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WEED SCIENCE

Sequential and Co-Application of Glyphosate and Glufosinate in Cotton

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ABSTRACT

Glufosinate controls GR Palmer amaranth (*Amaranthus palmeri* S. Wats.), but might be less effective than glyphosate on certain weeds. Glyphosate and glufosinate applications in tolerant cotton (*Gossypium hirsutum* L.) cultivars can broaden the spectrum of control and aid in resistance management. Research investigating sequential application and potential interactions with co-applications of glyphosate and glufosinate is limited. Field research at six locations evaluated Palmer amaranth and annual grass control with glyphosate and glufosinate co-applied or sequentially applied. Herbicides were applied to two-leaf cotton and repeated 14 d later. A GR biotype comprised 10 to 90% of the Palmer amaranth populations. Greater Palmer amaranth control was achieved following sequential applications of glufosinate compared with glyphosate. Co-application of glufosinate plus glyphosate was more effective than glyphosate alone at most locations but never more effective than glufosinate alone. Glyphosate controlled goosegrass (*Eleusine indica* [L.] Gaertn.) more than glufosinate and a similar response was observed for large crabgrass (*Digitaria sanguinalis* [L.] Scop.). Glufosinate and glyphosate co-applied were less effective than glyphosate alone on both grasses, but not more effective than glufosinate alone. Glufosinate followed by (fb) glyphosate was the preferred order for sequential application to control Palmer amaranth at most locations. With high percentages of the GR biotype, glufosinate applied sequentially was more effective than any other sequential applications. Glyphosate fb glufosinate and glufosinate fb glyphosate controlled large crabgrass similar to glyphosate applied sequentially and greater than glufosinate applied sequentially. For goosegrass, glyphosate fb glufosinate was more effective than the reverse. Seven days after the second postemergence application, sequential application of glyphosate fb glufosinate controlled goosegrass as well as glyphosate applied sequentially and better than glufosinate applied sequentially.

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is one of the most common and problematic weeds in cotton and other agronomic crops in the southern U.S. (Webster, 2013). The biology of this weed, its impact on cotton yield, and the difficulty of control in cotton were reviewed by Culpepper et al. (2010). Palmer amaranth has a high photosynthetic capacity, giving the weed the ability to grow rapidly (Ehleringer, 1983). Although crops suffer during dry conditions, Palmer amaranth has effective drought tolerance mechanisms allowing it to survive and grow (Ehleringer, 1983; Place et al., 2008; Wright et al., 1999). Palmer amaranth has been reported to reduce cotton yield up to 92% with eight weeds m^{-1} of row and interfere with mechanical harvest (MacRae et al., 2008; Morgan et al., 2001; Rowland et al., 1999; Smith et al., 2000). Prior to commercialization of herbicide-resistant cotton, few postemergence (POST) herbicides were available to control Palmer amaranth; thus, effective control required multiple applications of preplant, preemergence (PRE), and POST-directed herbicides (Wilcut et al., 1995). Cultivation usually supplemented chemical control. These programs were effective if PRE herbicides received timely rainfall for activation and POST-directed herbicides were applied to small weeds (Culpepper and York, 1997). However, the height differential necessary for POST-directed application was difficult to achieve due to rapid growth of Palmer amaranth.

Glyphosate-resistant (GR) cotton was commercialized in 1997, allowing growers to effectively and conveniently control Palmer amaranth with glyphosate (Culpepper and York, 1998, 1999; Scott et al.,

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2002). However, with widespread planting of GR crops and extensive reliance on glyphosate, resistant biotypes evolved. Resistance to glyphosate has been confirmed in 31 weed species (Heap, 2014). The first confirmation of resistance to glyphosate in an *Amaranthus* species occurred with Palmer amaranth in Georgia in 2005 (Culpepper et al., 2006). By the end of 2014, GR Palmer amaranth had been confirmed in 24 states (Heap, 2014). Palmer amaranth resistant to acetolactate synthase (ALS)-inhibiting herbicides is also prevalent, and multiple resistance to both glyphosate and ALS-inhibiting herbicides is common (Heap, 2014; Sosnoskie et al., 2011). In North Carolina, 95% of the Palmer amaranth populations contain at least some individuals resistant to both glyphosate and ALS-inhibiting herbicides (Poirier et al., 2014).

Herbicides for POST application in cotton to control biotypes of Palmer amaranth with multiple resistance are limited (Culpepper et al., 2006). However, glufosinate controls GR Palmer amaranth (Culpepper et al., 2006; Hoffner et al., 2012; Norsworthy et al., 2008; Whitaker et al., 2011), giving growers an option to control this weed in glufosinate-tolerant cotton (Barnett et al., 2013; Culpepper et al., 2009; Gardner et al., 2006; Whitaker et al., 2011). In addition to Palmer amaranth, glufosinate also controls a number of other weed species (Anonymous, 2014). However, glufosinate is less effective than glyphosate on grasses, sedges, and certain broadleaf species (Corbett et al., 2004; Hoss et al., 2003; Koger et al., 2007; Steckel et al., 1997; Whitaker et al., 2011; Wiesbrook et al., 2001). Application of both glyphosate and glufosinate to the same crop would broaden the spectrum of control due to glyphosate and glufosinate having different modes of action (Devine et al., 1993; Steirrucken and Amrhein, 1980). Use of both herbicides in the same crop also could be beneficial for resistance management (Diggle et al., 2003; Gressel and Segel, 1990; Norsworthy et al., 2012; Wrubel and Gressel, 1994).

