CHAPTER IV

EVALUATION OF TOLERANCE CHANGES OF COMMON TURFGRASS WEEDS TO GLYPHOSATE

ABSTRACT: The objectives of the experiment were to determine if exposure to glyphosate changes the population response to glyphosate over multiple generations among different populations of several turfgrass weed species. Populations of annual bluegrass (*Poa annua* L.) and large crabgrass (*Digitaria sanguinalis* L.) were individually planted (original population). Forty-three days after emergence, the number of seeds emerging was determined. Plants were sprayed with 0.00, 0.84 and 1.68 kg a.e. glyphosate ha^{-1} (RoundUp Pro® formulation) 45 days after first emergence. One hundred days after first emergence, surviving plants were determined if the plant contained any green, growing tissue that were increasing in biomass. Plants were allowed to grow and seed collected. Plants surviving glyphosate produced a subsequent population of plants with varied glyphosate survival rates. Survival from glyphosate application with plants collected from a population that was not exposed to glyphosate varied in survival in the subsequent generation. The viability of seed collected varied by species and generation. Although the results are preliminary and additional experiments are necessary for further interpretation, it appears glyphosate

applications could provide a selective pressure to shift the level of glyphosate tolerance in subsequent populations.

NOMENCLATURE: glyphosate; large crabgrass, *Digitaria sanguinalis* L.; wild oat, *Avena fatua* L.; annual bluegrass, *Poa annua* L. *var. annua*; dandelion, *Taraxacum oficinale* Weber.

ADDITIONAL INDEX WORDS: glyphosate, glyphosate-tolerance, percent 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. Advances in genetic engineering have developed glyphosate resistant crops for genetic markers and selective weed control (Marshall, 1998). Previous glyphosate resistant crops have been grown in annual cropping systems.

Glyphosate-resistance genes have been recently introduced into 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 turfgrass. The goals of weed control in annual cropping systems are related to the impact on crop yield. In a turfgrass system, higher levels of control are desired to ensure a high quality turf surface 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 and current use of glyphosate resistant crops along with increasing numbers of glyphosate resistant weed populations further substantiates the need to understand weed populations in herbicide resistant crops.

In addition, increased herbicide tolerance and/or resistance with time by specific weed populations has been documented (See Table 3.1, previous chapter). Research comparing the relative survival rates of various populations of weed species or comparing the effect of selective pressure from glyphosate applications on the glyphosate tolerance levels of subsequent generations is limited. Mengistu et. al. (2003) compared a population of wild oat (*Avena fatua* L.) sampled 36 yr apart for herbicide resistance. Since six herbicides (imazamethabenz, difenzoquat, diclofop, fenoxaprop-P, sethoxydim and tralkoxydim) were evaluated prior to their commercial release, the populations can be evaluated for their shift in susceptibility. In 1964 all 43% of the wild oat collections were susceptible, but in 2000 only 9% of the collections were susceptible to all six herbicides. The frequency of resistance increased between 1.7 to 11-fold to all herbicides was increased from 1964 to 2000 and often multiple herbicide mode of action resistance had developed.

The objectives of this 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) Department of Agronomy and Horticulture greenhouses. Experiments were initiated October 2002. Species included annual bluegrass (*Poa annua* L. *var. annua*; 3 populations), creeping bluegrass (*Poa annua* L. var. *reptans* cv. 'True Putt'), large crabgrass (*Digitaria sanguinalis* L.; 3 populations) and dandelion (*Taraxacum officinale* Weber.). Seed sources included *T. officinale* Weber. (from England, Illinois and California), *P. annua* (from England and Oregon), and *D. sanguinalis* (from England, Iowa and California). The history of the seed sources' exposure to glyphosate is unknown. Seed sources will be identified as indicated by the following seed source numbers.

P. annua

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)

'True Putt' *P. annua* var. *reptans* cv. 'True Putt'

T. officinale

1) England collected by Herbiseed

2) Illinois collected by V and J Seed Farms

3) California collected by Valley Seed Service

D. sanguinalis

1) England collected by Herbiseed

2) Iowa collected by V and J Seed Farms

3) California collected by Valley Seed Service

Twelve replicates of each population by glyphosate rate were seeded with twenty-five seeds placed 1 cm below soil surface in 10 cm x 10 cm x 7.6 cm black pots filled with 24:9:35:32 (v:v:v:v) sand, soil, peat moss, vermiculite soil mix, respectively.

