CHAPTER III

EVALUATION OF GLYPHOSATE TOLERANCE OF COMMON TURFGRASS WEED POPULATIONS

ABSTRACT: Research was conducted to determine if populations of common turfgrass weeds differ in tolerance to glyphosate. A greenhouse experiment was conducted with large crabgrass (*Digitaria sanguinalis* L.), dandelion (*Taraxacum officinale* Weber), annual bluegrass (*Poa annua* L. *var. annua*) and creeping bentgrass (*Agrostis stolonifera* L. cv. 'Penncross'). Seeds of each species from at least 2 populations and seeds collected from populations whose parental material was exposed to different glyphosate application regimes were planted in 35 cm x 60 cm x 15 cm black flats filled with 24:9:35:32 (v:v:v:v) sand, soil, peat moss, vermiculite soil mix, respectively. Survival counts were collected after one and two applications of 1.68 kg a.e. glyphosate ha^{-1} (Roundup Pro® Pro formulation with 356 g L^{-1} of glyphosate acid). Plant survival based on 1) percent of plants that survived a single glyphosate application per number germinated; 2) percent of plants that survived two applications per number of plants germinated; and 3) percent of plants that survived two applications based on number of plants that survived the first application. Plant survival from a single 1.68 kg a.e. glyphosate ha^{-1} application ranged from 0 to 72% for all populations of all species, with the dandelion populations exhibiting the greatest range. Some

populations of dandelion plants collected from plants surviving previous glyphosate applications had up to four times higher survival of a single glyphosate application than the parent population. In addition, some large crabgrass and dandelion populations that survived two glyphosate applications exhibited similar results. Survival numbers from these studies were higher than reported by others. Plant response to glyphosate based on plant survival appears to be more conservative than response based on control estimates.

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

ADDITIONAL INDEX WORDS: glyphosate, glyphosate-tolerance, survival, turfgrass weeds.

ABBREVIATIONS: DAS, d after seeding; LSD, least significant difference;

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 crops for selective weed control (Marshall, 1998). Previous glyphosate resistant crops have been grown in annual cropping systems.

Glyphosate-resistant genes recently have been introduced to several turfgrass species, such as creeping bentgrass (*Agrostis stolonifera* L.) and Kentucky bluegrass (*Poa pratensis* L.) (Kind, 1998). 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 is related to the impact on crop yield and harvest quality. In a turfgrass system, higher levels of control are required to ensure high quality turf surfaces without visible disruptions based on aesthetics. 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 glyphosate resistant crops and increasing numbers of glyphosate resistant weed populations further substantiates the need to understand the weed population dynamics in herbicide resistant crops (Heap, 1997).

In addition, increased herbicide tolerance and/or resistance with time by specific weed populations has been documented (Table 3.1). Previous research has compared the relative survival rates of various populations of weed species or the relative survival rates of various weed species whose parental material survived glyphosate applications.

The objectives of the research were 1) to compare glyphosate survival rates of several populations of common turfgrass weeds and 2) to determine if seeds collected from plants surviving glyphosate applications produced progeny populations with different glyphosate survival rates than the parent or preceding generation.

MATERIALS AND METHODS

Greenhouse research was conducted at the University of Nebraska (Lincoln, NE). Experiments were initiated 7 May, 17 May, 8 July, 11 July and 16 July 2002. Twenty-five seeds of each population of annual bluegrass (*Poa annua* L. *var. annua*), large crabgrass (*Digitaria sanguinalis* L.), dandelion (*Taraxacum officinale* Weber) and creeping bentgrass (*Agrostis stolonifera* L. cv. 'Penncross') seeds per were placed 1 cm below soil surface in straight 2.5 cm rows in 35 cm x 60 cm x 15 cm black flats filled with 24:9:35:32 (v:v:v:v) sand, soil, peat moss, vermiculite soil mix, respectively. Seed sources included dandelion (from England, Illinois and California), annual bluegrass (from England and Oregon), and large crabgrass (from England, Iowa and California). The history of the seed sources' exposure to glyphosate is unknown. In addition, seed was collected from a previous experiment using glyphosate. Creeping bentgrass populations were commercially available cultivars 'A-4', 'Providence' and 'Penncross'. For simplicity, seed sources will be identified as indicated by the following seed source numbers.

