CHAPTER V

PHYTOTOXICTY OF CLIPPINGS FROM CREEPING BENTGRASS TREATED WITH GLYPHOSATE[1]

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ABSTRACT: Recent advances in genetic engineering have developed glyphosate resistant (GR) crops for genetic markers and selective weed control. The effects of glyphosate residue on turfgrass clippings could be toxic to non GR species. The objective of this experiment was to determine if glyphosate would retain activity within clippings of creeping bentgrass when applied to Kentucky bluegrass and perennial ryegrass. Greenhouse grown 'Penncross' and GR ASR-368 were treated with glyphosate at 2.24 kg/ha. Clippings were collected 1, 3, 7 and 12 d after application and applied to greenhouse grown Kentucky bluegrass

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and perennial ryegrass. Kentucky bluegrass and perennial ryegrass dry weight and percent cover were reduced by clippings receiving glyphosate that were harvested 1 and/or 3 d after glyphosate application from both susceptible and resistant creeping bentgrass. Results indicate that glyphosate remains active in clippings for up to 3 d after treatment within creeping bentgrass clippings. Glyphosate-applied, creeping bentgrass clippings will need to be managed to prevent injury to susceptible species.

NOMENCLATURE: Glyphosate *N*-(phosphonomethyl)glycine; creeping bentgrass, *Agrostis stolonifera* L.; Kentucky bluegrass, *Poa pratensis* L.; perennial ryegrass, *Lolium perenne* L.

ADDITIONAL INDEX WORDS: Glyphosate, clippings, turfgrass, mulch.

ABBREVIATIONS: DAT, days after treatment; G, glyphosate; GR, glyphosate resistant; GRCB-G, glyphosate resistant creeping bentgrass treated with glyphosate; LSD, least significant differences; PCB-G, 'Penncross' creeping bentgrass treated with glyphosate; PCB-UTC, 'Penncross' creeping bentgrass untreated control; UTC, untreated control.

INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] blocks the aromatic amino acid biosynthetic pathway in plants and has been used for non-selective weed control in cropping and aquatic systems since the 1970's (Jaworski 1972). Recent advances in genetic engineering have developed glyphosate resistant (GR) crops for genetic markers and selective weed control (Marshall 1998). Glyphosate remains in an unaltered state for extended periods of time after application both in and on leaf tissue. Greater than 66% of applied ^{14}C-glyphosate was found in tissue up to 12 d after application to various weed and tree species (Schultz and Burnside1980; Tanphiphat and Appleby 1990; Putnam1976; Thompson et al. 1994; Sandberg et al. 1980; Devine and Bandeen 1983). The active form of glyphosate in and on leaf tissue may retain herbicidal activity on susceptible species. Rapid binding to soil colloids and low mammalian toxicity of glyphosate has contributed to rapid adoption of glyphosate in GR crops such as soybeans [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) (Torstennson 1985; Vencill, 2002). In addition, GR turfgrasses have also been developed (Kind 1998). Minimal research has been conducted on the effects of using glyphosate for selective weed control in a perennial turfgrass system. Soil colloid bound glyphosate leaves no phytotoxic residue in soil unless the glyphosate molecules are released into soil solution and activated.

