## CHAPTER II

# GLYPHOSATE RATE-RESPONSE OF COMMON TURFGRASS WEEDS

ABSTRACT: Research was conducted to determine the glyphosate rate response for common turfgrass weeds. Thirty day and one hundred day old plants grown under greenhouse conditions received glyphosate treatments of 0.000, 0.0075, 0.0098, 0.0188, 0.0375, 0.0563, 0.075, 0.188, 0.375, 0.563, 0.75, 1.68, 3.38, 7.5 and 37.5 kg a.e. glyphosate ha<sup>-1</sup>. Growth measurements were collected 30 and 70 days after treatment. Above ground biomass was collected 70 days after treatment. The experiment was conducted with large crabgrass (Digitaria sanguinalis L.), dandelion (Taracaxum officinale Weber), yellow nutsedge (Cyperus esculentus L.) annual bluegrass (Poa annua L. var. annua) and creeping bentgrass (Agrostis stolonifera L. cv. 'Penncross'). Plant survival was determined 70 days after treatment if the plant had any green, growing tissue remaining that was increasing biomass. The natural log of glyphosate rate was used to determine lethal rate required to kill 50 and 90% of each species population (LR<sub>50</sub> and LR<sub>90</sub>). The LR<sub>50</sub> was predicted as 1.3 for large crabgrass, 1.5 for dandelion, 3.9 for yellow nutsedge, 1.4 for annual bluegrass, and 2.6 kg a.e. glyphosate ha<sup>-1</sup> for creeping bentgrass for 30 day old plants and 7.6, 4.0, 3.6, 1.6, 3.4 kg a.e. glyphosate ha<sup>-1</sup>, respectively, for 100 day old plants. The LR<sub>90</sub> of 2.0, 3.5, 14.1, 3.3, and 10.4 kg a.e. glyphosate ha<sup>-1</sup>, respectively, were

predicted for 30 day old plants and 25.1, 22.7, 19.5, 7.2, and 12.8 kg a.e. glyphosate ha<sup>-1</sup>, respectively, for 100 day old plants. Using LR<sub>90</sub> values, 30 day old annual bluegrass, dandelion and large crabgrass plants could be adequately controlled at rates below 4 kg a.e. glyphosate ha<sup>-1</sup>. However, the same species receiving glyphosate 100 day after seeding required rates higher than 7.2 kg a.e. glyphosate ha<sup>-1</sup>. To prevent plant survival and minimize the risk of reproduction and increased tolerance within the subsequent populations, 4.0 kg a.e. glyphosate ha<sup>-1</sup> should be applied only to juvenile plants. Creeping bentgrass and yellow nutsedge required rates greater than 7.5 kg a.e. glyphosate ha<sup>-1</sup>. High rates of glyphosate required for 90% control in this study may indicate other options could provide better weed control. The future use of glyphosate for selective weed control in turfgrass systems will require the use of a wide variety of weed control options to ensure adequate control and to minimize the potential for herbicide resistance.

NOMENCLATURE: glyphosate; creeping bentgrass, *Agrostis stolonifera* L. cv. 'Penncross'; yellow nutsedge, *Cyperus esculentus* L.; large crabgrass, *Digitaria sanguinalis* L.; annual bluegrass, *Poa annua* L. *var. annua*; dandelion, *Taraxacum oficinale* Weber.

**ADDITIONAL INDEX WORDS:** Rate-response, glyphosate, herbicide-resistant, survival, turfgrass weeds.

**ABBREVIATIONS:** DAS, d after seeding, DAT, d after glyphosate treatment; G, glyphosate; GR, glyphosate-resistant; LR<sub>50</sub>, lethal rate required to kill 50% of the population; LR<sub>90</sub>, lethal rate required to kill 90% of the population; UTC, untreated control.

## INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] blocks the aromatic amino acid biosynthetic pathway in plants and has been used for weed control in cropping and aquatic systems since the 1970's (Jaworski 1972). The initial uses for glyphosate were non-selective applications for pre-plant, dormant grass, orchard, right-of-way, and ornamental weed control. Recent advances in genetic engineering have developed glyphosate resistant (GR) crops for selective weed control (Marshall, 1998). Previous GR crops have been grown in annual cropping systems.