Annual bluegrass

1) England collected by Herbiseed (New Farm, Mire Lane, West End, Twyford, RG10 0NJ, England)
2) Oregon collected by V and J Seed Farms (P.O. Box 82, Woodstock, IL 60098)
3) Oregon collected by Valley Seed Service (P. O. Box 9335, Fresno, California 93791)

Dandelion

1) England collected by Herbiseed
2) Illinois collected by V and J Seed Farms
3) California collected by Valley Seed Service

Large crabgrass

1) England collected by Herbiseed
2) Iowa collected by V and J Seed Farms
3) California collected by Valley Seed Service

In addition to the above weed populations, seed was collected from a previous experiment in which plants were treated with various glyphosate applications. In this previous experiment, seed was collected from surviving plants originating from population 1 after received the following treatments on 2 week intervals with the first application occurring 30 d after seeding (DAS). Seed sources will be identified as indicated by the following seed source letters. Numbers indicate a single application in kg a.e. glyphosate ha^{-1}.

A) 0.00 + 0.00 + 0.00 + 0.00 + 0.00 + 0.00 + 0.00

B) 0.00 + 0.00 + 0.00 + 0.00 + 0.84 + 0.84 + 2.24

C) 0.01 + 0.01 + 0.01 + 0.01 + 0.84 + 0.84 + 2.24

D) 0.05 + 0.05 + 0.05 + 0.05 + 0.84 + 0.84 + 2.24

E) 0.10 + 0.10 + 0.10 + 0.10 + 0.84 + 0.84 + 2.24

F) 0.50 + 0.50 + 0.50 + 0.50 + 0.84 + 0.84 + 2.24

G) 5.00 + 5.00 + 5.00 + 5.00 + 0.84 + 0.84 + 2.24

The arrangement of plants in previous experiments allowed for open pollination prior to collection of seed.

Number of germinated plants were determined 43 DAS. Glyphosate (Roundup® Pro formulation with 356 g L^{-1} of glyphosate acid) was applied 45 DAS at 2.24 kg ha^{-1} to all flats. The number of surviving plants with green, growing tissue that were increasing in biomass were counted 70 DAS. The two flats seeded on 7 and 17 May 2002 also received an additional glyphosate application 71 DAS. An additional survival count was made 90 DAS for those two flats. Glyphosate treatments were applied with a conveyor, pressurized (276 kPa) sprayer in 374 L ha^{-1} of water. 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. Newly emerging plants were removed by hand after glyphosate application and were not used for analysis.

Survival following the first glyphosate application for each population was determined by dividing the number of surviving plants by the total number of treated plants. Survival from the second glyphosate application was determined by dividing the number of surviving plants by both the total number of plants that germinated and the total number of plants that survived the first glyphosate application. Although data follow a binomial distribution, pooling means of survival within a pot allows the experiment to be analyzed with a Gaussian distribution due to the Central Limit Theorem (March and Seppalainen, 1997). Therefore, survival data were subjected to ANOVA analysis by population for each species using SAS PROC GLM procedures. Fisher's protected least significant differences (LSD) were computed. Significance levels of either 0.10 or 0.15 were used because of the relatively small number of experimental replicates used.

RESULTS AND DISCUSSION

The results of the experiment indicate trends for natural variability of survival of glyphosate applications between populations of a given weed species (Tables 3.2-3.4). However, none of the species showed different survival at the 0.05 significance level. Typically, binomial and ratio data require high number of experimental units to gain the same statistical power as continuous Gaussian data (Evans et. al., 2000). Additional experimental replicates were not conducted due to seed availability and time restrictions. Increasing replication may increase the statistical power of the study. Significant trends at the 0.10 and 0.15 significance levels will be used for the purposes of discussion.

Annual bluegrass, creeping bentgrass and large crabgrass populations evaluated did not differ in survival of a single glyphosate application (Table 3.2). Most of the populations had survival less than 23%. The exceptions were annual bluegrass collected from a population with no glyphosate history (40%), large crabgrass population 2 from California (25%) and large crabgrass population 1 from England subjected to treatment C (25%). Annual bluegrass survival ranged from 11 to 40%. Creeping bentgrass survival ranged from 15 to 23%. Large crabgrass survival ranged from 0 to 25%.

Dandelion populations did differ in survival of a single glyphosate application (Table 3.2; alpha = 0.10). Dandelion survival ranged from 0 to 72%. The two populations, population 2 from Illinois (0%) and population 1 from England (16%), had the lowest survival. Seed collected from population 1 exposed to treatments A,B and D-F did have higher survival, but only seeds from plants receiving treatment B and E were significantly higher than the parent population. These results may indicate a dandelion population's overall glyphosate tolerance is higher than other species investigated and may be different after survival of a previous glyphosate application.