Herbicide residue in turfgrass clippings and mulch has been studied to determine the effects on susceptible species. Thompson et al. (1984) found that 44 to 72% of the 2,4-D applied to Kentucky bluegrass (*Poa pratensis* L.) was readily dislodged by a cheesecloth wipe with distilled water from 9 h to 15 d after application. The readily dislodgeable residue could injure susceptible species. Total residue recovered was 82.4, 98.6, 85.6, 85.8, 80.9, 79.0 and 65.6% of total 2,4-D applied for 9 h, 1, 2, 4, 9, 12 and 15 d after application, respectively. Residue recovered on the leaf surface by cheesecloth wipe with distilled water was 61.2, 72.0, 58.3, 60.1, 57.4, 54.7 and 44.4% of total 2,4-D applied, respectively. Less than 10% of total 2,4-D applied was recovered within cuticle, within leaf and within thatch for the given times. Bahe and Peacock (1995) applied a mixture of 2,4-D (21.5%) + dicamba (3,6-dichloro-o-anisic acid; 11.5%) + MCPP [2-(2-methyl-4-chlorophenoxy) propionic acid; 2.3%] at 0.5 g total a.i/m^2 on tall fescue (*Festuca arundinacea* Schreb.). Clippings from tall fescue was applied on tomato (*Lycopersicon esculentum* L.), cucumber (*Cucumis sativus* L.), salvia (*Salvia splendens* F.), and marigold (*Tagetes tenuifolia* Cav.) grown in pots. Herbicide-treated mulch reduced dry weight by 80% for cucumber, 73% for tomato, 65% for marigold, and 34% for salvia compared to controls from 2 to 5 wk after mulching. The use of glyphosate on GR turfgrasses could result in herbicidal activity from the clippings on susceptible species. Jordan (1977) found up to 90.8% of ^{14}C-glyphosate remained in treated bermudagrass leaves and less than 8.7% remained on leaf surfaces 48 h after application. Glyphosate remaining amounts were highly dependent on temperature and relative humidity conditions.

The phytotoxicity of clippings obtained from glyphosate treated turfgrass has not been quantified. Specifically, no published research has been conducted examining the effects of glyphosate treated turfgrass clippings on susceptible species. In daily maintenance of turfgrass systems, turfgrass clippings are spread to surrounding turfgrass areas and vegetation through maintenance equipment, workers disposing of collected clippings and wind. Following a glyphosate application, the clippings should be managed to reduce the negative impact on susceptible species. Therefore, the objective of this experiment was to determine if glyphosate would retain activity within clippings of creeping bentgrass (*Agrostis stolonifera* L.) when applied to Kentucky bluegrass and perennial ryegrass (*Lolium perenne* L.).

MATERIALS AND METHODS

Greenhouse research was conducted at the University of Nebraska (Lincoln, NE). Experiments were initiated 29 Jan and 20 March 2002, organized in a completely randomized design within each species. Two replicates of each clipping treatment were used for each initiation date. 'Penncross' (glyphosate susceptible) and glyphosate-resistant ASR-368[3] creeping bentgrass were seeded into 35.5 by 50.8 by 15.2 cm flats with 24:9:35:32 (v:v:v:v) sand, soil, peat moss, vermiculite soil mix, respectively. The ASR-368 bentgrass was seeded at a rate of 36 g/m^2 and 'Penncross' was seeded at a rate of 18 g/m^2. Seeding rates between creeping bentgrasses differed because only 50% of ASR-368 was GR seed. The ASR-368 bentgrass was sprayed with 2.24 kg/ha glyphosate 40 d after seeding to remove non-resistant creeping bentgrass plants. All glyphosate treatments were applied with a conveyor, pressurized (276 kPa) sprayer in 374 L/ha of water. Creeping bentgrass was clipped to 19 mm every 7 d using scissors. Kentucky bluegrass (cv. 'Bluechip') and perennial ryegrass (cv. 'Prizm') were seeded into 11.4 by 11.4 cm pots filled with the above soil mix at rates of 7.4 and 14.7 g/m^2, respectively. Rates varied between species based on recommended seeding rates. Kentucky bluegrass and perennial ryegrass plants were clipped to 25 mm every 7 d using scissors. Plants were watered daily to soil

[3] Provided by Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041

saturation. Every 7 d, Peters[4] 20-10-20 (200 N ppm) fertilizer was applied. All plants were allowed to grow until 100% cover developed at 100 d after seeding.

