The *Journal of Cotton Science 16:88–95 (2012)*
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WEED SCIENCE

Evaluation of WideStrike® Flex Cotton Response to Over-the-Top Glufosinate Tank Mixtures

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ABSTRACT

Field studies were conducted in Louisiana, Mississippi, and Tennessee during 2010 to investigate tolerance of 'PHY 375 WRF' and 'PHY 485 WRF' cotton to post applications of glufosinate and glufosinate tank mixtures. Glufosinate, glyphosate, *S*-metolachlor, and dimethoate were applied alone and in all possible combinations of the four pesticides to two-leaf cotton. Glufosinate-only treatment injured cotton 18%, which was much higher than injury from *S*-metolachlor-, dimethoate-, and glyphosate-alone treatments. The tank mixture that delayed maturity the greatest was glufosinate plus *S*-metolachlor. The lowest yielding treatments were glyphosate plus glufosinate plus dimethoate plus *S*-metolachlor, glufosinate plus glyphosate plus *S*-metolachlor, and glufosinate plus glyphosate plus *S*-metolachlor. Yields were also reduced following application of glufosinate plus *S*-metolachlor and glufosinate plus glyphosate. When all tank mixtures that contained both glufosinate and *S*-metolachlor were compared to all tank mixtures containing glyphosate and *S*-metolachlor, injury was greater (21 vs 8%) with glufosinate-based treatments. This resulted in delayed maturity (4.1 vs 3.5 node above cracked boll [NACB]) and lower yield (1100 vs 1230 kg ha^{-1}) for glufosinate-based treatments. When we compared all the treatments that contained both glufosinate and dimethoate to those that contained both glyphosate and dimethoate, injury was greater (18 vs 5%), which resulted in delayed maturity (4.1 vs 3.3 NACB) but did not decrease yield. This data would suggest that growers should be cautious combining glufosinate with other pesticides and applying to WideStrike® cotton.

Managing glyphosate-resistant (GR) weeds in cotton has become a serious challenge for producers in the U.S. from the Mississippi Delta region to the Atlantic Coast. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S.Wats.) has only recently become problematic in this region because glyphosate has been used heavily on GR cotton varieties since their commercial introduction in 1997 (Duke and Powles, 2009; Gianessi, 2005; Owen and Zelaya, 2005). Glyphosate has been the dominant herbicide in cotton production because applications provide broad-spectrum control of most broadleaf and grass weed species (Askew et al., 2002; Baylis, 2000; Duke and Powles, 2009). Glyphosate systems are also less labor intensive (Culpepper and York, 1998) and are economical compared with conventional systems (Baylis, 2000; Duke and Powles, 2009; Gianessi, 2005). Glyphosate is an effective for control of weeds across a range of growth stages, so there is little need for timely applications as with conventional herbicides. In addition, glyphosate is relatively inexpensive and applying glyphosate alone two to three times post-emergence throughout the growing season is easy, effective, and ultimately profitable (Culpepper and York, 1998; Duke and Powles, 2009). However, this over-reliance on one herbicide has contributed to the introduction and spread of GR Palmer amaranth (Duke and Powles, 2008, 2009).

GR weeds did not appear until after GR crops were introduced, due to the heavy selection pressure placed on one herbicide (Culpepper et al., 2006; Duke and Powles, 2008; Powles, 2008). Consequently, areas where GR crops are grown are areas where GR weeds are developing most rapidly (Duke and Powles, 2008; Culpepper, 2006). GR Palmer amaranth was first confirmed in Georgia in 2005 (Culpepper et al., 2006), but can now be found

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throughout most of the U.S. cotton belt including Tennessee, Arkansas, Mississippi, Missouri, Alabama, North Carolina, South Carolina, and Louisiana (Heap, 2010). Palmer amaranth has a rapid growth rate and is competitive with many agronomic crops, including cotton (Culpepper et al., 2006). GR crops have been adopted quickly because glyphosate effectively controls glyphosate-susceptible Palmer amaranth; therefore the loss of this herbicide option where GR Palmer amaranth is widespread presents serious concerns for growers (Steckel et al., 2008).

Glufosinate is a glutamine synthetase inhibitor, an enzyme important for the conversion of glutamate and ammonia to the amino acid, glutamine (WSSA, 2007). This results in an accumulation of toxic ammonia and also inhibits photosynthesis (Coetzer and Al-Khatib, 2001; Wendler et al., 1990). Like glyphosate, glufosinate is a nonselective herbicide that is effective in controlling troublesome broadleaf and grass weed species such as GR Palmer amaranth (Culpepper et al. 2000; 2009; MacRae et al., 2007; Norsworthy et al., 2008). However, glufosinate is a contact herbicide and requires thorough coverage for effective control of GR Palmer amaranth (Tharp et al., 1999). Glufosinate can provide effective control of GR Palmer amaranth when applied at the appropriate time and is a good alternative to a glyphosate-based system when GR weeds are present (Culpepper et al., 2009; Everman et al., 2007; Steckel et al., 1997). In addition, there are no known glufosinate-resistant weeds at this time, making it an important tool in controlling GR weeds (Green, 2009).

