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A Glyphosate-Resistant Biotype of Annual Bluegrass in Tennessee

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Glyphosate is regularly used to control annual bluegrass populations in dormant bermudagrass turf. A population of annual bluegrass not controlled by glyphosate at 840 g ha^{-1} (glyphosate resistant, GR) was identified on a golf course in Humboldt, TN in 2010. Mature tillers of GR plants were established in a greenhouse and treated with glyphosate at 0, 210, 420, 840, 1,680, 3,360, and 6,720 g ha^{-1}. Mature tillers of a biotype known to be susceptible to glyphosate (SS) were also established in the greenhouse and subjected to the same treatments. At 14 d after treatment (DAT), glyphosate controlled the SS biotype > 95% at rates > 420 g ha^{-1}. Comparatively, the GR biotype was only controlled 76% with glyphosate at 6,720 g ha^{-1}. The rates required to provide 50% control (I_{50} values) for SS and GR biotypes were 236 and 2,812 g ha^{-1} respectively, resulting in a resistance factor of 12. Photochemical efficiency (F_v/F_m) values on SS plants treated with glyphosate at > 210 g ha^{-1} measured 0.000 at 14 DAT, whereas F_v/F_m values on GR plants were not significantly different from the untreated control with glyphosate rates ≤ 840 g ha^{-1} on the same date. In laboratory experiments, the SS biotype accumulated greater shikimate concentrations than the GR biotype 3 to 6 DAT. Future research should evaluate strategies for managing GR and SS annual bluegrass with alternative modes of action.
Nomenclature: Annual bluegrass, *Poa annua* L.; bermudagrass, *Cynodon dactylon* L. Pers.; glyphosate; photochemical efficiency.
Key words: bermudagrass, dormant, herbicide resistance, golf course, turf, weed control.

Annual bluegrass is a problematic winter annual weed on golf courses throughout the U.S. transition zone. In bermudagrass fairways and roughs, annual bluegrass infestations commonly occur during periods of dormancy (Toler et al. 2007). These infestations negatively affect bermudagrass quality because of the bunch-type growth habit, light green color, and poor stress tolerance of annual bluegrass compared with other species (Beard et al. 1978; Gaussion and Branham 1987; Hall and Carey 1992; Johnson and Murphy 1995).

Glyphosate is labeled for broadleaf and grassy weed control in dormant bermudagrass turf at rates of 0.32 to 1.16 kg ha^{-1} (Anonymous 2010). Toler et al. (2007) reported that an application of glyphosate in February at 0.56 kg ha^{-1} controlled annual bluegrass 93%. Glyphosate is commonly used to control annual bluegrass in dormant bermudagrass fairways, as efficacy with other herbicides, particularly those inhibiting acetolactate synthase (ALS), such as foramsulfuron and bispyribac-sodium, can be negatively affected by cold temperature conditions in winter and early spring (Hutto et al. 2008; Lycan and Hart 2006; Willis 2008). Additionally, glyphosate applications provide turf managers with a more economical option for broad-spectrum winter weed control than the aforementioned ALS inhibitors (D. Green, personal communication). Thus, many populations are under yearly glyphosate selection pressure and turfgrass managers have limited the diversity of herbicides used for control; both of these phenomena have been identified as principal factors in the development of glyphosate-resistant (GR) weeds (Duke and Powles 2009).

Annual bluegrass response to glyphosate applications has been shown to vary considerably. Goss et al. (2005) treated four unique populations of annual bluegrass with glyphosate at 840 g ha^{-1} and plant survival ranged from 15 to 60%. When the application rate was increased to 1,680 g ha^{-1}, plant survival ranged from 2 to 9%. Annual bluegrass biotypes resistant to atrazine, prodiamine, simazine, and diuron have been reported (Heap 2011; Hutto et al. 2004); however, only a single biotype of GR annual bluegrass has been confirmed (Binkholder et al. 2011). Selected from a zoysiagrass (*Zoysia* spp.) golf course in Missouri, glyphosate at 6.28 kg ha^{-1} reduced the biomass of this biotype by only 60%, whereas applications at 0.78 kg ha^{-1} and greater controlled a susceptible biotype 100% (Binkholder et al. 2011). Using dry-weight biomass data, this biotype yielded a resistance factor (RF) of 5.2.

The development of GR weed species has been termed the most important herbicide resistance issue globally (Powles 2008). To date, 19 different species with glyphosate resistance have been identified, all resulting from widespread use of glyphosate for broad-spectrum weed control (Heap 2011). GR annual bluegrass is of concern given this species' prolific seed production and long-term seed viability (Roberts and Feast 1973). A biotype of annual bluegrass at Humboldt Country Club (Humboldt, TN) was not controlled after treatment with glyphosate at 840 g ha^{-1} during bermudagrass dormancy. The golf course superintendent made a single application of glyphosate at 840 g ha^{-1} every year from 1990 to 2009 to control weeds during bermudagrass dormancy (D. Green, personal communication). The objective of this research was to determine the sensitivity of a potentially GR annual bluegrass biotype collected from this location.