Glyphosate-resistant genes recently have been introduced into several turfgrass species. Little is known regarding the use of glyphosate for selective weed control in a perennial grass system. Weed control strategies differ between annual cropping systems and perennial grasses. The goals of weed control in annual cropping systems are related to the impact on crop yield and quality of harvest. In a turfgrass system, higher levels of control are desired to ensure a quality surfaces without visible disruptions. The use rates and yearly application amounts could be greater in turfgrass systems due to the necessity for more complete control associated with aesthetics and the inability to use cultivation methods for weed control (Beard, 1973). The rapid adoption, current use of GR crops and increasing numbers of GR weed populations further substantiates the need to understand the best weed management options in herbicide resistant crops.

Turfgrass systems require specifically targeted herbicide applications for yearlong control of various weed types. Adequate weed control requires herbicide application to small weeds, before seed production is possible and when herbicides are more effective (Kapusta et al., 1994). Timing of annual weed control is based on the germination timing. However, each weed species germinates at a distinctly different times with many weed species germinating throughout the growing season. Any application will likely result in poor control of some weed species due to applications too early or too late. Therefore, proper weed control would require multiple applications for several years if a single herbicide is used. Multiple exposures to any herbicide increases the potential for herbicide resistant weeds to develop (Heap, 1997).

Required rates to control various weeds often change over time due to increased weed and herbicide use patterns (Table 2.1). For example, recommended label rates for weed control are changed over time as more research is conducted and further information gained including a better understanding of the herbicide and environment interactions. In addition, a better understanding of different glyphosate formulations has indicated rate comparisons are related to acidic properties of the herbicide rather than amount of active ingredients alone. Therefore, previous research often listed rates as a function of a.i. rather than a.e. Various authors who contributed to Grossbard and Atkinson (1985) provided a wide range of recommended rates for control of weed species depending on the application (Table 2.1). The range of rates required to control Cynodon dactylon (L.) Pers. was 0.8 to 4.8 kg a.i. glyphosate ha<sup>-1</sup>. Yellow nutsedge (Cyperus esculentus L.) was controlled with rates ranging from 1.0 to 2.0 kg a.i. glyphosate ha<sup>-1</sup>. However, the recommended use rates on Monsanto's (Monsanto Company, Agricultural Products; St. Louis, MO 63167) 1991 and 2001 Roundup Pro<sup>®</sup> label were 5.60 for *Cynodon dactylon* and 3.36 kg a.i. alyphosate ha<sup>-1</sup> for yellow nutsedge, respectively. Further, the recommended label rates changed from 0.56 to 1.12-4.48 kg glyphosate ha<sup>-1</sup> for *Digitaria* species from 1991 to 2001. Knowledge gained regarding glyphosate's effectiveness resulted in changes in recommended rates. In addition, increased herbicide tolerance and/or resistance with time by specific weed populations has been documented (Baerson et al., 2002; Boerboom et al., 1990; Boutsalis and Powles, 1995; Burnet et al., 1994; Christoffoleti et al, 1997; Darmency and Gasquez, 1990; DePrado et al., 1997; Dinelli, 2000; Fischer et al., 2000; Foes et al., 1999; Gill et al., 1996; Grossbard and Atkinson, 1985; Kelly et al., 1999; Kern et al., 1996; Lee and Ngim, 2000; Llewellyn and Powles, 2001; Mueller et al., 2003; O'Donovan et al., 1999; Powles et al., 1998; Perez and Kogan, 2003; Pratley et al., 1999; VanGessel, 2001; Vaughn et al., 1990; Walsh et al., 2001; Westwood and Weller, 1997).

The objective of the research were to determine the rate response for common turfgrass weeds at spray volumes used by turfgrass managers. Further

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understanding of the control of common turfgrass weeds could ensure proper management of GRTs.

### MATERIALS AND METHODS

Greenhouse research was conducted at the University of Nebraska (Lincoln, NE). Experiments were initiated 2 Oct 2001, 8 Mar 2002 and 1 Jun 2002. Five to 10 annual bluegrass (Poa annua var. annua L.), large crabgrass (Digitaria sanguinalis L.), dandelion (Taraxacum officinale Weber) and creeping bentgrass (Agrostis stolonifera L. cv. 'Penncross') seeds per replicate and 2 yellow nutsedge tubers per replicate were placed 1 cm below soil surface of 2.5 cm diam by 21 cm depth cone-tainers (Stuewe & Sons, Inc.; 2290 SE Kiger Island Drive; Corvallis, OR 97333-9425) filled with 24:9:35:32 (v:v:v:v) sand, soil, peat moss, vermiculite soil mix, respectively, leaving 2.5 cm headspace above soil surface. Plants were thinned to 2 plants per replicate after germination with additional emerging seedlings being removed throughout the experiment. Glyphosate (Roundup<sup>®</sup> Pro formulation with 489 g L<sup>-1</sup> of active ingredient or 356 g L<sup>-1</sup> of glyphosate acid) was applied 30 or 100 d after seeding (DAS) at rates of 0.000, 0.0075, 0.0098, 0.0188, 0.0375, 0.0563, 0.075, 0.188, 0.375, 0.563, 0.75, 1.68, 3.38, 7.5 and 37.5 kg a.e. glyphosate ha<sup>-1</sup>. All glyphosate treatments were applied with a conveyor, pressurized (276 kPa) sprayer in 374 L ha-1 of water. Six to eight replicates of each treatment level were used per experiment. Large crabgrass plants receiving glyphosate 100 DAS were not analyzed, except for the 2 Oct 2001 initiation date, because the plants were too large to fit under the

