rating unit decrease compared with the one, two, and three LS application timing. Glyphosate applied at 0.58 kg·ha⁻¹ a.e. only resulted in discoloration (less than 7.0) at the four LS timing. At 60 DAS, glyphosate applied at 0.15 and 0.29 kg·ha⁻¹ a.e. produced color ratings greater than 8.0, regardless of application timing. This indicates that the PRG injury noted at the four LS (0.29 kg·ha⁻¹ a.e. rate) at 48 DAS was transient because plots nearly fully recovered by the 60 DAS rating

date. Glyphosate applied at 0.58 kg·ha⁻¹ a.e. at the one LS, two LS, and three LS resulted in color ratings 8.0 or greater. Color ratings dropped to 4.6 when an application was made at the four LS. Unlike the application at the 0.29 kg·ha⁻¹ a.e. rate where recovery was noted by 60 DAS, it appears an application at the highest rate tested resulted in permanent injury that would likely require reseeding.

It appears both cultivars are sensitive to glyphosate applications at the four LS. Numerous reports detailing the seedling tolerance of various turfgrass species response to numerous herbicides and PGRs have also been reported (Dernoeden et al., 2008; Kaminski et al., 2004; Perry, 2011). The four LS is the approximate time when PRG starts to mature and produce tillers. It is at this point during the growth and development phases of both cultivars when they are most sensitive to glyphosate. Baldwin et al. (2012) noted both cultivars were able to tolerate a glyphosate application rate up to 0.81 kg·ha⁻¹ a.e. with minimal injury ≈4 months after seeding. Meanwhile, Flessner et al. (2014) noted both cultivars showed 50% population inhibition after a glyphosate application (≈2.6 kg·ha⁻¹ a.e.) when applied at the three- to four-tiller stage for both cultivars. Several overseeding trials with 'Replay' were initiated in the Phoenix, AZ, area during the winter of 2010–11. At these trial locations, glyphosate applications at the four LS did not show injury that is reported in this current research (C. M. Baldwin, unpublished data). As a result of these observations, field trials in multiple locations are required to determine how the maturity tolerance differs when seeding PRG on bare ground vs. overseeding into bermudagrass.

Determining the maturity tolerance of both cultivars is a critical piece of information when considering a PRG renovation. Applying glyphosate before the four LS could become an important part of a conversion process that will help ensure minimal weed pressure during the establishment phase. Previous research with other herbicides has shown the importance of weed control and having suitable herbicide tolerance in newly emerging turfgrass species so as to minimize weed pressure during establishment (Hoiberg et al., 2012; Venner, 2011).

This is the first reported research in the literature describing the effect of a fall application response and seedling tolerance after glyphosate applications at various rates and locations. The cover and color data suggest that these glyphosate-tolerant PRG cultivars can tolerate glyphosate up to 0.58 kg·ha⁻¹ a.e. when applied at the one LS, two LS, or three LS with minimal stand loss and injury. However, glyphosate applications greater than 0.29 kg·ha⁻¹ a.e. should be avoided at the four LS and in the fall season because a permanent reduction in stand density and browning would require reseeding. Future trials should investigate applications between 1 month and 4 months after seeding to determine when glyphosate can be safely applied at rates greater than 0.29 kg·ha⁻¹ a.e. In this trial, only

Table 6. Color of 'JS501' and 'Replay' perennial ryegrass after various glyphosate application rates and application timing when seeded on 7 May 2010 and 5 Aug. 2011 in Post Falls, ID.

Main factors	Timing	Perennial ryegrass color ²		
		32 DAS	48 DAS	60 DAS
Cultivar	JS501	8.7	8.4	8.7
	Replay	8.6	8.4	8.5
Rate (kg-ha ⁻¹ a.e.)	0	9.0	9.0	9.0
	0.15	9.0	9.0	9.0
	0.29	8.8	8.2	8.8
	0.58	7.9	7.5	7.7
	1 LS	8.6	8.8	9.0
Timing	2 LS	9.0	8.8	8.8
	3 LS	8.0	8.5	8.9
	4 LS	9.0	7.6	7.7
ANOVA				
Cultivar (C)		NS ³	NS	NS
Rate (R)		NS	**	**
Timing (T)		NS	**	**
C*R		NS	NS	NS
C*T		NS	NS	NS
R*T		NS	**	**
C*R*T		NS	NS	NS

²Color based on a scale of 1 to 9, where 1 = brown, dead turf, 7 = acceptable injury, and 9 = green, no injury.

³NS = nonsignificant; *significant at 0.05 *P* level; **significant at 0.01 *P* level.

DAS = days after seeding; LS = leaf stage; ANOVA = analysis of variance.

Table 7. Color of 'JS501' and 'Replay' perennial ryegrass after various glyphosate application rates and application timing when seeded on 7 May 2010 and 5 Aug. 2011 in Post Falls, ID.

Rate (kg-ha ⁻¹ a.e.)	Timing	Perennial ryegrass color ²	
		48 DAS	60 DAS
0	1 LS	9.0	9.0
	2 LS	9.0	9.0
	3 LS	9.0	9.0
	4 LS	9.0	9.0
0.15	1 LS	9.0	9.0
	2 LS	9.0	9.0
	3 LS	9.0	9.0
	4 LS	9.0	9.0
0.29	1 LS	9.0	9.0
	2 LS	9.0	9.0
	3 LS	8.5	9.0
	4 LS	6.3	8.2
0.58	1 LS	8.3	9.0
	2 LS	8.2	8.0
	3 LS	7.4	8.6
	4 LS	6.0	4.6
LSD _{0.05}		1.72	1.47

²Color based on a scale of 1 to 9, where 1 = brown, dead turf, 7 = acceptable injury, and 9 = green, no injury.

DAS = days after seeding; LS = leaf stage; LSD = least significant difference.

air temperatures were considered; therefore, future trials should also investigate the impact of soil temperatures and daylengths on glyphosate tolerance as well. Other trials of interest would include investigating tank-mixing glyphosate with other herbicides, PGRs, and soluble fertilizer sources to determine the safety to both PRG cultivars.

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