Total number of germinated plants was determined 43 d after first emergence (DAE). Glyphosate (Roundup® Pro formulation with 356 g/L of glyphosate acid) was applied 45 DAE at 0.00, 0.84 and 1.68 kg ha^{-1} to each population. 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. The number of surviving plants with green, growing tissue that was increasing in biomass was counted 100 DAE. Percent survival of the first glyphosate application for each population was determined by dividing the number of surviving plants by the total number of plants that germinated (number-surviving ÷ number-germinated x 100% = % survival). Comparison of the survival rate of each population were computed using Fischer's protected least significant differences (LSD; alpha=0.05). 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, an experimental unit for survival is considered to be the average percent survival for each experimental run. T-tests were performed to compare F1 treatments against their respective, original parent population.

RESULTS AND DISCUSSION

Original population germination rates varied with species and population from 18 to 95% (Tables 4.1 and 4.3). Germination rates were similar for each population within a species from the original population to F1 generation;

however, no statistical comparisons were made (Tables 4.1-4.4). An exception was observed for *D. sanguinalis* population 2 in which germination rates were halved from original population to F1 generations. All original populations of *P. annua* and *D. sanguinalis* had highest survival when receiving 0.00 and 0.84 kg a.e. glyphosate ha^{-1} (Tables 4.1 and 4.3). Survival percentages when 1.68 kg a.e. glyphosate ha^{-1} was applied were lower.

Only some original populations produced enough seed by the time of analysis to analyze F1 generations (Tables 4.2 and 4.4). Experiments will need to be continued to compare other F1 and future F2 and F3 populations. Germination rates of the F1 population varied with species, source population and glyphosate treatment from 11 to 70%.

P. annua population 1 showed an increase in survival from original population to F1 generation when glyphosate was not applied to either generation (Table 4.2). *P. annua* population 1 showed no change in survival from original population to F1 generation when the original population received 0.84 kg a.e. glyphosate ha^{-1} and the F1 generation received either 0.00 or 0.84 kg a.e. glyphosate ha^{-1}. A reduction in survival was observed when the original population received 0.84 kg a.e. glyphosate ha^{-1} and the F1 generation received 1.68 kg a.e. glyphosate ha^{-1}. *P. annua* population 'True Putt' exhibited an increase in survival when the original population generation did not receive a glyphosate application and the F1 generation received either 0.00 or 0.84 kg a.e. glyphosate ha^{-1}. In addition, an increase was observed when the original population generation received 1.68 kg a.e. glyphosate ha^{-1} and the F1 generation did not receive a glyphosate application.

D. sanguinalis F1 survival rates were decreased or were unchanged compared to the original population whenever the original population did not receive any glyphosate for all treatments of the F1 generation (Table 4.4). For *D. sanguinalis* population 2, survival rates were doubled when the parental generation received 0.84 kg a.e. glyphosate ha^{-1} for all treatments of the F1 generation. Populations of *D. sanguinalis* and *P. annua* varied in their germination and survival rates from glyphosate applications. Due to the various sources of seed and potential differences in ages of seed, variability would be expected. In most cases, exposure to glyphosate in the parental generation did not affect the germination rates in the F1 generation. In terms of germination, a fitness cost due to glyphosate survival was not observed except for a population of *D. sanguinalis*.

Mengistu et. al. (2003) showed increase levels of resistance in wild oat populations after 36 yr of crop use for several different herbicide classes. Although the time period was not specifically identified for development of some populations to gain herbicide resistance when selective pressure was applied, herbicide tolerance was increased and herbicide resistance was developed in some populations within 36 yr. Several populations in these experiments changed their survival rates after one generation of selective pressure. Increases in survival was observed from the 'True Putt' *P. annua* population when the parent population was not exposed to glyphosate and from the *D. sanguinalis* population 2 when the parent population survived 0.84 kg a.e. glyphosate ha^{-1}. The *D. sanguinalis* population 2 appears to have selected for glyphosate tolerance after only one generation. However, since the 'True Putt' *P. annua* population did not receive any selective pressure, these results require further experimentation for appropriate interpretation. However, *P. annua* cross-pollinates and the experiment did not attempt to limit any potential cross-pollination. Pollination could have occurred from other *P. annua* plants surviving glyphosate applications. If crossing between populations did occur, then any individuals that contained a trait for increased glyphosate tolerance would have a selective advantage over more susceptible individuals.