sprayer. These plants would not have received adequate spray coverage. All species receiving the same glyphosate rate were sprayed at the same time 30 or 100 DAS. Before and after application, plants were organized in a completely randomized design within each species and glyphosate application timing. Plants were watered daily until soil saturation except 24 hr following glyphosate application. Every 7 d, Peters 20-10-20 fertilizer (200 N ppm; J.R. Peters, Inc., 6656 Grant Way, Allentown, PA 18106) was applied through the irrigation water. Plant height, longest leaf length and visual percent brown tissue were evaluated 30 and 70 d after glyphosate treatment (DAT) for annual bluegrass, large crabgrass, yellow nutsedge and creeping bentgrass plants. Dandelion plants were evaluated for plant height, widest leaf width and percent brown tissue 30 and 70 DAT. Above ground biomass was collected 70 DAT. Dry weight was determined after samples were placed in 60 C oven for 72 hr.

Dry weight, percent brown tissue, plant height, longest leaf length and widest leaf width were subjected to ANOVA analysis by treatment for each species and application timing using SAS PROC MIXED procedures. Fisher's protected least significant differences (LSD) were computed at P=0.05. Hartley's F-max test (P = 0.05) was performed for test of homogeneity of variance between experiments (Hartley, 1950). Lethal rate required to kill 50 and 90% (LR<sub>50</sub> and LR<sub>90</sub>) of each treatment population and associated 95% confidence interval was determined using SAS PROBIT analysis and using the natural log of glyphosate rate for analysis. Plants were classified as living with the presence of any remaining green, growing tissue that was increasing in biomass, regardless of general plant health.

#### **RESULTS AND DISCUSSION**

Hartley's F-max indicated experiments could be combined for all collected data within each species. Predicted LR<sub>50</sub> and LR<sub>90</sub> were determined. Although dry weight, percent brown tissue, plant height, longest leaf length and widest leaf width were collected and aided in living status determination, these data will not be discussed. However, data showed typical rate response patterns with increased growth at lower rates, decreased growth at higher, species-specific rates and complete kill at extremely high, species-specific rates (Data not shown; See Appendix A).

In general, glyphosate rates required to control each species were higher than recommended label rates and previous rate response experiments (Tables 2.1 and 2.2), especially results from predicted LR<sub>90</sub> rates on plants treated 100 DAS. The experiment conservatively determined lethal rate if any green, growing tissue that was increasing in biomass. Herbicide control is usually deemed acceptable when 90 to 95% of the population is controlled or to the point where yield is minimally affected in cropping systems. However, control is often based on LR<sub>50</sub> values due the smallest amount of variation and smallest confidence interval located at the mid-point of a sigmoidal, rate-response curve (Pratley et al., 1999). Acceptable control and LR<sub>50</sub> values are often directly related but are not equal. Weed threshold levels are much lower in turfgrass systems compared to cropping systems because any weed presence detracts from overall turfgrass quality. In addition, the methods used in the experiment conservatively estimate plant survival based on an individual plant's ability to maintain green, growing tissue that was increasing in biomass. A majority of the surviving plants exhibited herbicide injury at recommended glyphosate use rates.

Experimental conditions could have increased the overall required LR<sub>90</sub> values including higher spray volumes and lack of spray additives used in turfgrass systems compared to cropping systems. Different species populations and greenhouse growing conditions could attribute to the higher rates than previous work. Finally, the experiment did not compare plant competition nor plant fitness. Although the aforementioned factors could have influenced the results of the experiment, the results ultimately indicate higher levels of glyphosate will be required. In addition, conservative estimates of survival could reduce the risk of GR weed population development if plants are not allowed to reproduce, despite the likely reduction in plant fitness.

