The *Journal of Cotton Science* 20:86–93 (2016)
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WEED SCIENCE

Glufosinate Rate and Timing for Control of Glyphosate-Resistant Rhizomatous Johnsongrass (*Sorghum halepense*) in Glufosinate-Resistant Cotton (*Gossypium hirsutum*)

Randall L. Landry, Daniel O. Stephenson, IV*, and Brandi C. Woolam

ABSTRACT

Johnsongrass was documented as resistant to glyphosate in 2010. Consequentially, strategies to mitigate and/or manage glyphosate-resistant (GR) johnsongrass in Louisiana cotton are needed. Field experiments were conducted in Louisiana from 2011 through 2013 to evaluate the number and timing of glufosinate applications for control of GR johnsongrass in glufosinate-resistant (GLR) cotton. Treatments included two or three glufosinate applications during the season with the initial application applied 2 or 4 wk after planting, and sequential applications applied 2 or 3 wk after the initial application. In treatments with two applications, 880 g ha^{-1} of glufosinate followed by (fb) 590 g ha^{-1} of glufosinate were applied. Glufosinate at 590 g ha^{-1} was sequentially applied for treatments with three applications. Following two applications, delaying the sequential application from 2 to 3 wk after the initial application reduced control from 95 to 85% 14 d after final glufosinate treatment (DAT), but no differences in control with sequential timings was observed following three applications. Similar results were observed 21 DAT, except with three applications separated by 3 wk, which controlled johnsongrass 97% compared to 86% when sequential applications were spaced 2 wk apart. In addition, three applications spaced 3 wk apart reduced johnsongrass heights to 22% of the nontreated. Cotton yield was increased following two applications that were initiated 4 wk after application (WAP) (1000 kg ha^{-1}) compared to 2 WAP (620 kg ha^{-1}). Data indicate three glufosinate applications separated by 3 wk will provide season-long GR johnsongrass control and maximize cotton yield in GLR cotton.

R.L. Landry, D.O. Stephenson, IV*, and B.C. Woolam. LSU AgCenter, Dean Lee Research and Extension Center, 8105 Tom Bowman Drive, Alexandria, LA 71302
*Corresponding author: dstephenson@agcenter.lsu.edu

Surveys conducted in Arkansas, Louisiana, and Mississippi during 1978 to 1991 indicated 55 to 90% of cotton (*Gossypium hirsutum* L.) fields were infested with johnsongrass [*Sorghum halepense* (L.) Pers.], which reduced the estimated value of harvested cotton $5.8 ±1.9 million (McWhorter, 1993). Johnsongrass can influence commodity yields negatively by competition, allelopathy, and hosting diseases or insects (Bendixon, 1986; Warwick and Black, 1983). Johnsongrass has an upright growth habit and its reproductive ability can make this perennial grass an issue for producers (Anderson et al., 1960; Holm et al., 1977; McWhorter, 1961). Johnsongrass can reach heights of 3.5 m and reproduce by both rhizomes and seeds (Holm et al., 1977; Ingle and Rogers, 1961; McWhorter, 1971a, b; Oyer et al., 1959). Anderson et al. (1960) and Talbot (1928) reported johnsongrass rhizomes produce new plants 1 yr after development.

Johnsongrass is native to the Mediterranean region, specifically the political boundary between Syria and Turkey (Holm et al., 1977; Spencer, 1974). Johnsongrass range has been expanded by environmental (e.g., animals, wind, water), human (e.g., sowing as a forage), and mechanical means (e.g., harvesters, cultivators). Current range of johnsongrass is from latitude 55° N to latitude 45° S, where it infests six continents, excluding Antarctica (Holm et al., 1977). Expansion to colder climates is limited because rhizomes are not tolerant to freezing temperatures (Warwick and Black, 1983). Information is limited on introduction into North America; however, McWhorter (1971a) reported johnsongrass was planted extensively as a forage plant in the 1830s. By 1975, johnsongrass was reported in 59 of the 64 parishes in Louisiana (Allen, 1975).

