Effects of Temperature and Relative Humidity on the Toxicity of Glyphosate to Bermudagrass (*Cynodon dactylon*)[1]

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Abstract. Glyphosate [*N*-(phosphonomethyl)glycine] toxicity to bermudagrass [*Cynodon dactylon* (L.) Pers.] increased significantly with each rate increase from 0.14 to 1.12 kg/ha. Under greenhouse conditions approximately 50% bermudagrass control was obtained at 0.56 kg/ha glyphosate. Visible toxicity and fresh wt of treated plants and regrowth of plants clipped at the soil surface 24 h after treatment were used as indices for penetration and translocation of glyphosate. Visible injury to bermudagrass with 0.56 kg/ha glyphosate was greater at 100% than at 40% relative humidity (RH) at both 22 and 32 C. Fresh wt data indicated that 0.56 kg/ha glyphosate was more toxic at 32 C than at 22 C at 40% RH, but no difference was observed at 100% RH. Less than 10% of the applied ^{14}C-glyphosate penetrated the treated bermudagrass leaf at 22 C and 40% RH; whereas, more than 70% penetrated the treated leaf at 32 C and 100% RH. Five to six times more ^{14}C-label was translocated into the plant at 100% than at 40% RH. Significantly more ^{14}C-label translocated out of the treated leaf and into the plant at 32 C than at 22 C at 40% RH but no significant increase was observed at 100% RH.

INTRODUCTION

Bermudagrass is a creeping herbaceous plant that reproduces primarily by vegetative means. The rapid lateral spread of bermudagrass forms a dense sod that makes this plant highly competitive with crops for water and soil nutrients. Bermudagrass was introduced into the United States, probably from Africa, as a warm season forage grass. By 1800, bermudagrass was considered one of the most important lawn and pasture grasses in the south (1).

Regardless of its beneficial or esthetic value, bermudagrass has become a serious problem weed in cotton fields of the southeastern United States. The increase in bermudagrass infestation in cotton fields has been brought about by a combination of factors which include changes in tillage practices, above normal rainfall, skip-row planting patterns, and failure to properly maintain weed-free perimeters around fields (5).

Environmental changes alter the activity of herbicides on weeds (7). Translocation of simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] and terbutryn [2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine] was altered by increased temperature which promoted high transpiration in plants (4, 10). High levels of relative humidity increased dalapon (2,2-dichloropropionic acid) uptake into plants by hydrating the cuticle (2, 9).

Glyphosate has been used to successfully control bermudagrass (5, 6). However, our field test results have shown glyphosate control can be inconsistent. This study was conducted to investigate the influence of air temperature and relative humidity on glyphosate absorption and translocation in bermudagrass.

MATERIALS and METHODS

Rate response to bermudagrass. Greenhouse experiments were conducted to study the response of bermudagrass to increasing rates of glyphosate and to establish a rate that would give approximately 50% visible toxicity. Bermudagrass plants were grown from a single 16- to 20-cm stolon that was rooted at three to four nodes. The bermudagrass plants were cultured in 10-cm plastic pots containing Bosket silt loam and watered one to two times daily. Nitrogen was applied at 112 kg N/ha weekly. Plants were 6 to 8 weeks old and 10 to 16 cm tall with 6 to 10 aerial shoots when treated. The greenhouse was maintained at 25 to 35 C, 40 to 75% RH, and a 12 h light period. Glyphosate was applied at 0.14, 0.28, 0.56, 0.84, and 1.12 kg/ha over-the-top in water at 187 L/ha. All glyphosate treatments contained 0.25% (v/v) nonoxynol (9.5) [α-(*p*-nonylphenyl)ω-hydroxypoly(oxyethylene)] surfactant. The experimental design was a randomized complete block with four replications. The experiment was repeated. Visual evaluation of plant injury was made at 14 and 21 days after treatment using a 0 to 100 scale whereby 0 = no injury and 100 = complete kill.