Glyphosate was directly applied at a rate of 2.24 kg/ha glyphosate to two flats of both ASR-368 and 'Penncross' creeping bentgrass 3 d after previous clipping and 100 d after seeding. Plants had approximate height of 27 mm at time of glyphosate application. Creeping bentgrass clippings harvested with scissors at a height of 22 mm were collected 1, 3, 7 and 12 d after glyphosate treatment (DAT) from each flat. Clippings from two untreated 'Penncross' flats were also collected. Clippings from each flat were immediately divided into equal portions by weight and placed in the top 2 cm of shoot canopy of a Kentucky bluegrass and a perennial ryegrass pot. Weights of clipping fresh weights were recorded. Flats and pots were watered daily. In each experiment, six Kentucky bluegrass and six perennial ryegrass plants did not receive clipping or glyphosate treatments. Kentucky bluegrass and perennial ryegrass pots were evaluated 7, 12 and 22 DAT for herbicide injury. Visual turfgrass percent cover (0-100%) was estimated visually. Aboveground, green tissue was collected using scissors 22 DAT. Tissue was dried for 72 h at 60 C and dry weights recorded. Plants were not watered until immediately following collection of 1 DAT clipping removal. Plants were then watered daily using overhead irrigation until soil saturation.

Clipping fresh weight (non-transformed) was linearly regressed against

[4] J.R. Peters, Inc., 6656 Grant Way, Allentown, PA 18106

herbicide injury ratings and dry weight using PROC REG procedures of SAS[5] to determine if clipping fresh weight of collected clippings affected injury ratings or dry weight. Hartley's F-max test (P = 0.05) was performed for test of homogeneity of variance between experiments (Hartley 1950). Dry weight and visual herbicide injury ratings were analyzed by each clipping harvest DAT and by treatment using SAS PROC MIXED procedures to obtain ANOVA and treatment comparisons. Fisher's protected least significant differences (LSD) were computed at P=0.05. Identical untreated Kentucky bluegrass and perennial ryegrass experimental units that did not receive glyphosate or clipping applications were used for all DAT comparisons.

RESULTS AND DISCUSSION

Hartley's F-max indicated experiments could be combined for herbicide injury ratings and dry weight (data not shown). Clipping weights from creeping bentgrass flats did not correlate with Kentucky bluegrass or perennial ryegrass herbicide injury ratings nor dry weight for any day of clipping application treatments (data not shown). Clipping weights were 3.81 to 10.89 g for 'ASR-368' and 1.17 to 8.87 g for 'Penncross'. All R^2 values were less than 0.21 Therefore, herbicide injury nor reduction in dry weight cannot be solely attributed to the amount of bentgrass clippings applied to Kentucky bluegrass or perennial ryegrass.

[5] SAS Institute, Inc., 100 SAS Campus Drive, Cary, NC, 27513-2414

Clippings obtained 1 DAT from 'Penncross' creeping bentgrass treated with glyphosate (PCB-G) and ASR-368 treated with glyphosate (GRCB-G) reduced Kentucky bluegrass dry weight compared to untreated control (UTC). Kentucky bluegrass dry weight did not differ between clipping of PCB-G and untreated 'Penncross' creeping bentgrass (PCB-UTC) for clippings obtained 1 DAT. No significant differences were observed between the UTC and Kentucky bluegrass receiving clippings that were obtained 3 and 7 DAT. Similar patterns were observed for Kentucky bluegrass receiving clippings obtained 12 DAT except for an increase in dry weight when PCB-G were applied. Perennial ryegrass dry weight followed similar patterns as Kentucky bluegrass except the reduction in dry weight from PCB-G and GRCB-G was found for clippings obtained 3 DAT. In addition, a reduction in perennial ryegrass percent cover was observed 7 and 12 DAT when PCB-UTC clippings were harvested and applied 1 DAT. Also, dry weight from PCB-G and GRCB-G was not different from PCB-UTC at 3 DAT. An increase in dry weight when PCB-G were applied compared to control was also observed with perennial ryegrass.