FiberMax and Stoneville cotton cultivars with both GlyTol® and LibertyLink® traits and Phytogen cultivars with the WideStrike® trait are commercially available and allow topical application of both glyphosate and glufosinate. These cultivars were planted on 70 to 75% of the hectares in Arkansas, Mississippi, North Carolina, South Carolina, and Tennessee; 48 to 61% of the hectares in Alabama, Louisiana, and Missouri; and 35% of the hectares in Georgia in 2013 (USDA-AMS, 2014). The increasing problem with GR Palmer amaranth has been a major reason for the transition to these cultivars.

Tolerance to glyphosate in GlyTol LibertyLink cultivars is due to insertion of the *2mepsps* gene (USDA-APHIS, 2008). The 2mepsps protein has decreased binding affinity for glyphosate and continues to function in the presence of the herbicide. Tolerance to glufosinate is due to insertion of the bialaphos resistance (*bar*) gene (USDA-APHIS, 2003), which encodes for phosphinothricin acetyltransferase, an enzyme that catalyzes the conversion of lethal L-phosphinothricin into nonlethal *N*-acetyl-L-phosphinothricin (Devine et al., 1993; Hinchee et al., 1993). Cotton with the WideStrike trait expresses two delta-endotoxins, which confer resistance to lepidopteran pests. This cotton was produced by cross breeding a transgenic line that produces the insecticidal protein Cry1Ac and a transgenic line that produces the insecticidal protein Cry1F (USEPA-OPP, 2005). Both of these events contain the phosphinothricin acetyltransferase (*pat*) gene, which was inserted for use as a selectable marker during plant transformation. The *pat* gene also confers resistance to glufosinate, although expression of the gene is incomplete and injury usually occurs when glufosinate is applied topically to WideStrike cotton (Culpepper et al., 2009; Steckel et al., 2012; Whitaker et al., 2011). Injury appears as necrosis on exposed cotton leaves shortly following application. The cotton typically recovers and yield is unaffected (Culpepper at al., 2009; Sweeney and Jones, 2015; Whitaker et al., 2011; Wright et al., 2014) although exceptions have occurred, especially with three applications per season, applications to larger cotton (Barnett et al., 2015), or applications to cotton stressed by early season insects (Stewart et al., 2013). The WideStrike trait is typically stacked with the Roundup Ready® Flex trait, which imparts tolerance of glyphosate.

Cultivars with tolerance to both glyphosate and glufosinate offer growers increased flexibility in managing GR Palmer amaranth and other weeds and also can be useful in management programs to reduce selection pressure for further resistance. However, research on the order of sequential application and potential interactions with co-applications of glyphosate and glufosinate is limited. The objective of this study was to determine cotton response and Palmer amaranth and annual grass control following glyphosate and glufosinate co-applied or applied sequentially.

MATERIALS AND METHODS

The experiment was conducted at six sites in North Carolina during 2011 and 2012. Sites included the Upper Coastal Plain Research Station near Rocky Mount, private farms near Micro and Mount Olive, and three separate fields at the Central Crops Research Station near Clayton. Soils at each site are described in Table 1. All sites were naturally infested with Palmer amaranth (Table 2). In addition, four and three sites were also infested with goosegrass and large crabgrass, respectively.

Cotton cultivar PHY 375 WRF (Dow AgroSciences, Indianapolis, IN) was planted on 12 May 2011 at both Clayton sites and 6 May 2011 at Rocky Mount. Cotton cultivar PHY 499 WRF (Dow AgroSciences, Indianapolis, IN) was planted 2 and 22 May 2012, at Clayton and Micro, respectively. Cotton cultivar FM 1944GLB2 (Bayer CropScience, Research Triangle Park, NC) was planted 7 May 2012 at Mount Olive. Cotton was planted in conventionally prepared seedbeds at all locations except Rocky Mount, which was in a strip-tillage system (Meijer and Edmisten, 2014). The potassium salt of glyphosate (Roundup PowerMax® Herbicide, Monsanto, St. Louis, Missouri) at 868 g ae ha^{-1} and paraquat (Parazone® 3SL Herbicide, Makhteshim Agan of North America, Raleigh, NC) at 561 g ai ha^{-1} were applied 24 and 0 d before planting, respectively, at Rocky Mount to control winter vegetation and emerged summer annual weeds. Cotton was planted in 91-cm rows at Rocky Mount and 97-cm rows at other locations. Plots were 4 rows by 9 m. The experimental design was a randomized complete block with treatments replicated four times.