Annual bluegrass, creeping bentgrass, large crabgrass and dandelion populations did not differ in survival based on the original number of treated plants surviving two glyphosate applications (Table 3.2). Since survival was determined from only two experimental replicates, trends are not evident. In addition, the two experimental replicates used for the second glyphosate application had the highest germination rates of all of the runs (data not shown). Therefore, some of the survival data are higher than for a single glyphosate application. Survival of two glyphosate applications would be expected to be lower than a single glyphosate application (Culpepper et al., 2000).

Survival of annual bluegrass, creeping bentgrass and large crabgrass receiving two glyphosate applications was less than 13% (Table 3.3). Exceptions included annual bluegrass population 1 from Oregon subjected to treatment A (50%) and treatments D (13%) and G (25%). In addition, the 'A-4' creeping bentgrass (27%) and large crabgrass population 1 from England subjected to treatment A (13%) and treatment C (63%) had survival above 13%. Dandelion survival ranged from 0 to 75%, with a majority of the treatments above 40%. A trend to increased survival of two glyphosate applications for plant populations collected from plants surviving glyphosate application is present, but requires further experimentation to confirm the trend. The high level of tolerance to two glyphosate applications further indicates a dandelion population's overall glyphosate tolerance is higher than other species investigated and may be increased after survival of a previous glyphosate application.

Survival of two glyphosate applications by all populations of annual bluegrass and creeping bentgrass are not different when survival is based on the number of plants surviving the first glyphosate application (Table 3.4). Survival ranged from 0 to 50% for annual bluegrass and 12 to 42% for creeping bentgrass populations. Although none of the parent annual bluegrass population 1 from England survived two glyphosate applications, populations produced from seed collected from plants exposed to glyphosate had 25 to 50% survival. In addition, population 1 from England subjected to treatment A showed 50% survival. Although the trend is not significant, the increased survival of plants from seed collected from the population 1 from England that did not receive glyphosate could be attributed to cross pollination and exchange of genetic material from other plants surviving glyphosate. Further experiments would be required to interpret the trend.

Large crabgrass and dandelion populations are different (alpha = 0.15) when survival is based on the number of plants surviving the first glyphosate application (Table 3.4). Large crabgrass survival ranged from 0 to 100%. The highest survival populations resulted from population 1 from England receiving treatment C (100%), which is not significantly different than populations 1 from England receiving treatment A (63%). Dandelion survival ranged from 0 to 100%. The highest survival resulted from population 1 from England receiving treatments D (100%), E (86%) and F (100%). Each of these populations were not significantly different than their parent population 1 (67%) or population receiving 1 receiving treatment B (50%). Population 2 did not survive.

Percent control is typically measured by visual cover over a plot area. In this experiment, plant survival was measured on a plant count basis. Johnson et.

al. (1998) measured the number of large crabgrass plants m^{-2}, but found glyphosate applied at a full label rate contained 35 plants m^{-2}, while the control plots only contained 23 plants m^{-2}. Application timing could have influenced survival rates from glyphosate, but should not directly increase the number of plants per area. Most previous experiments measuring control of the species investigated using glyphosate showed high levels of control using lower rates of glyphosate. Buhler et. al. (1990) applied 1.1 kg a.e. glyphosate ha^{-1} to dandelion and showed 94, 96 and 63% control 20, 80, and 350 DAT, respectively. The loss of control 350 DAT may be attributed to new plants or new growth from previously injured plants. Culpepper et. al. (2001) found at least 99% control of large crabgrass 2 and 5 weeks after treatment when glyphosate was applied at rates of 0.56, 0.84 and 1.12 kg a.i. ha^{-1}. Culpepper and York (1998) found large crabgrass was not adequately controlled with one application, but control was acceptable when a second glyphosate application was made 4 weeks after the initial application. Gimenez et. al. (1998) obtained 100% control of large crabgrass 1 and 3 weeks after treatment when 0.42, 0.56 and 0.84 kg a.e. glyphosate ha^{-1} was applied. Moseley and Hagood (1991) obtained 91 and 96% control of large crabgrass with applications of 0.11 and 0.55 kg a.i. glyphosate ha^{-1}, respectively, were made in addition to 0.04 kg chlorimuron $ha^{-1}$and 0.46 kg linuron ha^{-1}.

In these studies, survival was based on a plant count basis. Survival numbers were larger than would be obtained from experiments based on percent control. For example, much lower glyphosate application rates provided 91 and 96% control of large crabgrass (Moseley and Hagood, 1991). However, this experiment indicated 10-25% of the plants may still survive at much higher rates. Although plants are injured, regrowth and future reproduction are possible, especially in perennial systems. The weakness of the method is the requirement for many experimental units to obtain the same level of precision as percent control estimations. In this experiment, alpha levels of 0.10 and 0.15 allowed for statistical separation of treatments. Some populations of dandelion plants collected from plants surviving previous glyphosate applications had higher survival of a single glyphosate application than the parent population. In addition, some large crabgrass and dandelion populations that survived two glyphosate applications had higher survival from two glyphosate applications than the parent population.