Materials and Methods

Plant Culture. Three cores (930 cm^2) were removed from the third fairway at Humboldt Country Club (Humboldt, TN) on February 17, 2010 using a standard golf course cup cutter. Each core contained mature annual bluegrass plants suspected to be GR. These GR plants had not been treated with any herbicide after emerging in the fall of 2009. A biotype known to be susceptible to glyphosate (hereafter referred to as SS) was harvested in the same manner from the East Tennessee Research and Education Center (Knoxville, TN).

Considering that annual bluegrass is a self-pollinated species (Ellis 1973) and seed was limited, individual tillers of the GR and SS biotypes were used in greenhouse and laboratory experiments. Individual GR and SS tillers were

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removed from cores harvested in the field and transplanted into 164 cm^3 conetainers (SC10 Super Cell Conetainer, Steuwe & Sons, 31933 Rolland Dr., Tangent, OR 97389) filled with a peat moss growing medium (Farfard Super Fine Germinating Mix, 770 Silver St., Agawam, MA 01001). Tillers of the GR and SS biotypes were maintained under controlled greenhouse conditions for 5 wk before initiating research. During the 5-wk acclimation period plants were irrigated to prevent the onset of wilt and clipped daily at a height of ~ 6 cm. Plants were fertilized with a complete fertilizer (Howard Johnson's Triple Twenty Plus Minors, 700 W. Virginia St., Milwaukee, WI 53204) to promote active growth. Average daily maximum/minimum temperatures in the greenhouse measured 27/16 C and natural lighting provided an average photoperiod of 11 h 36 min.

Greenhouse Experiments. Both GR and SS plants were treated with glyphosate (Roundup ProMax, Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, MO 63167) at 0, 210, 420, 840, 1,680, 3,360, and 6,720 g ha^{-1} using a CO_2- powered backpack boom sprayer containing flat-fan nozzles (Teejet 8002 flat-fan spray nozzle, P.O. Box 7900, Wheaton, IL 60187) calibrated to deliver 280 L ha^{-1} of spray volume. Annual bluegrass control was evaluated visually on a 0 (no injury) to 100% (complete kill) scale at 7 and 14 d after treatment (DAT). Measurements of photochemical efficiency (F_v/F_m) were made on each evaluation date to provide a quantitative assessment of plant response to glyphosate treatment. F_v/F_m was measured twice per conetainer from different newly emerged leaves using a pulse-modulated fluorometer (OS1-FL Pulse Modulated Fluorometer, Opti-sciences, Inc., 8 Winn Ave., Hudson, NH 03051). F_v/F_m was determined by subtracting F_o, the minimal level of fluorescence, from F_m, the maximum level of fluorescence. This difference ($F_m - F_o$) is then divided by F_m to calculate F_v/F_m, with a larger number, indicating greater photosynthetic efficiency (Maxwell and Johnson 2000).

Statistical Analysis of Greenhouse Data. Experimental design was a randomized complete block with four replications. Two experimental runs were conducted with data from each subjected to ANOVA in SAS. No significant interactions with experimental run were detected; thus, data from each experimental run were combined. Log-logistic regression analysis was conducted to evaluate GR and SS control after glyphosate treatment using the model proposed by Seefeldt et al. (1995):

$$y = C + \left([D - C] \Big/ \left[1 + \{x/I_{50}\}^b \right] \right) \quad [1]$$

where y is annual bluegrass control, x is glyphosate rate in g ha^{-1}, D is the upper limit for y (i.e., 100% annual bluegrass control), C is the lower limit for y (i.e., 0% annual bluegrass control), I_{50} is rate of glyphosate giving a 50% response (i.e., 50% annual bluegrass control), and b is the slope of the line at the I_{50}. Data from the untreated control (0 g ha^{-1}) were excluded from analysis. All regression analyses were conducted using Prism (Prism 5.0 for Mac OSX, GraphPad Software, 2236 Avenida de la Playa, La Jolla, CA 92037), a software package used by researchers evaluating nonlinear treatment responses (Brosnan et al. 2010; Molulsky and Christopoulos 2004). F_v/F_m data were analyzed using ANOVA in SAS with Fisher's Protected LSD values used to separate treatment means at the $P \leq 0.05$ level.

Laboratory Experiments. Both GR and SS biotypes were treated with a labeled rate of glyphosate (420 g ha^{-1}) using a CO_2- powered backpack boom sprayer containing flat-fan nozzles calibrated to deliver 280 L ha^{-1} of spray volume. Untreated plants were also examined.