Treatments consisted of glyphosate and the ammonium salt of glufosinate (Liberty® 280 SL Herbicide, Bayer CropScience, Research Triangle Park, NC) applied sequentially or co-applied. Glyphosate and glufosinate rates were based upon the manufacturers' suggested use rates (1X rates) of 868 and 543 g ae ha^{-1}, respectively. Sequential applications included glyphosate applied to two-leaf cotton 18 to 22 d after planting (POST-1) followed by (fb) glufosinate applied 14 d later to six-leaf cotton (POST-2) and glufosinate applied POST-1 fb glyphosate applied POST-2. Co-applications included mixtures of glyphosate plus glufosinate at 1X plus 1/2X, 1/2X plus 1X, and 1X plus 1X rates applied POST-1 and repeated

Table 1. Description of soils at experiment sites.

Site	Year	Soil series[z]	Soil texture	Soil pH	Soil humic matter[y] %
Clayton 1	2011	Norfolk	Loamy sand	5.4	0.60
Clayton 2	2011	Lynchburg	Sandy loam	5.4	1.08
Rocky Mount	2011	Aycock	Sandy loam	5.5	0.41
Clayton 3	2012	Norfolk	Loamy sand	5.2	0.46
Micro	2012	Faceville	Sandy loam	5.4	0.56
Mount Olive	2012	Wagram	Loamy sand	5.2	0.30

[z] Norfolk, fine-loamy, kaolinitic, thermic, Typic Knadiudults; Lynchburg, fine-loamy, siliceous, thermic Aeric Paleaquults; Aycock, fine-silty, siliceous, subactive, thermic Typic Paleudults; Faceville, fine, kaolinitic, thermic Typic Knadiudults; Wagram, loamy, kaolinitic, thermic Arenic Kandiudults.

[y] Soils characterized by the Agronomic Services Division of the North Carolina Department of Agriculture and Consumer Services. Soil humic matter determined according to Mehlich (1984).

Table 2. Weed species and density at experiment sites[z].

Weed species	Weed density					
	2011			2012		
	Clayton 1	Clayton 2	Rocky Mount	Clayton 3	Micro	Mount Olive
	------ no. m^{-2} ------					
Goosegrass	25	24	5	32	0	0
Large crabgrass	12	0	40	18	0	0
Palmer amaranth	5	> 100	12	74	> 100	> 100

[z] Weed density in nontreated control determined at second POST application.

at POST-2. For comparison, glyphosate and glufosinate were applied sequentially at 1X rates at the POST-1 and POST-2 application timings. A nontreated control was included for comparison. Excluding the nontreated control, all plots received a directed layby application of diuron (Direx®, Makhteshim Agan of North America, Raleigh, NC) at 840 g ai ha^{-1} plus MSMA (MSMA 6 Plus, Drexel Chemical Co., Memphis, TN) at 2240 g ai ha^{-1} plus non-ionic surfactant (Induce®, Helena Chemical Co., Collierville, TN) at 0.25% (v/v) 2 to 3 wk after POST-2 when cotton had 12 nodes and was 45 to 55 cm tall. Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with flat-fan nozzles (DG11002 TeeJet® Drift Guard flat-spray nozzles, TeeJet Technologies, Wheaton, IL) set to deliver 140 L ha^{-1} at 165 kPa for the POST-1 and POST-2 applications. The layby application was applied with a single flood nozzle (TK-VS2 FloodJet® wide angle flat-spray nozzle, TeeJet Technologies, Wheaton, IL) per row middle set to deliver 140 L ha^{-1} at 210 kPa.

Percent weed control and cotton injury were estimated visually using a scale of 0 to 100, where 0 = no weed control or cotton injury and 100 = complete weed control or cotton death (Frans et al., 1986) 7 d after POST-1 (DAP1), 7 d after POST-2 (DAP2), 7 d after layby, and late in the season. Cotton in herbicide-treated plots was mechanically harvested in mid-October to mid-November using a spindle picker modified for small-plot harvesting. Cotton in nontreated controls could not be harvested due to the severe weed infestations. Data for cotton injury, weed control, and seed cotton yield were subjected to analysis of variance using the PROC MIXED procedure of Statistical Analysis Systems (SAS) (version 9.2; SAS Institute Inc., Cary, NC). Visual estimates of weed control and cotton injury were arcsine square-root transformed before analysis (Grafen and Hails, 2002). Non-transformed data are presented, with means separation based upon analysis of transformed data. Data for nontreated controls were not included in analyses. Herbicide treatments and location were fixed factors, whereas replications were treated as random. Means were separated using Fisher's Protected LSD at $p = 0.05$.

RESULTS AND DISCUSSION

Site/year combinations (environments) by herbicide treatment interactions were significant for each response recorded, hence data were analysed by location.