United States crop producers planted 6.0, 6.0, 4.2, and 4.6 million ha of cotton in 2011, 2012, 2013, and 2014, respectively (Anonymous, 2015a), indicating that it is an important crop in the U.S. It is also susceptible to early-season weed competition. Bridges and Chandler (1987) reported full-season competition of johnsongrass at densities of 1, 2, 4, 8, 16, and 32 plants 9.8 m^{-1} of row reduced average seed cotton yield 1, 4, 14, 40, 65, and 70%, respectively. Furthermore, Keeley and Thullen (1989) found that competition between johnsongrass and cotton for 6, 9, 12, and 25 wk after emergence reduced cotton yields 20, 60, 80, and 90%, respectively. In Oklahoma, johnsongrass reduced stripper-harvest efficiency in cotton 0.3 and 0.6% per weed in 15 m of row in 1996 and 1997, respectively (Wood et al., 2002).

Effective weed control in agronomic crops requires combinations of cultural, mechanical, and chemical strategies (Anderson, 1996; Nalewaja, 1999). Keeley and Thullen (1981) reported cultivation alone was not sufficient to prevent johnsongrass from impacting seed cotton yields. Crop rotations, such as a soybean-cotton or corn-cotton, in conjunction with high herbicide rates of acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicides and residual herbicides with different modes of action, controlled johnsongrass (Dale and Chandler, 1979; Frans et al., 1991). Introduction of ACCase- and acetolactate synthase (ALS)-inhibiting herbicides provided an effective postemergence (POST) option for control of johnsongrass in cotton and soybean [*Glycine max* (L.) Merr.] (Banks and Tripp, 1983; Tranel and Wright, 2002). Johnson et al. (1991) found that johnsongrass was controlled 70 to 90% when the ACCase-inhibiting herbicides clethodim, sethoxydim, fluazifop, haloxyfop, or quizalofop were applied POST as split applications in soybean. Likewise, Banks and Tripp (1983) reported similar results with sequential applications of ACCase-inhibiting herbicides at lower rates when compared to a single application at a higher rate. Unfortunately, clethodim- and fluazifop-resistant johnsongrass was documented in Louisiana in 1997 (Heap, 2015), but those populations have been effectively controlled by glyphosate.

Glyphosate applied POST-directed controlled johnsongrass in cotton (Banks and Santelmann, 1977). Glyphosate applied via a rope wick in cotton reduced johnsongrass shoots 78% compared to cultivation only (Keeley et al., 1984). Introduction of GR crops allowed POST broadcast applications of glyphosate to manage grass and broadleaf weeds without crop injury (Ateh and Harvey, 1999; Delannay et al., 1995; Nelson and Renner, 2013; Webster et al., 1999). Consequently, 82% of GR cotton in the U.S. was treated with glyphosate in 2005 (Xiu, 2012). However, excessive use of a single herbicidal site of action for weed control has been the likely cause of documented resistance to that site of action (Green and Owen, 2011). Excessive use of glyphosate in Argentina and in the U.S. in Arkansas, Louisiana, and Mississippi has led to the evolution of GR johnsongrass (Heap, 2015; Johnson et al., 2014a; Riar et al., 2011; Vila-Aiub et al., 2007). Currently, there are 15 GR species in the U.S. (Heap et al., 2015). With continued planting of GR cotton in the U.S. and increasing number of GR weeds, review of weed-control practices is needed to mitigate and manage GR weeds (Dill, 2005; Duke, 2005).

GLR cotton allows producers to apply glufosinate POST without injury to the cotton (Blair-Kerth et al., 2001). Glufosinate interrupts essential amino acid biosynthesis by inhibiting the glutamine synthetase enzyme, which is responsible for converting glutamate and ammonia to glutamine (Duke, 1990). This inhibition causes a buildup of ammonia in susceptible plants that rapidly destroys cells and tissue (Sauer et al., 1987; Tachibana et al., 1986). Glufosinate alone and in mixtures controls many grass and broadleaf weeds (Corbett et al., 2004; Everman et al., 2007; Tharp et al., 1999). In GLR soybean, sequential applications of 450, 590, or 790 g ha^{-1} of glufosinate provided 80, 95, or 97% GR johnsongrass control, respectively, 28 d after treatment (Johnson et al., 2014b). In GLR cotton, if more than 590 g ha^{-1} of glufosinate is applied in any single application, the season total of glufosinate applied may not exceed 1800 g ha^{-1}, but if a rate between 590 and 880 g ha^{-1} is applied as a single application, then the season total of glufosinate applied may not exceed 1500 g ha^{-1} (Anonymous, 2015b); therefore, the maximum amount of glufosinate allowed in-crop can influence application rates and the number of applications available to a cotton producer.