At 1, 2, 3, and 6 DAT, all aboveground biomass was harvested at the soil surface, bagged, and placed into a −10 C freezer. Since GR and SS tillers did not provide enough tissue to analyze individually (< 0.10 g), tissues from all four replications were combined and experimental runs served as a means of replication. Shikimate concentrations in GR and SS biotypes were determined using procedures similar to Mueller et al. (2003) for horseweed [*Conyza canadensis* (L.) Cronq.]. Plant tissues were finely ground in liquid nitrogen before being weighed and placed into 20-ml vials with 5 ml of 1 M HCl (Fisher Scientific, 1 Liberty Lane, Hampton, NH 03842). Vials were then placed on a reciprocating shaker (Fisher Scientific, 1 Liberty Lane, Hampton, NH 03842) at 80 rpm for 15 h. Each HCl extract was then passed through a 0.45-µm syringe filter into a 4-ml vial for liquid chromatography analysis using a chromatograph (Waters Corporation, 34 Maple Street, Milford, MA 01757) equipped with a UV detector using a wavelength of 215 nm. A Phenomenex (Phenomenex Luna NH_2 100A Column, 411 Madrid Ave., Torrance, CA. 90501) Luna NH_2 100A column (250 by 4.6 mm; 5-µm particle size) was used with an injection volume of 10 µl. The isocratic mobile phase consisted of 15% (H_2O + 0.1% H_3PO_4) + 85% acetonitrile. Data capture was performed using Chemstation software (Chemstation software, Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051. Total run time was 20 min with a shikimic acid retention time of 6.9 min. All samples were analyzed < 1 d after extraction.

Results and Discussion

Greenhouse Experiments. Responses of the GR and SS biotypes to increasing rates of glyphosate varied (Figure 1). At 14 DAT, glyphosate controlled the SS biotype > 95% at rates greater than 420 g ha^{-1}. Comparatively, the GR biotype was only controlled 76% with glyphosate at 6,720 g ha^{-1}. I_{50} values for GR and SS biotypes were 2,812 and 236 g ha^{-1} respectively, resulting in an RF of 12. RF values for other grassy weed species resistant to glyphosate range from 2 to 11 (Baerson et al. 2002; Powles et al. 1998; Pratley et al. 1999; Vila-Aiub et al. 2007).

GR and SS response in F_v/F_m varied at 7 and 14 DAT (Table 1). F_v/F_m values on GR plants were not significantly different from the untreated control with glyphosate rates ≤ 840 g ha^{-1} at 14 DAT, suggesting that photosynthesis was not affected by glyphosate at ≤ 840 g ha^{-1}.

On the SS biotype, significant reductions in F_v/F_m were observed with glyphosate at rates > 210 g ha^{-1} on each rating date. By 14 DAT, F_v/F_m values on SS plants treated with glyphosate at > 210 g ha^{-1} measured 0.000, indicating that plants had been killed (i.e., no photosynthesis was occurring).

Laboratory Experiments. Shikimate concentrations in untreated plants measured < 50 µg g^{-1} of fresh-weight tissue on

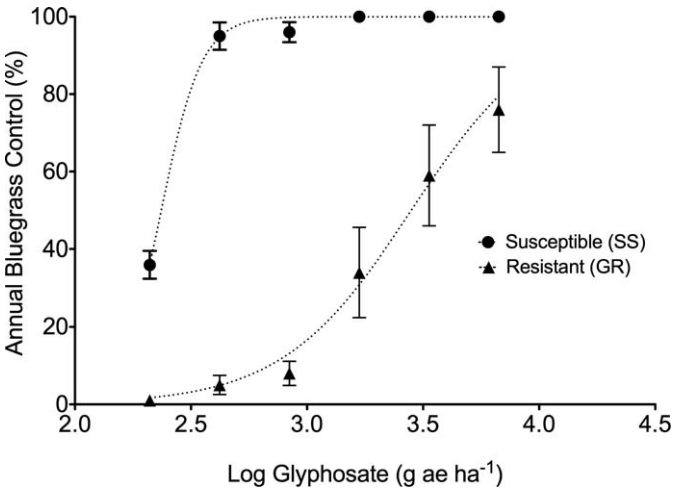

Figure 1. Effect of herbicide treatment on control of glyphosate-resistant (GR) and glyphosate-susceptible (SS) annual bluegrass 14 d after treatment. Points represent treatment means and lines represent the predicted response as determined by log-logistic regression analysis. I_{50} values for the GR and SS biotypes were 2,812 and 236 g ha^{-1}, respectively.