Palmer amaranth control. Glyphosate applied alone controlled Palmer amaranth 94 to 97% and 85 to 91% at 7 DAP1 and 7 DAP2, respectively, at Clayton 1, Clayton 2, Rocky Mount, and Mount Olive (Tables 3 and 4). The few plants at these locations that survived sequential glyphosate applications were assumed to be a GR biotype. A larger percentage of the population was GR at Clayton 3, where glyphosate alone controlled Palmer amaranth only 75 and 64% at 7 DAP1 and 7 DAP2, respectively. Almost all plants at Micro were a GR biotype; glyphosate controlled Palmer amaranth ≤ 10% at Micro.

Table 3. Palmer amaranth control 7 d after first postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]	Palmer amaranth control					
	2011			2012		
	Clayton 1	Clayton 2	Rocky Mount	Clayton 3	Micro	Mount Olive
	%					
Glyphosate (1X)	**97 a**	**94 c**	**94 a**	**75 b**	**10 c**	**95 a**
Glufosinate (1X)	**97 a**	**97 b**	**94 a**	**96 a**	**93 a**	**99 a**
Glyphosate (1X) + glufosinate (1/2X)	**96 a**	**97 b**	**86 b**	**89 a**	**73 b**	**97 a**
Glyphosate (1/2X) + glufosinate (1X)	**97 a**	**97 b**	**95 a**	**93 a**	**94 a**	**98 a**
Glyphosate (1X) + glufosinate (1X)	**97 a**	**99 a**	**92 a**	**94 a**	**94 a**	**100 a**

[z] **Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.**

[y] **Herbicides were applied to 2-leaf cotton 18 to 22 d after planting. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively.**

Table 4. Palmer amaranth control 7 d after the second postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]		Palmer amaranth control					
		2011			2012		
POST-1	POST-2	Clayton 1	Clayton 2	Rocky Mount	Clayton 3	Micro	Mount Olive
		%					
Glyphosate (1X)	Glyphosate(1X)	91 b	85 d	87 cd	64 c	8 c	87 b
Glyphosate (1X)	Glufosinate (1X)	100 a	97 ab	91 abc	88 b	12 c	99 a
Glufosinate (1X)	Glufosinate (1X)	99 a	98 ab	92 ab	99 a	93 a	96 ab
Glufosinate (1X)	Glyphosate (1X)	99 a	93 c	93 a	96 ab	73 b	96 a
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	98 a	97 bc	80 e	89 b	76 b	95 ab
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	100 a	99 a	88 bcd	100 a	97 a	99 a
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	100 a	100 a	86 de	99 a	97 a	100 a

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively.

No differences in Palmer amaranth control among treatments were noted 7 DAP1 at Clayton 1 and Mount Olive, with control ranging from 96 to 97% and 95 to 100%, respectively (Table 3). Co-applied glyphosate 1X plus glufosinate were 3 to 5% and 14 to 19% more effective than glyphosate alone at Clayton 2 and Clayton 3, respectively. Glufosinate 1/2X co-applied with glyphosate POST-1 reduced control 8% at Rocky Mount, but all other treatments provided at least 92% Palmer amaranth control 7 DAP1 (Table 3). At Micro, glyphosate alone controlled Palmer amaranth only 10%, but glufosinate at the 1/2X and 1X rates co-applied with glyphosate increased control to 73 and 94%, respectively, 7 DAP1. At all locations, Palmer amaranth control following co-applications of glyphosate at 1/2X or 1X with glufosinate 1X was similar to glufosinate alone 7 DAP1 (Table 3).

Palmer amaranth was controlled 92 to 99% 7 DAP2 by sequential glufosinate applications POST-1 fb POST-2 (Table 4). Glufosinate applied sequentially (92 to 99%) was more effective than glyphosate applied sequentially (8 to 91%) at all locations except Mount Olive, and a similar trend was noted at Mount Olive. Compared to glyphosate alone POST-1 fb POST-2, sequential applications of glufosinate 1/2X co-applied with glyphosate increased Palmer amaranth control 7, 12, 25, and 68% at Clayton 1, Clayton 2, Clayton 3, and Micro, respectively. However, sequential applications of glufosinate at the 1X rate co-applied with glyphosate was needed to increase control at Mount Olive 7 DAP2 (Table 4). The only negative response from co-applying glufosinate and glyphosate occurred at Rocky Mount, where sequential applications of glufosinate 1/2X plus glyphosate controlled Palmer amaranth 7% less than sequential glyphosate applications, and, in contrast to all other locations, glufosinate 1X co-applied with glyphosate applied sequentially did not improve control at Rocky Mount. At Rocky Mount, sequential applications of glyphosate 1X plus glufosinate 1X reduced control 6% compared with glufosinate applied alone sequentially. Whitaker et al. (2011) observed equivalent Palmer amaranth control with co-applied glufosinate and glyphosate relative to glufosinate applied alone. However, Reed et al. (2014) noted the addition of glyphosate to glufosinate reduced Palmer amaranth control 20 to 25% compared to glufosinate alone in 1 of 2 yr.