Johnsongrass populations resistant to both glyphosate and ACCase-inhibitors have not been discovered in Louisiana. Additionally, current utilization of only ACCase-inhibiting herbicides to control GR johnsongrass populations by Louisiana crop producers could potentially lead to populations with multiple resistance to glyphosate and ACCase-inhibitors. Therefore, alternative strategies need to be investigated for management of current GR johnsongrass populations and mitigation of potential multiple-resistant populations. Furthermore, glufosinate use restrictions might influence GR johnsongrass management decisions for GLR cotton producers. Therefore, the objective of this research was to evaluate number and timing of glufosinate applications for control of GR johnsongrass in GLR cotton.

MATERIALS AND METHODS

Field experiments were conducted 2011, 2012, and 2013 at Louisiana State University Agricultural Center, Dean Lee Research and Extension Center near Alexandria, LA. Soil was a Coushatta silt loam (fine-silty, mixed, superactive, thermic Fluventic Eutrudepts) with a pH of 8.0 and 1.5% organic matter. An augmented factorial arranged in a randomized, complete block design with four replications was used in all experiments. Factor one consisted of two or three glufosinate (Liberty 280 SL, Bayer CropScience LP, Triangle Park, NC) applications in the growing season. The second and third factors were initial glufosinate application timing of 2 or 4 wk after application (WAP) and sequential glufosinate application timing of 2 or 3 wk after the initial glufosinate, respectively. Based upon labeling (Anonymous, 2015b), glufosinate rates utilized in the initial applications were 880 or 590 g ha^{-1} for treatments prescribed to receive two or three glufosinate applications, respectively, and glufosinate at 590 g ha^{-1} was applied in all sequential applications. A nontreated control was included for comparison. Table 1 presents actual application timings. Table 2 provides cotton leaf number and height and johnsongrass leaf number, height, and density at each glufosinate application.

Plot size was 9-m long with four, 0.97-m rows. All treatments were applied with a tractor-mounted, compressed-air sprayer calibrated to deliver 187 L ha^{-1} at 145 kPa using TeeJet 11002DG, flat fan nozzles (Spraying Systems Co., Wheaton, IL). 'Phytogen 375 WRF', 'Stoneville 5445 LLB2', and 'Fibermax 1944 GLB2' cotton were seeded in 2011, 2012, and 2013, respectively. Cotton was seeded at 102, 800, 102,000, and 101,800 seeds ha^{-1} in 2011, 2012, and 2013, respectively. All studies were conducted using conventional-tillage methods and standard cotton production practices.

Cotton injury and johnsongrass control (0% no control or injury to 100% complete control or crop death) were assessed 14 and 21 d after final glufosinate application (DAT) and at harvest. Johnsongrass heights were determined by measuring five live plants per plot 28 d after the final application and at harvest. In all years, johnsongrass heights and densities in the nontreated plot averaged 117 and 143 cm and 190 and 200 shoots m^{-2} 28 DAT and at harvest, respectively (data not shown). Prior to analysis, johnsongrass heights were converted to percentage of the nontreated plants. Yield was determined by harvesting the center two rows of plots using conventional harvesting equipment. High johnsongrass densities in the nontreated plots prohibited machine harvest of these plots in all years, thus the nontreated yields were excluded from analysis. Seed cotton yields were adjusted to 40% lint turnout prior to analysis.

All data were subjected to analysis of variance using PROC GLIMMIX in SAS (release 9.3, SAS Institute, Cary, NC). Type III statistics were used to test all possible fixed effects (total glufosinate applications, initial and sequential glufosinate application timings) or interactions among the fixed effects. Random effects were years and replications nested within in years (Blouin et al., 2011). Considering year and replication an environmental or random effect permits inferences about treatments to be made over a range of environments (Blouin et al., 2011; Carmer et al., 1989). All data were subject to arcsine square-root transformation to test for normality (Ahrens et al., 1990), but nontransformed means are presented. Least square means were calculated and means were separated using Tukey's honest significant difference test at $p \leq 0.05$.

Table 1. Treatments evaluated in 2011, 2012, and 2013

Herbicide and total number of applications	Application rate, g ha^{-1}	Application timing, WAP[z]
glufosinate fb[z] glufosinate	880 fb 590	2 fb 4
glufosinate fb glufosinate	880 fb 590	2 fb 5
glufosinate fb glufosinate	880 fb 590	4 fb 6
glufosinate fb glufosinate	880 fb 590	4 fb 7
glufosinate fb glufosinate fb glufosinate	590 fb 590 fb 590	2 fb 4 fb 6
glufosinate fb glufosinate fb glufosinate	590 fb 590 fb 590	2 fb 5 fb 8
glufosinate fb glufosinate fb glufosinate	590 fb 590 fb 590	4 fb 6 fb 8
glufosinate fb glufosinate fb glufosinate	590 fb 590 fb 590	4 fb 7 fb 10

[z] Abbreviations: WAP, weeks after planting; fb, followed by.