Table 3.1. Documented weed populations with herbicide tolerance and/or resistance.

Scientific Name	Common Name	Researcher
Avena fatua L.	wild oat	Kern et al., 1996
Chenopodium album L.	common lambsquarters	Darmency and Gasquez, 1990
Convolvulus arvensis L.	field bindweed	Westwood and Weller, 1997
Conyza canadensis L. Cronq.	horseweed	Mueller et al., 2003
Conyza canadensis L. Cronq.	horseweed	VanGessel, 2001
Echinochloa oryzoides (Ard.) Fritsch	early watergrass	Fischer et al., 2000
Echinochloa phyllopogon (Stapf) Koss	late watergrass	Fischer et al., 2000
Eleusine indica (L.) Gaertn.	goosegrass	Lee and Ngim, 2000
Eleusine indica (L.) Gaertn.	goosegrass	Vaughn et al., 1990
Kochia scoparia (L.) Schrad.	Kochia	Christoffoleti et al, 1997
Kochia scoparia (L.) Schrad.	Kochia	Foes et al., 1999
Lolium rigidum Gaudin.	rigid ryegrass	Burnet et al., 1994
Lolium rigidum Gaudin.	rigid ryegrass	Gill et al., 1996
Lolium rigidum Gaudin.	rigid ryegrass	Llewellyn and Powles, 2001
Lolium rigidum Gaudin.	rigid ryegrass	Powles et al., 1998
Lolium rigidum Gaudin.	rigid ryegrass	Pratley et al., 1999
Lotus corniculatus L.	birdsfoot trefoil	Boerboom et al., 1990
Poa annua L.	annual bluegrass	Kelly et al., 1999
Raphanus raphanistrum L.	wild radish	Walsh et al., 2001
Sisymbrium orientale Torn.	Indian hedgemustard	Boutsalis and Powles, 1995
Sonchus oleraceus L.	common sowthistle	Boutsalis and Powles, 1995
various	various	Heap, 1997

Table 3.2. Percent survival of annual bluegrass, creeping bentgrass, dandelion, and large crabgrass surviving 1.68 kg a.e. glyphosate ha^{-1} from several seed sources and seed collected from a previous experiment.

	Poa annua	*Agrostis stolonifera*	*Digitaria sanguinalis*	*Taraxacum officinale*
Seed Source	---------------- % (number-surviving glyphosate per number-germinated per hundred) ----------------			
Population 1 †	18 a ‡	-	14 a	16 bc
Population 2	20 a	-	10 a	0 c
Population 3	16 a	-	25 a	-
1 + treatment A §	40 a	-	16 a	53 ab
1 + treatment B	-	-	-	72 a
1 + treatment C	11 a	-	25 a	-
1 + treatment D	15 a	-	0 a	47 ab
1 + treatment E	-	-	0 a	68 a
1 + treatment F	-	-	-	50 ab
1 + treatment G	18 a	-	-	-
A-4 #	-	22 a	-	-
Providence	-	23 a	-	-
Penncross	-	16 a	-	-

† Population 1 (All populations from England), Population 2 (*P. annua* from Oregon, *D. sanguinalis* from Iowa, *T. officinale* from Illinois), Population 3 (*P. annua* from Oregon, *D. sanguinalis* and *T. officinale* from California).

‡ Treatments in a column followed by the same letter are not significantly different at 0.10 probability level.

§ Treatments A-G were collected from surviving plants orginating from population 1 and received the following treatments on 2 week intervals (kg glyphosate ha-1): A) 0 + 0 + 0 + 0 + 0 + 0; B) 0 + 0 + 0 + 0 + 1.12 + 1.12 + 2.24; C) 0.01 + 0.01 + 0.01 + 0.01 + 1.12 + 1.12 + 2.24; D) 0.05 + 0.05 + 0.05 + 0.05 + 1.12 + 1.12 + 2.24; E) 0.10 + 0.10 + 0.10 + 0.10 + 1.12 + 1.12 + 2.24; F) 0.50 + 0.50 + 0.50 + 0.50 + 1.12 + 1.12 + 2.24; G) 5 + 5 + 5 + 5 + 1.12 + 1.12 + 2.24

A-4, Providence and Penncross are commercially available creeping bentgrass cultivars

Table 3.3. Percent survival of annual bluegrass, creeping bentgrass, dandelion, and large crabgrass surviving 2x1.68 (3.36 total) kg a.e. glyphosate ha^{-1} from several seed sources and seed collected from a previous experiment.