Results following sequential applications of glyphosate and glufosinate varied by location. Regardless of order of application, Palmer amaranth control 7 DAP2 following sequential applications of glyphosate and glufosinate (88 to 100%) was greater than glyphosate applied POST-1 fb POST-2 (64 to 91%) at Clayton 1, Clayton 2, Clayton 3, and Mount Olive (Table 4). At Rocky Mount and Micro, similar control was obtained with glyphosate POST-1 fb glufosinate POST-2 and glyphosate applied sequentially. However, glufosinate POST-1 fb glyphosate POST-2 was 6 and 65% more effective than glyphosate applied sequentially at Rocky Mount and Micro, respectively. At Clayton 1, Rocky Mount, and Mount Olive, glyphosate and glufosinate applied sequentially (91 to 100%) were as good as, but never better, than glufosinate applied sequentially (92 to 99%). Glyphosate POST-1 fb glufosinate POST-2 and glufosinate applied sequentially were similar at Clayton 1, Clayton 2, Rocky Mount, and Mount Olive. In contrast, sequential applications of glyphosate fb glufosinate controlled Palmer amaranth 11 and 81% less than glufosinate applied sequentially at Clayton 3 and Micro, respectively. Clayton 3 and Micro were the locations with a larger percentage of the GR Palmer amaranth biotype.

Glufosinate controls Palmer amaranth only if applied when the plant is small ($\leq$10 cm) (Barnett et al., 2013; Corbett et al., 2004; Culpepper et al., 2009; Gardner et al., 2006). Following poor control of Palmer amaranth by glyphosate POST-1, Palmer amaranth plant heights reached 20 to 30 cm, rendering glufosinate applied POST-2 ineffective. Similar control was obtained by glufosinate POST-1 fb glyphosate POST-2 and glufosinate applied sequentially at Clayton 1, Rocky Mount, Clayton 3, and Mount Olive. Sequential application of glufosinate POST-1 fb glyphosate POST-2 was 5 and 20% less effective than glufosinate applied sequentially at Clayton 2 and Micro. Both of these locations had more than 100 Palmer amaranth plants m^{-2} with a portion resistant to glyphosate and Palmer amaranth emerged several times during the season. Therefore, a second application of glufosinate was more beneficial than glyphosate applied POST-2 at these locations.

Trends in Palmer amaranth control 7 d after layby were generally similar to those observed 7 DAP2. No differences in control were noted among treatments at Rocky Mount (Table 5). Treatments containing glufosinate provided greater Palmer amaranth control than sequential applications of glyphosate at all locations except Mount Olive and Rocky Mount. At Mount Olive, no treatment was less effective than glyphosate applied sequentially (Table 5). Glyphosate and glufosinate co-applied or sequentially applied were more effective than glyphosate alone at Mount Olive. Glyphosate and glufosinate applied sequentially, regardless of order of application, were as effective as glufosinate applied sequentially at all locations except Micro. At Micro, glufosinate POST-1 fb glyphosate POST-2 controlled Palmer amaranth 17% less than glufosinate applied sequentially, but control was 57% greater than glyphosate POST-1 fb glufosinate POST-2. Co-application of glufosinate 1X plus glyphosate 1/2X or 1X was also as effective as glufosinate applied sequentially.

Table 5. Palmer amaranth control by glyphosate, glufosinate, and co-applications of glyphosate and glufosinate 7 d after layby application[z].

Postemergence herbicides[y]		Palmer amaranth control					
		2011			2012		
POST-1	POST-2	Clayton 1	Clayton 2	Rocky Mount	Clayton 3	Micro	Mount Olive
		%					
Glyphosate (1X)	Glyphosate(1X)	93 b	86 d	82 a	62 b	5 d	84 c
Glyphosate (1X)	Glufosinate (1X)	100 a	99 a	87 a	90 a	19 c	96 a
Glufosinate (1X)	Glufosinate (1X)	100 a	97 bc	88 a	97 a	93 a	87 bc
Glufosinate (1X)	Glyphosate (1X)	100 a	93 c	87 a	97 a	76 b	92 ab
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	100 a	98 ab	78 a	88 a	80 b	95 ab
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	100 a	100 a	88 a	98 a	97 a	92 ab
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	100 a	100 a	83 a	94 a	95 a	94 a

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively. All treatments received a postemergence-directed layby application of diuron plus MSMA at 841 plus 2240 g ha^{-1}, respectively, 2 to 3 wk after POST-2 application.