Table 2. Cotton and johnsongrass leaf number, height, and density at each application timing averaged across years

Application timing	Cotton		Johnsongrass		
	Leaf no.[z]	Height, cm	Leaf no.[z]	Height, cm	Density, shoots/m^2
2 WAP[z]	1	3	4	24	38
4 WAP	4	13	5	45	140
5 WAP	5	16	5	36	172
6 WAP	8	33	4	47	27
7 WAP	9	44	4	44	43
8 WAP	12	60	4	78	40
10 WAP	15	70	6	63	54

[z] Abbreviations: no., number; WAP, weeks after planting.

RESULTS AND DISCUSSION

No cotton injury was observed following glufosinate applications (data not shown). Others have observed glufosinate injury on cotton cultivars containing the WideStrike® trait (Culpepper et al, 2009; Steckel et al., 2012), but no injury was observed on PHY 375 WRF in this experiment in 2011. WideStrike cultivars were injured 15 to 20% 5 d after glufosinate was applied at 430 to 860 g ha^{-1}, but cotton yield was not reduced (Culpepper et al, 2009; Steckel et al., 2012). Blair-Kerth et al. (2001) found GLR cultivars were not injured by glufosinate. Furthermore, Gardner et al. (2006) reported 2% or less injury on cotton following POST application of glufosinate and injury was not reported later in growing season.

An interaction of total glufosinate applications and sequential application timing was detected for johnsongrass control 14 and 21 DAT and johnsongrass height as a percentage of the nontreated 28 DAT (Table 3). The main effect of total glufosinate applications was significant for johnsongrass control and height as a percentage of the nontreated at cotton harvest.

No differences in johnsongrass control were observed between 2 and 4 WAP initial application timing, with control averaging 93, 85, and 78% 14 and 21 DAT and at harvest, respectively (data not shown). Similarly, the initial glufosinate application timing did not influence johnsongrass heights with heights ranging from 52 to 62% of the nontreated 28 DAT (data not shown). Following two glufosinate applications, johnsongrass control 14 DAT was decreased when the sequential glufosinate application was delayed from 2 (93%) to 3 (85%) wk after the initial application (Table 4). Following three glufosinate applications, sequential application timing did not influence johnsongrass control 14 DAT, with control ranging from 94 to 98%. Regardless of the total number of glufosinate applications, a sequential application 2 wk after the initial glufosinate application controlled johnsongrass 83 to 86% 21 DAT. However, three glufosinate applications applied sequentially 3 wk apart controlled johnsongrass 97% 21 DAT, whereas two total applications applied 3 wk apart provided only 71% control. Similar to control observations 21 DAT, johnsongrass heights were 22% of the nontreated following three total glufosinate applications applied 3 wk apart, but johnsongrass heights ranged from 57 to 82% of the nontreated for all other treatments 28 DAT. At harvest, glufosinate applied three times controlled johnsongrass 89%, whereas two applications provided 66% control with no effect of initial or sequential glufosinate application timings (data not shown). Likewise, glufosinate applied three times reduced johnsongrass height to 37% of the nontreated at harvest, but two applications reduced height to only 78% of the nontreated (data not shown). These data indicate that three total glufosinate applications applied sequentially 3 wk apart provides the greatest johnsongrass control and height reduction.

Total glufosinate applications and initial application timing interaction was significant for cotton yield (Table 3). Regardless of the initial glufosinate application timing, cotton yield following three glufosinate applications ranged from 1060 to 1100 kg ha^{-1} when the initial treatment was applied 2 or 4 WAP, and cotton yield was similar following two applications with the initial application 4 WAP (Table 5). Cotton yield was 620 kg ha^{-1} following two applications where the initial application was at 2 WAP (Table 5). Although johnsongrass control and height as a percentage of the nontreated and cotton yield were influenced by initial and sequential application timing in these experiments, all parameters were maximized following three applications of glufosinate.