Effect of environment on glyphosate toxicity. Two separate experiments were conducted to determine the effects of temperature and relative humidity on the toxicity of glyphosate to bermudagrass. Plants were grown in the greenhouse as previously described. Three days before treatment all plants were conditioned in growth chambers at either 22 or 32 C and 40 or 100% RH. Plants were treated with 0.56 kg/ha glyphosate over-the-top in water at 187 L/ha. The treated plants were immediately returned to the growth chambers. Plants in the first experiment (regrowth study) were maintained under the appropriate environmental conditions for 24 h after treatment, then all treated and untreated plants were clipped at the soil surface and moved to the greenhouse. Glyphosate toxicity was determined by visual evaluation and fresh wt of regrowth 8 weeks after glyphosate application.

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Plants in the second experiment (toxicity study) were maintained under the appropriate environmental conditions for 6 days after treatment. They were then moved to the greenhouse where all treated and untreated plants were maintained for the duration of 8 weeks. Glyphosate toxicity was evaluated in the same manner as in the clipping experiment. Each experiment was designed as a randomized complete block with three replications. All experiments were repeated.

Effects of environment on ^{14}C-glyphosate movement. Uniformly selected bermudagrass stolons were propagated in the greenhouse in 5-cm plastic cups containing silica sand. The plants were watered daily with one-half strength Hoagland's No. 1 solution. Each cup contained a single fully developed bermudagrass plant with 9 to 12 leaves. Two μl of a 0.1% (v/v) surfactant solution was applied with a microsyringe to the upper surface of the fifth or sixth leaf from the top of the plant and allowed to dry. ^{14}C-glyphosate (specific activity, 1.87 mCi/mM) was then applied (0.15 μCi in 7.5 μl water) over the surfactant-treated leaf. Treated plants were maintained for 48 h at 22 or 32 C and 40 or 100% RH. Translocation of the ^{14}C of glyphosate was determined by autoradiographic techniques similar to those described by Crafts and Yamaguchi (3) and by liquid scintillation assaying. The ^{14}C-glyphosate remaining on leaf surface after treatment was washed from the detached leaf in 4 ml water. Each side of the leaf was rinsed in 1 ml water, and the wash and the rinse were combined. This process was repeated. A 200 μl aliquot of each wash was used for determining the amount of ^{14}C-glyphosate that remained on the leaf surface. The leaf sections and the wash aliquots were combusted and the $^{14}CO_2$ collected in 15 ml liquid scintillation fluid.

Counting data were corrected for background, quenching, and dilution factors. Quantitative data for ^{14}C are presented as the ^{14}C of glyphosate. All treatments were replicated four times, and the individual experiments were repeated. The data are expressed as percent dpm of a 7.5 μl ^{14}C-glyphosate standard.

Table 1. Bermudagrass response to over-the-top applications of glyphosate in greenhouse studies.

Rate of applications (kg/ha)	Injury ratings[a] 14 days (%)	Injury ratings[a] 21 days (%)
0.14	0 b[b]	11 e
0.28	3 b	29 d
0.56	8 b	53 c
0.84	67 a	69 b
1.12	73 a	91 a

[a]Injury ratings were based on 0 = no injury and 100 = complete kill.
[b]Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

RESULTS and DISCUSSION

Rate response to bermudagrass. Glyphosate toxicity to bermudagrass increased up to 91% with increasing herbicide rates (Table 1) as measured by visual ratings 21 days after treatment. Approximately 50% visible control was obtained with 0.56 kg/ha glyphosate. Thus, this rate was used for all environmental studies to determine how temperature and relative humidity altered the toxicity of glyphosate.

Effect of environment on glyphosate toxicity. Visible injury ratings of the toxicity study indicated that 0.56 kg/ha glyphosate was twice as toxic when bermudagrass plants were maintained at 100% RH than when they were maintained at 40% RH regardless of the temperature (Table 2). Visual ratings based on regrowth showed a more pronounced difference be-

Table 2. Bermudagrass response to 0.56 kg/ha glyphosate 8 weeks after treatment as affected by temperature and relative humidity.