Annual grass control. Goosegrass was present at Rocky Mount and at each of the Clayton locations. Glyphosate controlled this weed 98 to 100% 7 DAP1 and was 10 to 19% more effective than glufosinate (Table 6). Others have reported that glyphosate is more effective than glufosinate on goosegrass (Corbett et al., 2004; Culpepper et al., 2000). Glyphosate co-applied with glufosinate and glufosinate applied alone were similarly effective at all locations except Clayton 2. At this location, compared to glufosinate alone, glyphosate 1X plus glufosinate 1/2X controlled goosegrass slightly greater. However, glyphosate 1X plus glufosinate 1X was no better than glufosinate applied sequentially. Additionally, co-applied glufosinate and glyphosate controlled goosegrass less than glyphosate applied alone except for glyphosate 1X plus glufosinate 1X at Clayton 3. Glufosinate antagonism of glyphosate has been reported on goosegrass, giant foxtail (*Setaria faberi* Herrm.), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* [Lam.] Husnot.), rye (*Secale cereal* L.), and wheat (*Triticum aestivum* L.) (Bethke et al., 2013; Chahal et al., 2012; Chuah et al. 2008).

Glyphosate applied POST-1 and POST-2 controlled goosegrass 98 to 100% compared with 87 to 88% control by glufosinate applied sequentially 7 DAP2 (Table 7). Similar control at all locations was noted with glyphosate applied sequentially (98 to 100%) and glyphosate fb glufosinate (100%). Glufosinate fb glyphosate (93 to 99%) controlled goosegrass similar to glyphosate applied sequentially (98 to 100%) at Clayton 2, Clayton 3, and Rocky Mount, but 5% less than two applications of glyphosate at Clayton 1. Glyphosate POST-1 fb glufosinate POST-2 controlled goosegrass 100% and was more effective than glufosinate applied sequentially (87 to 88%) at all locations. Glufosinate POST-1 fb glyphosate POST-2 (95 to 99%) was more effective than glufosinate applied sequentially (87 to 88%) at the Clayton locations. Compared to glyphosate alone, goosegrass control was reduced 5 to 11% and 12 to 20% by co-application of glyphosate and glufosinate at Clayton 1 and Rocky Mount, respectively. At Clayton 2, glufosinate 1X plus glyphosate 1/2X or 1X was less effective than glyphosate alone. Compared to glyphosate alone, co-applied glyphosate and glufosinate reduced goosegrass control 13 to 22% at Rocky Mount, except for glyphosate 1X plus glufosinate 1X. Glyphosate at 1/2X co-applied with glufosinate did not improve control compared to glufosinate alone. However, glyphosate at 1X co-applied with glufosinate increased control at two of four locations.

Diuron plus MSMA applied layby masked some of the differences in goosegrass control noted 7 DAP2 (Table 8). At 7 d after layby, all treatments at Clayton 1 and Clayton 2 controlled goosegrass 97 to 100%. At Clayton 3, sequential applications of glyphosate fb diuron plus MSMA controlled goosegrass 95% compared with 80 to 87% control by all other treatments. At Rocky Mount, sequential applications of glyphosate or glufosinate , and glufosinate fb glyphosate controlled goosegrass 93 to 98%. Other treatments controlled goosegrass 86 to 89%. Similar trends were noted for goosegrass control late in the season (data not shown).

Table 6. Goosegrass control 7 d after first postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]	Goosegrass control			
	2011			2012
	Clayton 1	Clayton 2	Rocky Mount	Clayton 3
	%			
Glyphosate (1X)	99 a	100 a	99 a	98 a
Glufosinate (1X)	80 b	87 c	89 b	84 b
Glyphosate (1X) + glufosinate (1/2X)	85 b	93 b	85 b	84 b
Glyphosate (1/2X) + glufosinate (1X)	82 b	88 bc	86 b	85 b
Glyphosate (1X) + glufosinate (1X)	86 b	89 bc	87 b	91 ab

[z] **Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.**

[y] **Herbicides were applied to 2-leaf cotton 18 to 22 d after planting. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively.**

Table 7. Goosegrass control 7 d after the second postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]		Goosegrass control			
		2011			2012
POST-1	POST-2	Clayton 1	Clayton 2	Rocky Mount	Clayton 3
		%			
Glyphosate (1X)	Glyphosate(1X)	100 a	98 ab	100 a	100 a
Glyphosate (1X)	Glufosinate (1X)	100 a	100 a	100 a	100 a
Glufosinate (1X)	Glufosinate (1X)	88 c	88 c	88 bc	87 b
Glufosinate (1X)	Glyphosate (1X)	95 b	99 a	93 ab	98 a
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	91 c	93 bc	80 c	78 b
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	89 c	89 c	88 bc	87 b
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	95 b	88 c	80 c	98 a

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively.

Table 8. Goosegrass control by glyphosate, glufosinate, and co-applications of glyphosate and glufosinate 7 d after layby application[z].