Table 3. Significance of the main effects of total glufosinate applications, initial glufosinate application timing, and sequential glufosinate application timing and interactions among main effects pooled across environments for johnsongrass control and height at each evaluation date and cotton yield[z,y]

Variable	Johnsongrass control			Johnsongrass heights		Cotton yield
	14 DAT	21 DAT	Harvest	28 DAT	Harvest	
	p-value					
TAPP	0.0009	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
IAPP	0.4615	0.2964	0.6105	0.1473	0.6277	0.0094
SAPP	0.2661	0.8321	0.6882	0.2522	0.4056	0.9303
TAPP x IAPP	0.4612	0.3937	0.2906	0.3364	0.3794	0.0019
TAPP x SAPP	0.0017	< 0.0001	0.2102	0.0011	0.1798	0.2379
IAPP x SAPP	0.0687	0.5591	0.7557	0.1463	0.2557	0.7411
TAPP x IAPP x SAPP	0.5970	0.3691	0.3202	0.4561	0.3133	0.7355

[z] Abbreviations: IAPP, initial glufosinate application timing; DAT, days after treatment; SAPP, sequential glufosinate application timing; TAPP, total glufosinate applications.

[y] Main effects and interactions considered significant for Type III error if $p \leq 0.05$.

Table 4. Johnsongrass control 14 and 21 d after treatment and johnsongrass heights as a percentage of the nontreated 28 DAT as influenced by number of glufosinate applications and sequential glufosinate application timing

Number of glufosinate applications	Sequential glufosinate application timing					
	Johnsongrass control				Johnsongrass height	
	14 DAT[Z]		21 DAT[Z]		28 DAT[Z]	
	2 wk after initial application	3 wk after initial application	2 wk after initial application	3 wk after initial application	2 wk after initial application	3 wk after initial application
	%					
Two	93 a[y]	85 b	83 b	71 c	67 a	82 a
Three	94 a	98 a	86 b	97 a	57 a	22 b

[z] Abbreviation: DAT, days after treatment.

[y] Data pooled over initial glufosinate application timing. Means followed by the same letter within each evaluation date are not significantly different based on Tukey's honest significant difference test at $p \leq 0.05$.

Table 5. Cotton lint yield as influenced by number of glufosinate applications and initial glufosinate application timing[z]

Number of glufosinate applications	Initial glufosinate application timing	
	2 wk after planting	4 wk after planting
	kg ha^{-1}	
Two	620 b	1000 a
Three	1100 a	1060 a

[z] Data pooled over sequential glufosinate application timing. Means followed by the same letter are not significantly different based on Tukey's honest significant difference test at $p \leq 0.05$.

Steckel et al. (1997) observed that efficacy of glufosinate on several annual weed species was influenced by weed height and glufosinate rate at application. A single application of glufosinate at 450, 590, or 740 g ha^{-1} controlled johnsongrass 65 to 96% 14 DAT with control inversely related to johnsongrass heights of 15, 30, 45, and 60 cm at time of application (Johnson and Norsworthy, 2014). Furthermore, glufosinate applied sequentially at 450, 590, or 740 g ha^{-1} to 15- to 25-cm johnsongrass 3 wk after soybean emergence fb another application 3 wk later to 45- to 70-cm johnsongrass provided 80, 95, and 97% control, respectively , 28 DAT (Johnson et al., 2014b). Results of Johnson and Norsworthy (2014) and Johnson et al. (2014b) supported the findings of Steckel et al. (1997) that glufosinate efficacy is influenced by weed height and rate at time of application. However, johnsongrass leaf number and height at time of each application in our research (Table 2) do not suggest that either of these influenced control or cotton yield. Reductions in johnsongrass density might be the reason for control observations in these experiments.

The purpose of these experiments was to determine if glufosinate application rates and timings specified by the glufosinate label (Anonymous 2015b) would provide season-long control of GR johnsongrass, not to determine glufosinate efficacy based upon johnsongrass growth variables. These data indicate that three applications spaced 3 wk apart is a good option for management of GR johnsongrass in GLR cotton. Although data show that glufosinate will control GR johnsongrass, utilization of only one herbicidal site of action (glufosinate in this case) for weed management is not proper implementation of a herbicide-resistance management program. Therefore, we suggest the use of glufosinate as part of a herbicide-resistance management program that contains ACCase-inhibiting herbicides, MSMA, and residual herbicides such as fluometuron applied PRE, early- and mid-POST, and as a directed-POST application in GLR cotton to manage GR johnsongrass and, potentially, other problematic weeds.

ACKNOWLEDGEMENTS

Thanks to the staff at the LSU AgCenter Dean Lee Research and Extension Center for help in crop management.

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