Further experimentation is necessary to determine other trends in population shifts of glyphosate tolerance. The experiment will continue at the University of Nebraska to compare F1, F3 and possibly F4 generations. Furthermore, after several generations, broad-sense heritability estimates will be determined to identify the how selective pressure from glyphosate applications affect common turfgrass weeds' glyphosate tolerance.

Table 4.1. *Poa annua* population germination and survival of the original populations following glyphosate applications.

	Original Population		
Population[†]	Treatment (kg a.e. ha^{-1})	Germination (%)	Plant Survival of glyphosate application (%)
1	0.00	52	30 cd [‡]
	0.84	42	60 a
	1.68	52	9 gh
2	0.00	73	25 de
	0.84	74	21 ef
	1.68	70	3 h
3	0.00	94	20 ef
	0.84	93	15 fg
	1.68	95	2 h
'True Putt'	0.00	65	33 c
	0.84	69	46 b
	1.68	60	8 gh

† Population 1: England (Herbiseed), Population 2: Oregon (V and J Seed Farms) Population 3: Oregon (Valley Seed Service), 'True Putt' creeping bluegrass cultivar from University of Minnesota.

‡ Letters not followed by the same letter are significantly different at the P=0.05 level.

Table 4.2. *Poa annua* survival rate comparison from original parent population and F1 populations that were collected from original populations after glyphosate application.

	Original Population		F1 Generation			
Population†	Treatment (kg a.e. ha^{-1})	Plant survival of glyphosate application (%)	Treatment (kg a.e. ha^{-1})	Germinate (%)	Plant survival of glyphosate application (%)	T-Test ‡
1	0.00	30	0.00	50	51	**
			0.84	57	27	ns
			1.68	55	17	*
1	0.84	60	0.00	21	60	ns
			0.84	24	57	ns
			1.68	23	44	**
'True Putt'	0.00	33	0.00	32	89	**
			0.84	32	54	**
			1.68	31	43	ns
'True Putt'	1.68	8	0.00	70	100	**
			0.84	69	16	ns
			1.68	70	11	ns

† Population 1: England (Herbiseed), Population 2: Oregon (V and J Seed Farms) Population 3: Oregon (Valley Seed Service), 'True Putt' creeping bluegrass cultivar from University of Minnesota.

‡ T-test performed between original and F1-treatment generations for survival. 'ns' indicates not significantly different; '*' and '**' indicates significant differences at alpha < 0.05 and alpha < 0.01, respectively.

Table 4.3. Large crabgrass (*Digitaria sanguinalis*) population germination and survival of the original populations following glyphosate applications.

	Original Generation		
Population†	Treatment ($kg\ a.e.\ ha^{-1}$)	Germination (%)	Plant Survival of glyphosate application (%)
1	0.00	21	75 b ‡
	0.84	19	21 e
	1.68	18	8 f
2	0.00	44	47 c
	0.84	39	36 d
	1.68	42	12 ef
3	0.00	19	100 a
	0.84	18	50 c
	1.68	21	6 f

† Population 1: England (Herbiseed), Population 2: Iowa (V and J Seed Farms) and Population 3: California (Valley Seed Service).

‡ Letters not followed by the same letter are significantly different at the P=0.05 level.

Table 4.4. Large crabgrass (*Digitaria sanguinalis*) survival rate comparison from original parent population and F1 populations that were collected from original populations after glyphosate application.

	Original Population		F1 Generation			
Population†	Treatment (kg a.e. ha^{-1})	Plant survival of glyphosate application (%)	Treatment (kg a.e. ha^{-1})	Germinate (%)	Plant survival of glyphosate application (%)	T-Test ‡
1	0.00	75	0.00	11	73	ns
			0.84	14	66	ns
			1.68	16	42	**
2	0.00	47	0.00	19	60	ns
			0.84	18	60	ns
			1.68	22	42	ns
2	0.84	36	0.00	12	87	**
			0.84	13	69	**
			1.68	14	81	**
3	0.00	100	0.00	24	55	**
			0.84	23	39	**
			1.68	25	36	**

† Population 1: England (Herbiseed), Population 2: Iowa (V and J Seed Farms) and Population 3: California (Valley Seed Service).

‡ T-test performed between original and F1-treatment generations for survival. 'ns' indicates not significantly different; '**' indicates significant differences alpha < 0.01.

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