Postemergence herbicides[y]		Goosegrass control			
		2011			2012
POST-1	POST-2	Clayton 1	Clayton 2	Rocky Mount	Clayton 3
		%			
Glyphosate (1X)	Glyphosate(1X)	100 a	99 a	98 a	95 a
Glyphosate (1X)	Glufosinate (1X)	100 a	100 a	87 c	87 b
Glufosinate (1X)	Glufosinate (1X)	98 a	98 a	97 ab	82 b
Glufosinate (1X)	Glyphosate (1X)	100 a	100 a	93 ab	87 b
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	99 a	97 a	86 c	82 b
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	98 a	100 a	87 bc	80 b
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	99 a	98 a	89 bc	85 b

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively. All treatments received a postemergence-directed layby application of diuron plus MSMA at 841 plus 2240 g ha^{-1}, respectively, 2 to 3 wk after POST-2 application.

Large crabgrass was present at the Clayton 1, Clayton 3, and Rocky Mount locations. All herbicides and herbicide combinations applied POST-1 controlled this weed 98 to 100% (data not shown). Following POST-2 application, all treatments at Clayton 1 and Clayton 3 controlled large crabgrass 98 to 100% (Table 9). At Rocky Mount, glyphosate alone and glyphosate plus glufosinate applied sequentially, regardless of order of application, controlled large crabgrass 100%. Glufosinate applied POST-1 fb POST-2 and all sequential co-applications of glufosinate and glyphosate were 4 to 10% less effective than glyphosate applied sequentially. However, following the layby application of diuron and MSMA, all treatments controlled large crabgrass 95 to 99% (data not shown).

Table 9. Large crabgrass control 7 d after the second postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]		Large crabgrass control		
		2011		2012
POST-1	POST-2	Clayton 1	Rocky Mount	Clayton 3
		%		
Glyphosate (1X)	Glyphosate(1X)	100 a	100 a	99 a
Glyphosate (1X)	Glufosinate (1X)	100 a	100 a	100 a
Glufosinate (1X)	Glufosinate (1X)	100 a	96 b	100 a
Glufosinate (1X)	Glyphosate (1X)	100 a	100 a	100 a
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	100 a	94 b	100 a
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	100 a	94 b	98 a
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	100 a	90 b	100 a

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively.

Cotton injury and yield. The 'FM 1944GLB2' cotton at Mount Olive sustained no injury from either glyphosate or glufosinate. This cultivar contains both the GlyTol and LibertyLink traits and is resistant to both glyphosate and glufosinate (Wallace et al., 2011). Injury at the other locations, which included WideStike cotton, was expressed as foliar necrosis. Glufosinate alone caused 5 to 10% and 4 to 10% necrosis 7 DAP1 and 7 DAP2, respectively (data not shown). At both locations in 2011, but not in 2012, co-application of glyphosate with glufosinate increased necrosis 2 to 7% 7 DAP1 (data not shown). Steckel et al. (2012) also observed greater injury with co-applied glufosinate and glyphosate applied to WideStrike cotton. Cotton injury in the current study was transient with little to no injury observed at layby. The amount of necrosis in this study was similar to that reported previously with glufosinate applied to WideStrike varieties (Culpepper et al., 2009; Whitaker et al., 2011). Utilization of the rates and number of applications investigated in this study, glufosinate would be expected to have no adverse effect on yield of WideStrike cotton (Culpepper et al., 2009; Steckel et al., 2012; Whitaker et al., 2011).

No cotton yield differences were observed among herbicide treatments at Clayton 2, Rocky Mount, or Mount Olive (Table 10). Although differences in Palmer amaranth control were observed at these locations, all treatments controlled Palmer amaranth at least 80% 7 DAP2 and 7 d after layby (Tables 4 and 5). At Clayton 1, cotton yield was similar following glyphosate only and sequential application of glyphosate and glufosinate alone or as a co-application (Table 10). In addition, cotton yield following glufosinate only was greater than glyphosate only and glyphosate plus glufosinate co-applications treatments. These yield responses did not correlate with Palmer amaranth control (Tables 4 and 5). Cotton yield at Clayton 3 following glyphosate POST-1 and POST-2 was less than yield with all other treatments (Table 10). Palmer amaranth was controlled only 64% at 7 DAP2 by this treatment compared with at least 88% control by all other treatments (Table 4). Glyphosate only or glyphosate fb glufosinate treatments at Micro could not be harvested due to severe weed infestations; thus, yields were assumed to be zero (Table 10). Yields were similar with any treatment that included glufosinate 1X applied sequentially and greater than yield of cotton receiving only one application of glufosinate or the 1/2X rate of the herbicide.

Table 10. Cotton yield following postemergence application of glyphosate, glufosinate, and co-applications of glyphosate and glufosinate[z].