Percent cover ratings support the results found with dry weight (Table 2). Clippings obtained from both PCB-G and GRCB-G reduced Kentucky bluegrass and perennial ryegrass cover ratings when obtained 1 and 3 DAT, respectively, compared to UTC for all three rating dates. No differences between GRCB-G, PCB-G or PCB-UTC were observed for Kentucky bluegrass and perennial ryegrass receiving clippings harvested 7 and 12 DAT.

These data indicate glyphosate remains in the active, parent form for up to 3 d in and on creeping bentgrass tissue. The length of herbicidal activity is shorter than the observed length of unaltered, parent residues found in previous experiments and may indicate glyphosate becomes bound to organic matter after 3 d. Schultz and Burnside (1980) recovered 93 to 96% of applied ^{14}C-glyphosate in original form 12 d after application on hemp dogbane (*Apocynum cannabinum* L.). Tanphiphat and Appleby (1990) found up to 66.6% of the absorbed ^{14}C-glyphosate 3 d after application on treated leaves of bulbous oatgrass (*Arrhenatherum elatius* var. *bulbosum*). The untreated leaves and underground organs had up to 17.4 and 19.3% of absorbed ^{14}C-glyphosate, respectively. Field bindweed (*Convolvulus arvensis* L.), Canada thistle [*Cirsium arvense* (L.) Scop], tall morningglory [*Ipomoea purpurea* (L.) Roth] and wild buckwheat (*Polygonum convolvulus* L.) had greater than 85% of the total ^{14}C-glyphosate in parent form 3 d after application (Sandberg et al. 1980). Glyphosate absorption did not increase on *Agoropyron repens* (L.) Beauv. beyond 3 d after application, however translocation to rhizomes continued for up to 7 d (Devine and Bandeen 1983). Up to 20% of the applied glyphosate was recovered the following spring with 34% of the glyphosate recovered in rhizomes being remobilized into new, aerial shoots. Ninety-seven to ninety-nine percent of recovered glyphosate from common bermudagrass (*Cynodon dactylon*) remains in foliage 2 d after application (Whitwell et al. 1980). The previous work indicates glyphosate can remain in and on plant tissue in the parent glyphosate form for several days or more.

After a period of time, the effect of the glyphosate application is reduced. Clippings obtained 1 DAT from PCB-G and GRCB-G injured both Kentucky bluegrass and perennial ryegrass. However, only perennial ryegrass was injured from clippings obtained 3 DAT. Possible deactivation mechanisms include binding to organic matter or lignin fraction of degrading clippings, binding to soil, metabolism by microorganisms or loss in daily irrigation solution. Metabolism is not a likely mechanism as Gottrup et. al. (1976) did not detect ^{14}C-glyphosate metabolites 7 DAT with Canada thistle or leafy spurge (*Euphorbia* esula L.). However, binding to organic matter may be a possibility, as metribuzin and *S*-ethyl metribuzin was adsorbed on wheat (*Triticum aestivum* L.) straw more than soil with the lignin fraction believed to have the highest sorptive capacity of intact straw (Dao 1991).

Another potential source of a reduction in glyphosate activity is rainfall or irrigation following glyphosate application. Bariuan et. al. (1999) found purple nutsedge (*Cyperus rotundus* L.) shoot growth reduction 3 weeks after glyphosate application to be decreased by 30% when rainfall was applied 24 hr after application. In our experiment, the first clipping material was removed prior to any irrigation. Subsequently all plants were watered daily. Thus clippings collected after 1 d received daily overhead irrigation, which may contribute to the reduction in glyphosate activity. Potential quantities of freely available and absorbed glyphosate on glyphosate treated turfgrass leaves are poorly understood.