Temperature (C)	Relative humidity (%)	Toxicity study[a] Visual rating[c] (%)	Toxicity study[a] Fresh wt (%)	Regrowth study[b] Visual rating[d] (%)	Regrowth study[b] Fresh wt (%)
22	40	43 b[e]	63 a	76 a	68 a
	100	95 a	13 c	4 b	2 c
32	40	51 b	48 b	80 a	52 b
	100	96 a	5 c	2 b	7 c

[a]Toxicity data based on plant response to 0.56 kg/ha glyphosate while under the appropriate environmental conditions for 6 days, then under greenhouse conditions for the remainder of 8 weeks.
[b]Regrowth data based on plant response to 0.56 kg/ha glyphosate while under the appropriate environmental conditions for 24 h, then all foliage clipped at the soil surface and the plants allowed to regrow under greenhouse conditions for the remainder of 8 weeks.
[c]Visual toxicity rating based on 0 = no injury; 100 = complete kill.
[d]Visual regrowth rating based on 0 = no regrowth; 100 = complete regrowth.
[e]Means within a column followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 3. Absorption and translocation of 0.15 μCi ^{14}C-glyphosate in bermudagrass after 48 h at 22 or 32 C and 40 or 100% relative humidity.

		Percent of applied ^{14}C activity (dpm) distribution		
Temperature (C)	Relative humidity (%)	Remaining in treated leaf (%)	Remaining on surface (%)	Translocated[a] (%)
22	40	90.8 a[b]	0.4 h	8.8g
	100	39.8 d	2.1 h	58.1 c
32	40	84.7 b	1.3 h	14.0 f
	100	29.4 e	8.7 g	61.9 c

[a]Individual values obtained by subtraction of the sum of the values in the two columns to the left from 100%.

[b]Means followed by the same letter do not differ significantly at the 5% level using Duncan's multiple range test.

tween relative humidity than did the toxicity ratings. Fresh wt measurements of the treated bermudagrass plants indicated that glyphosate toxicity was significantly greater at 32 C than 22 C at 40% RH, but was not different at 100% RH.

McWhorter and Jordan (7) and Prasad et al. (9) found that dalapon absorption and translocation into test species were greater at high (95 to 100%) RH than at low (25 to 35%) RH. As suggested by the results with dalapon, the effect of relative humidity on cuticle density and hydration may partially account for the increase in penetration and toxicity of glyphosate at higher relative humidity in the present study.

Effect of environment on ^{14}C-glyphosate movement. At both 22 and 32 C, translocation of the ^{14}C-label out of the treated leaf into the plant was five to six times greater at 100% RH than at 40% RH (Table 3). More than 90% of the applied herbicide remained on the leaf surface at 22 C and 40% RH; whereas, 29% of the total applied herbicide remained on the leaf surface at 32 C and 100% RH. More ^{14}C-glyphosate was absorbed at 32 C than at 22 C, for each of the relative humidities. Likewise, more ^{14}C of glyphosate was translocated at 32 C than at 22 C at 40% RH. However, there was no difference in the amount of translocation of the ^{14}C-label at 100% RH. McWhorter and Jordan (8) reported that ^{14}C-dalapon translocation was greater at 100% RH than at 35% RH at temperatures of 21 and 32 C. The results reported in this study agree with the ^{14}C-dalapon data. Even though slightly more ^{14}C-glyphosate absorption and translocation was obtained at 32 C than at 22 C for a given relative humidity, the greater environmental influence was relative humidity. Regardless of temperature, between two and three times more ^{14}C-glyphosate was absorbed at 100% RH than at 40% RH.