For the purposes of most of the discussion, LR<sub>90</sub> values will be discussed due to the higher correlation to desirable turfgrass weed control and potential for plant survival and reproduction. Weed control was determined by plant survival, not impact on yield or quality of harvest

All species had lower  $LR_{50}$  and  $LR_{90}$  values for plants sprayed 30 DAS compared to 100 DAS, except yellow nutsedge  $LR_{50}$  values (Table 2.2). Younger plants are often controlled more completely due to higher amount of herbicide per unit plant tissue and thinner cuticles (Levene and Owen, 1995). Yellow nutsedge LR<sub>50</sub> values may have increased slightly with the plant age due to increased leaf interception of herbicide application. However, no comparisons of leaf area or application coverage were estimated in these experiments.

Field observations by turfgrass managers and turfgrass scientists indicate creeping bentgrass and yellow nutsedge are not adequately controlled with a single glyphosate application at rates of 1.68 kg a.e. glyphosate ha<sup>-1</sup>. Creeping bentgrass is not specifically addressed on the RoundUp Pro<sup>®</sup> label. Results from the experiments indicate 7.8 to 12.8 kg a.e. glyphosate ha<sup>-1</sup> rates would be required to kill 90% of the creeping bentgrass population. The recommended label rate for yellow nutsedge is 3.36 kg a.i. glyphosate ha<sup>-1</sup> or 2.5 kg a.e. glyphosate ha<sup>-1</sup> (Table 2.1). Results indicate 14.1 to 19.5 kg a.e. glyphosate ha<sup>-1</sup> would be required to kill 90% of the yellow nutsedge population investigated (Table 2.2). The high use rates required for adequate control of creeping bentgrass and yellow nutsedge indicate other management options, including alternative chemistries, are better suited for control of creeping bentgrass and yellow nutsedge.

The recommended rate of control for annual bluegrass in Grossbard and Atkinson (1985) was 0.3 kg a.i. glyphosate ha<sup>-1</sup> (Table 2.1). Annual bluegrass is not listed on Monsanto's 1991 or 2001 herbicide label. A related species, *P. pratensis*, has a recommended label rate of 1.68 kg a.e. glyphosate ha<sup>-1</sup>. However, the results of these experiments indicate a rate of 3.3 and 7.2 kg a.e. glyphosate ha<sup>-1</sup> for plants sprayed 30 and 100 DAS, respectively, would be required to kill 90% of the population investigated (Table 2.2). The label rate for

*P. pratensis* would not adequately control annual bluegrass for selective control in turfgrass systems.

In 1991, recommended use rates for *Digitaria* species were 0.56 kg a.i. glyphosate ha<sup>-1</sup> (Table 2.1). However in 2001, label rates were changed to 1.12 to 4.48 kg a.i. glyphosate ha<sup>-1</sup>. Results from these experiments indicate large crabgrass would be adequately controlled at the 2001 label rates if they were sprayed 30 DAS. At 100 DAS, large crabgrass had the highest LR<sub>90</sub> value of species investigated (Table 2.2). Adequate control may have been reduced due to increased cuticle thickness, increased public control of large crabgrass with glyphosate, applications must be made while the plants are small.

Recommended use rates of glyphosate for dandelion control ranges from 2.36 to 5.60 kg a.i. ha<sup>-1</sup> (Table 2.1). In Grossbard and Atkinson (1985), recommended use rates were 2.2 kg a.i. glyphosate ha<sup>-1</sup>. As with large crabgrass, dandelion LR<sub>90</sub> values were below the high label rate when applied at 30 DAS, but were much higher than label rates when applied at 100 DAS (Table 2.2). Selective dandelion control with glyphosate will require applications to be made when plants are juvenile. Larger plants may require additional glyphosate applications and/or additional herbicides for proper control due to plant size, change in plant physiology and anatomy, and general plant health.

Results indicate the use of glyphosate as the only means of selective control in turfgrass systems may not adequately control some species or may require higher than label rates for control for other species. Current label rates of glyphosate could control several species investigated if applications were made to relatively young plants. However, older plants receiving glyphosate application were not adequately controlled.