Seed Source	*Poa annua*	*Agrostis stolonifera*	*Digitaria sanguinalis*	*Taraxacum officinale*
	% (number-surviving glyphosate per number-germinated per hundred)			
Herbiseed (H) †	0 a ‡	-	9 a	17 a
V and J Seed Source	2 a	-	10 a	0 a
Valley Seed	2 a	-	8 a	-
H + no glyphosate	50 a	-	13 a	63 a
H + treatment A §	-	-	-	50 a
H + treatment B	10 a	-	63 a	-
H + treatment C	13 a	-	0 a	67 a
H + treatment D	-	-	0 a	42 a
H + treatment E	-	-	-	75 a
H + treatment F	25 a	-	-	-
A-4 #	-	27 a	-	-
Providence	-	12 a	-	-
Penncross	-	10 a	-	-

† Population 1 (All populations from England), Population 2 (P. annua from Oregon, D. sanguinalis from Iowa, T. officinale from Illinois), Population 3 (P. annua from Oregon, D. sanguinalis and T. officinale from California).

‡ Treatments in a column followed by the same letter are not significantly different at 0.15 probability level.

§§ Treatments A-G were collected from surviving plants orginating from population 1 and received the following treatments on 2 week intervals (kg glyphosate ha-1): A) 0 + 0 + 0 + 0 + 0 + 0; B) 0 + 0 + 0 + 0 + 1.12 + 1.12 + 2.24; C) 0.01 + 0.01 + 0.01 + 0.01 + 1.12 + 1.12 + 2.24; D) 0.05 + 0.05 + 0.05 + 0.05 + 1.12 + 1.12 + 2.24; E) 0.10 + 0.10 + 0.10 + 0.10 + 1.12 + 1.12 + 2.24; F) 0.50 + 0.50 + 0.50 + 0.50 + 1.12 + 1.12 + 2.24; G) 5 + 5 + 5 + 5 + 1.12 + 1.12 + 2.24

A-4, Providence and Penncross are commercially available creeping bentgrass cultivars

Table 3.4. Percent survival of annual bluegrass, creeping bentgrass, dandelion, and large crabgrass surviving 2x1.68 (3.36 total) kg a.e. glyphosate ha^{-1} from several seed sources and seed collected from a previous experiment.

Seed Source	*Poa annua*	*Agrostis stolonifera*	*Digitaria sanguinalis*	*Taraxacum officinale*
	--------- % (number-surviving glyphosate per number-surviving 1st application per hundred) ---------			
Herbiseed (H) †	0 a ‡	-	33 bc	67 ab
V and J Seed Source	2 a	-	25 bc	0 c
Valley Seed	8 a	-	17 bc	-
H + no glyphosate	50 a	-	63 ab	25 bc
H + treatment A §	-	-	-	50 abc
H + treatment B	25 a	-	100 a	-
H + treatment C	50 a	-	0 c	100 a
H + treatment D	-	-	0 c	86 a
H + treatment E	-	-	-	100 a
H + treatment F	40 a	-	-	-
A-4 #	-	42 a	-	-
Providence	-	15 a	-	-
Penncross	-	12 a	-	-

† Population 1 (All populations from England), Population 2 (P. annua from Oregon, D. sanguinalis from Iowa, T. officinale from Illinois), Population 3 (P. annua from Oregon, D. sanguinalis and T. officinale from California).

‡ Treatments in a column followed by the same letter are not significantly different at 0.15 probability level.

§ Treatments A-G were collected from surviving plants orginating from population 1 and received the following treatments on 2 week intervals (kg glyphosate ha-1): A) 0 + 0 + 0 + 0 + 0 + 0; B) 0 + 0 + 0 + 0 + 1.12 + 1.12 + 2.24; C) 0.01 + 0.01 + 0.01 + 0.01 + 1.12 + 1.12 + 2.24; D) 0.05 + 0.05 + 0.05 + 0.05 + 1.12 + 1.12 + 2.24; E) 0.10 + 0.10 + 0.10 + 0.10 + 1.12 + 1.12 + 2.24; F) 0.50 + 0.50 + 0.50 + 0.50 + 1.12 + 1.12 + 2.24; G) 5 + 5 + 5 + 5 + 1.12 + 1.12 + 2.24

A-4, Providence and Penncross are commercially available creeping bentgrass cultivars

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