Postemergence herbicides[y]		Seed cotton yield					
		2011			2012		
POST-1	POST-2	Clayton 1	Clayton 2	Rocky Mount	Clayton 3	Micro	Mount Olive
		---------- kg ha^{-1} ----------					
Glyphosate (1X)	Glyphosate(1X)	3910 bc	2190 a	1340 a	2620 b	0 c	3360 a
Glyphosate (1X)	Glufosinate (1X)	3960 abc	2890 a	1580 a	3380 a	0 c	4180 a
Glufosinate (1X)	Glufosinate (1X)	4200 a	2540 a	1710 a	3350 a	2630 a	3690 a
Glufosinate (1X)	Glyphosate (1X)	4090 ab	2810 a	1220 a	3750 a	2090 b	4030 a
Glyphosate (1X) + glufosinate (1/2X)	Glyphosate (1X) + glufosinate (1/2X)	3910 bc	2780 a	1540 a	3330 a	1900 b	4180 a
Glyphosate (1/2X) + glufosinate (1X)	Glyphosate (1/2X) + glufosinate (1X)	3880 bc	2900 a	1730 a	3680 a	2760 a	4060 a
Glyphosate (1X) + glufosinate (1X)	Glyphosate (1X) + glufosinate (1X)	3750 c	2620 a	1800 a	3640 a	2700 a	3860 a

[z] Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[y] POST-1 herbicides were applied to 2-leaf cotton 18 to 22 d after planting. POST-2 herbicides were applied 14 days later. The 1X rates of glyphosate and glufosinate were 868 and 543 g ha^{-1}, respectively. All treatments received a postemergence-directed layby application of diuron plus MSMA at 841 plus 2240 g ha^{-1}, respectively, 2 to 3 wk after POST-2 application.

One component of a resistance management strategy is use of multiple, effective modes of action against species most prone to herbicide resistance (Norsworthy et al., 2012). Rotation of a GR crop with a glufosinate-tolerant crop would allow use of both glyphosate and glufosinate within the rotation. For resistance management, this would be preferable to continuous use of either herbicide alone. However, use of both herbicides within the same crop year would more effectively delay resistance evolution than annual herbicide rotation (Norsworthy et al., 2012). Thus, cotton cultivars with tolerance to both glyphosate and glufosinate, such as those with the GlyTol LibertyLink traits or those with the WideStrike trait, could be useful in a management program to avoid selection for resistance. The question that arises is whether the two herbicides should be applied sequentially or as a mixture. In fields currently without resistant biotypes, co-applications of the two herbicides theoretically would be more effective in preventing resistance selection than sequential applications (Diggle et al., 1993; Powles et al., 1997). Co-applications of glyphosate and glufosinate would have characteristics deemed essential for prevention of resistance (Norsworthy et al., 2012). Glyphosate and glufosinate are both effective on Palmer amaranth (assuming susceptible biotypes and timely application of glufosinate), and they have different modes of action (Devine et al., 1993; Steinrucken and Amrhein, 1980). However, glufosinate mixed with glyphosate might antagonize glyphosate on glyphosate-susceptible Palmer amaranth (Reed et al., 2014) and grasses (Bethke et al., 2013; Chahal et al., 2012; Chuah et al. 2008). Because of antagonism, Norsworthy et al. (2012) suggested that sequential applications would be superior to co-applications for reducing risks of resistance.

Most Palmer amaranth populations in North Carolina, and probably across the Southeast and Mid-South, already have GR biotypes comprising at least part of the population (Poirier et al., 2014). In the current study, control by glyphosate alone indicated 10 to 15% of the population at Clayton 1, Clayton 2, Rocky Mount, and Mount Olive; about 35% at Clayton 3; and more than 90% at Micro was resistant to glyphosate (Table 4). Large populations of Palmer amaranth can build up quickly (Norsworthy et al., 2014). That, along with the devastation caused by the weed in cotton and the difficulty of control, especially when it is resistant to commonly used herbicides (Culpepper et al. 2010), means Palmer amaranth must be the primary focus in formulating weed management programs. In fields with Palmer amaranth, and especially GR Palmer amaranth, the primary objective of a management program should be reducing the seedbank (Norsworthy et al., 2012). Considering the fecundity of Palmer amaranth, essentially complete control is necessary to reduce the seedbank (Culpepper et al., 2010; Norsworthy et al., 2014). At every location in this study, regardless of the percentage of the population thought to be GR, sequential applications of glufosinate was more effective than glyphosate applied sequentially (Table 4). In fields where a GR biotype comprises a large percentage of the population, such as the Micro location, glyphosate and glufosinate applied sequentially would be much less effective than glufosinate applied sequentially. And, similar to observations by Wiggins et al. (2013), there was no improvement in control when glyphosate is co-applied with glufosinate, because most individuals were resistant to glyphosate. Glyphosate would do little in this situation to reduce selection pressure on glufosinate.

At locations other than Micro, where most of the Palmer amaranth was still susceptible to glyphosate, glyphosate could substitute for one of the glufosinate applications without sacrificing Palmer amaranth control. Residual soil-applied herbicides are recommended in cotton management programs for Palmer amaranth (Culpepper et al., 2013; Scott and Smith, 2011; Wilson et al., 2011; York, 2014). Good Palmer amaranth control by soil-applied herbicides would increase the success with sequential POST applications of glufosinate and glyphosate. Because glufosinate added to glyphosate reduced large crabgrass and goosegrass control compared to glyphosate alone, sequential applications would be more effective on grasses.

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