Herbicide resistant populations could develop if herbicide tolerant individuals are allowed to reproduce and the herbicide is continually used (Heap, 1997). Prolific seeders like annual bluegrass, large crabgrass and dandelion are highly susceptible to selective pressure due to the large number of potential offspring produced. Higher rates would be necessary to adequately control tolerant weed species to the desired management threshold level. Current knowledge in herbicide resistance indicates the use of multiple applications of a high rate of the herbicide increases the developmental rate of herbicide resistance for some herbicides (Gressel, 1995a; Gressel, 1995b). Conversely, additional resistance has been developed to other herbicides from multiple low rate applications. Glyphosate-resistant plants have resulted from both procedures (Boerboom et al., 1990; Hartzler and Battles, 2001; Johnston et al., 1989; Johnston and Faulkner, 1991; Loux et al., 1987; Marshall et al., 1989; Marshall et al., 1987; Perez and Kogan, 2003; Westwood et al., 1997; Westwood and Weller, 1997). Therefore, turfgrass managers should use caution and use the proper rate of glyphosate at the most effective timing to minimize the potential for resistant development. Turfgrass managers should continue to utilize integrated pest management practices to minimize the potential development of herbicide resistant populations. These greenhouse experiments

could be used as a base line for recommended use rates for GRT, especially if field observations and experiments support these findings.

	Grossbard and Atk	inson, 1985 <sup>†</sup>	Monsanto	o Labels <sup>+</sup>	Greenhous	e Rate Response"
	Susceptibility/Control <sup>§</sup>	Effective Rates	1991	2001	active ingredient	acid equivalent
			kg glyphe	osate ha <sup>-1</sup>	*****	kg a.e. glyphosate ha <sup>-1</sup>
Agrostis spp.	S/P	1.7	1.68	1.68	10.4 - 17.0	7.8-12.8
Cynodon dactylon	G	0.8-4.8	5.60	5.60	n/a	n/a
Cyperus esculentus	Р	1.0-2.0	3.36	3.36	18.8 - 26.0	14.1-19.5
Cyperus rotundus	Ч	up to 9	3.36	3.36	n/a	n/a
Digitaria spp.	n/a	2.2	0.56	1.12-4.48	2.7 - 33.5	2.0-25.1
Poa annua	S	0.3	n/a	n/a	4.4 - 9.6	3.3-7.2
Poa pratensis	ď	1.5	2.24	2.24	n/a	n/a
Taraxacum officinale	ш	2.2	3.36-5.60	3.36-5.60	4.6 - 30.3	3.5-22.7
Zoysia japonica	n/a	2.2	n/a	n/a	n/a	n/a

Table 2.1. Previous rate response experiments and recommended label rates for control in cropping situations and results from greenhouse experiments.

† Grossbard, E. and D. Atkinson. eds. 1985. The Herbicide Glyphosate. Butterworths: London.

‡ Roundup Pro labels; Monsanto Company, St. Louis, MO § Susceptibility (S= susceptible), Control (P=poor control, G=good control and E=excellent control) and n/a = not available. # Rate response rates are based on LD<sub>90</sub> values from the greenhouse experiments.

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of greenhouse-grown annual bluegrass, creeping bentgrass, dandelion, large crabgrass and yellow nutsedge 30 and 70 d after seeding. Table 2.2. Predicted lethal glyphosate rate required to kill 50 and 90 percent of populations (LR50 and LR90)

0						
		30 DAS - LH	50	3	0 DAS - LH	90
		95% Confid	lence Limits		95% Confid	dence Limits
	Predict	Lower	Upper	Predict	Lower	Upper
	kg a.	e. glyphosat	e ha <sup>-1</sup>	kg a.e	e. glyphosat	te ha <sup>-1</sup>
Poa annua	1.3	1.2	1.6	3.3	2.6	4.8
Agrostis stolonifera	2.5	2.1	3.2	7.8	5.5	13.1
Taraxacum officinale	1.5	1.3	1.8	3.4	2.6	5.3
Digitaria sanguinalis	1.3	1.2	1.4	2.0	1.8	2.5
Cyperus esculentus	3.9	3.1	5.4	14.1	9.2	26.9
	Ŧ	00 DAS - LF	150	10	0 DAS - LF	390
		95% Confid	lence Limits		95% Confic	fence Limits
	Predict	Lower	Upper	Predict	Lower	Upper
	kg a.	e. glyphosat	e ha <sup>-1</sup>	kg a.e	e. glyphosat	e ha <sup>-1</sup>
Poa annua	1.6	1.3	2.0	7.2	4.6	15.8
Agrostis stolonifera	3.4	2.6	4.7	12.8	8.1	26.3
Taraxacum officinale	4.0	3.0	6.1	22.7	12.5	61.3
Digitaria sanguinalis	7.6	4.7	18.5	25.1	12.0	134.2
Cyperus esculentus	3.5	2.7	5.2	19.5	11.2	48.2

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