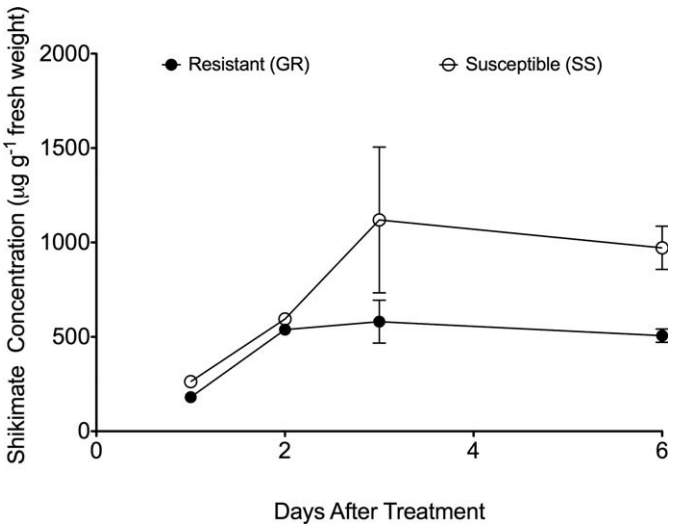

Figure 2. Shikimate concentrations in glyphosate-resistant (GR) and glyphosate-susceptible (SS) annual bluegrass tillers treated with glyphosate at 420 g ha^{-1}. Error bars represent standard error. Shikimate concentrations in untreated GR and SS plants measured $<$ 50 µg g^{-1} fresh weight.

each harvest date (data not shown). Although both biotypes initially accumulated shikimate 1 and 2 DAT, the SS biotype accumulated higher concentrations of shikimate than the GR biotype at 3 and 6 DAT (Figure 2). For example, shikimate concentrations in the SS and GR biotypes measured 1,119 and 580 µg g^{-1}, respectively, at 3 DAT. Shikimate concentrations did not increase in the GR or SS biotype after 3 DAT. The reason for this response is not clear. Mueller et al. (2008) reported similar responses after treating SS biotypes of common lambsquarters (*Chenopodium album* L.), common cocklebur (*Xanthium strumarium* L.), and broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster] with glyphosate at 840 g ha^{-1}. Concentrations of shikimate detected by Mueller et al. (2008) were greater than those reported in this research. This could be due to not only differences between species evaluated but environmental conditions as well. Shikimate concentrations in Palmer amaranth (*Amaranthus palmeri* S. Wats.) have been reported to be 10 times greater under field conditions than the glasshouse (Mueller et al. 2008). Additional studies are needed to confirm that the mechanism conferring glyphosate resistance in the GR biotype from Tennessee is an altered 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme similar to what has been reported for other annual grassy weeds resistant to glyphosate (Baerson et al. 2002). However, multiple mechanisms may be responsible for the evolution of herbicide resistance (Powles and Yu 2010).

Table 1. Photochemical efficiency (F_v/F_m) of annual bluegrass biotypes after treatment with increasing rates of glyphosate. Means were combined after two experimental runs in a greenhouse.

		Photochemical efficiency	
Biotype	Rate	7 DAT[a]	14 DAT
	g ha^{-1}	F_v/F_m	
Resistant (GR)	0	0.607	0.501
	210	0.549	0.537
	420	0.554	0.552
	840	0.431	0.531
	1,680	0.221	0.088
	3,360	0.195	0.069
	6,720	0.119	0.050
Susceptible (SS)	0	0.464	0.456
	210	0.399	0.604
	420	0.161	0.000
	840	0.119	0.000
	1,680	0.110	0.000
	3,360	0.103	0.000
	6,720	0.111	0.000
$LSD_{0.05}$		0.163	0.161

[a] Abbreviation: DAT, days after treatment.

This research represents the first instance of a weed species having glyphosate resistance in bermudagrass turf. Although not compared directly, the level of resistance in the GR biotype from Tennessee is greater than that reported for a GR annual bluegrass biotype infesting zoysiagrass turf in Missouri, and higher than what has been reported for many other grassy weeds in nonturf settings. Although glyphosate resistance has developed in other fecund species, transfer of resistance traits through pollen dispersal or seed movement is not likely in self-pollinated species such as annual bluegrass where gene flow in managed turfgrass settings is limited (Ellis 1973; Ng et al. 2004; Sweeney and Danneberger 1995). Rather, GR biotypes may emerge at specific locations after repeated use of glyphosate for POST weed control in dormant bermudagrass turf. Such was the case at this location, where the golf course superintendent regularly applied glyphosate at 840 g ha^{-1} for nearly 20 yr. Future research should evaluate strategies for managing GR annual bluegrass with alternative modes of action, as well as programs for managing SS annual bluegrass with alternative chemistries to prevent the evolution of new resistant biotypes.

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