The difference in the degree of absorption and translocation of the ^{14}C of glyphosate at 32 C and 100% RH and that of 22 C and 40% RH is shown by autoradiograms of the treated plants (Figure 1). At 22 C and 40% RH, the ^{14}C of glyphosate moved from the treated leaf into the root system

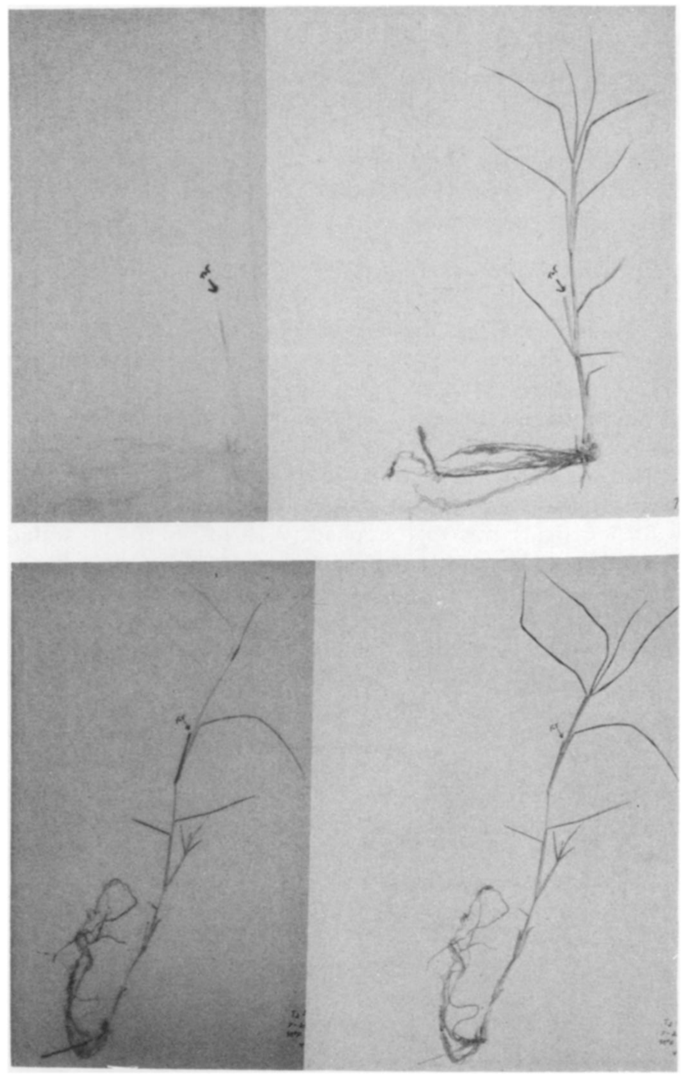

Figure 1. Translocation of ^{14}C-glyphosate in bermudagrass as affected by temperature and relative humidity. (top) Distribution of ^{14}C-glyphosate at 22 C and 40% RH. Autoradiograph left, plant right. (bottom) Distribution of ^{14}C-glyphosate at 32 C and 100% RH. Autoradiograph left, plant right.

of bermudagrass; whereas, at 32 C and 100% RH, the movement was into the entire plant system.

Sprankle et al. (11) reported that ^{14}C-glyphosate rapidly translocated into the rhizomes and untreated shoots of quackgrass [*Agropyron repens* (L.) Beauv.]. They also reported that for other annual and perennial species glyphosate moved to the area of highest metabolic activity. The autoradiograms obtained from treated bermudagrass in this study showed that the ^{14}C-label concentrated in developing rhizomes, stolons, and inflorescence (data not shown).

The results of these experiments indicate that glyphosate toxicity to bermudagrass is influenced by both temperature and relative humidity. Increasing relative humidity from 40 to

100% increased glyphosate absorption, translocation, and toxicity. Increasing the temperature from 22 to 32 C also increased glyphosate activity, but not to the extent to which relative humidity affected bermudagrass.

Bermudagrass is an increasing weed problem in crops throughout the southern United States. Glyphosate, when properly applied, can be used to control this weed. The level of bermudagrass control with glyphosate has at times been erratic. The results of this study can partially explain these erratic field results. Applying glyphosate at a time when the temperature and relative humidity favor increased translocation could greatly aid in controlling bermudagrass.

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