Clippings obtained 3 DAT from PCB-G and GRCB-G injured perennial ryegrass more than Kentucky bluegrass. Differences in susceptibility have been documented but are not well understood. In addition, lack of differences in the effect of PCB-G and GRCB-G is consistent with previous findings. Absorption and translocation of glyphosate are generally similar between susceptible and tolerant/resistant species. Westwood et al. (1997) found tolerant and susceptible biotypes of field bindweed did not display differences in glyphosate adsorption or translocation. Wyrill and Burnside (1976) found higher absorption of ^{14}C-glyphosate by susceptible common milkweed (*Asclepias syriaca* L.) compared to tolerant hemp dogbane, but no differences in translocation were observed. In most cases, absorption and translocation does not appear to differ between susceptible and tolerant species.

Clippings from both glyphosate-resistant and susceptible creeping bentgrass retained herbicide activity when collected up to 3 d after glyphosate application on susceptible species. Kentucky bluegrass was less susceptible to the herbicide activity than perennial ryegrass. Clippings from glyphosate treated creeping bentgrass may need be managed to prevent injury to susceptible species. Glyphosate treated clippings should not be placed on susceptible species, including adjacent turfgrasses, until the phytotoxic levels are reduced. Management options could include composting the clippings or returning the clippings to glyphosate resistant turfgrass swards.

Table 5.1. Kentucky bluegrass and perennial ryegrass dry weight 22 days after glyphosate application.

	Dry Weight			
	1 DAT[a]	3 DAT	7 DAT	12 DAT
	g dry weight/m^2			
Kentucky bluegrass				
UTC[b]	151 a [c]	151 a	151 b	151 b
PCB-UTC	142 ab	167 a	163 ab	151 b
PCB-G	124 bc	167 a	173 a	191 a
GRCB-G	109 c	145 a	166 ab	159 b
Perennial ryegrass				
UTC	127 a	127 a	127 a	127 b
PCB-UTC	129 a	114 ab	153 a	141 ab
PCB-G	66 b	128 ab	142 a	161 a
GRCB-G	55 b	92 b	129 a	143 ab

[a] Days after glyphosate treatment that clippings were harvested

[b] UTC, untreated control; PCB-UTC, 'Penncross' creeping bentgrass clippings without glyphosate; PCB-G, 'Penncross' creeping bentgrass clippings with glyphosate; GRCB-G, glyphosate resistant creeping bentgrass clippings with glyphosate

[c] Treatments followed by the same letter are not significantly different within column and species (α=0.05).

Table 5.2. Percent Cover[a] Remaining of Kentucky bluegrass and perennial ryegrass after clipping treatments.

	Percent Cover Remaining								
	7 DAT[b]		12 DAT			22 DAT			
	DAT that clippings were harvested								
	1	3	1	3	7	1	3	7	12
	percent cover								
Kentucky Bluegrass									
UTC[c]	99 a [d]	99 a	99 a	99 a	99 a	97 a	97 a	97 a	97 a
PCB-UTC	89 a	95 ab	88 a	99 a	99 a	95 a	98 a	95 a	96 a
PCB-G	50 b	88 b	68 b	93 a	99 a	48 b	90 a	96 a	99 a
GRCB-G	50 b	85 b	66 b	93 a	98 a	41 b	90 a	93 a	96 a
Perennial Ryegrass									
UTC	98 a	98 a	99 a	99 a	99 a	97 a	97 a	97 a	97 a
PCB-UTC	74 b	81 ab	74 b	85 ab	98 a	83 a	84 a	95 a	95 a
PCB-G	36 c	73 b	28 c	61 b	88 a	26 b	49 b	94 a	88 a
GRCB-G	38 c	85 ab	24 c	61 b	88 a	25 b	59 b	93 a	95 a

[a] Percent cover ratings were estimated visually; 0-100% of complete turfgrass cover of pot area.

[b] Days after glyphosate treatment

[c] UTC, untreated control; PCB-UTC, 'Penncross' creeping bentgrass clippings without glyphosate; PCB-G, 'Penncross' creeping bentgrass clippings with glyphosate; GRCB-G, glyphosate resistant creeping bentgrass clippings with glyphosate

[d] Treatments followed by the same letter are not significantly different within species and rating date ($\alpha=0.05$). Letters indicate LSD letters within column and species.

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