Varieties of glufosinate-resistant cotton (LibertyLink®) were introduced as an alternative to GR cotton varieties. LibertyLink cotton was developed through insertion of the bar gene derived from *Streptomyces hygroscopicus* (Jensen) Waksman & Henrici (Castle et al., 2006; Green, 2009; Tan et al., 2006). This bar gene expresses the phosphinothricin acetyltransferase (*pat*) enzyme that acetylates $_{\mathrm{L}}$-phosphinothricin, and confers tolerance to glufosinate (Herouet et al., 2005; OECD, 2002). Glufosinate can be applied to LibertyLink varieties for broad-spectrum weed control with no crop injury, and glufosinate is a good option for controlling GR weeds. Despite the need for an alternative technology to help control GR weeds, LibertyLink varieties have not been adopted quickly by growers in the Mid-South. Current varieties are not glyphosate-resistant, limiting herbicide options for non-GR weed species, but more importantly, these varieties have not performed as well as other cotton varieties in the Mid-South (UT, 2010).

WideStrike® cotton varieties contain two genes that confer resistance to lepidopteran pests (Castle et al., 2006; Dow Chemical Company, 2006). These varieties express the Cry1Ac and Cry1F insecticidal proteins; however, both of these genes also contain the *pat* gene. The *pat* gene is used as a selectable marker gene for determining the presence of the Cry1Ac and Cry1F genes, but also confers tolerance to the herbicide glufosinate. However, when the *pat* enzyme is used as a selectable marker for plant transformation, as with the WideStrike varieties, there are lower levels of *pat* activity, so the tolerance to glufosinate is incomplete compared with LibertyLink varieties (OECD, 2002; Tan et al., 2006).

Injury from glufosinate applied to WideStrike varieties can reach 15 to 25% with one to two applications without decreasing yield (Barnett et al., 2011; Culpepper et al., 2009; Dodds et al., 2011; Whitaker et al., 2011). WideStrike varieties designated as WRF also contain the CP4 EPSPS enzyme, which confers resistance to glyphosate. This gives growers the option of using both glyphosate and glufosinate as a part of their weed control program. These varieties have performed well in the Mid-South and as a result, are being used by a large percentage of the growers in this region (USDA-AMS, 2010). In Tennessee, ‘Phytogen 375 WRF’ and ‘Phytogen 485 WRF’ were the top two yielding varieties at six different locations in 2010 (UT, 2010). WideStrike varieties comprised 63% of the Tennessee cotton acres, 19% of the Louisiana cotton acres, and 3.5% of the Mississippi cotton acres in 2010 (USDA-AMS, 2010). Not only do these varieties perform well agronomically, they also allow growers to apply both glyphosate and glufosinate as part of their weed control program without reducing yields (Culpepper et al., 2009; UT, 2010). Growers have come to realize this and, although the application of glufosinate on WideStrike cotton varieties is not supported by the manufacturer of the herbicide nor the marketers of the cotton seed, many have used glufosinate in WideStrike cotton to control Palmer amaranth (authors personal experience).

Thrips (Thysanoptera), primarily *Frankliniella* spp. and *Thrips tabaci* Lindeman, are common pests of seedling cotton (Bournier, 1994; Leigh et al., 1996). At-planting treatments such as in-furrow aldicarb or insecticide seed treatments (thiamethoxam, imidacloprid) are used ubiquitously to prevent thrips injury and yield loss. Despite the widespread use of at-planting treatments, supplemental foliar applications of insecticides are often required for thrips control on seedling cotton. Commonly recommended insecticides include acephate, dicrotophos, and dimethoate (Stewart et al., 2010). Some "leaf burn" can be caused by the use of these insecticides, with dimethoate often causing some visual signs of injury (personal observation).

As GR Palmer amaranth continues to spread across the U.S. cotton belt, applying combinations of herbicides to control these weeds is becoming more common. Glufosinate can be an effective tool; however more timely applications (Steckel et al., 1997), as well as residual herbicides, are needed to control GR Palmer amaranth (Culpepper et al,. 2007, 2009; Everman et al., 2009; Whitaker et al., 2008). This presents a need to reduce the number of pesticide applications in cotton. In addition, tank-mixing herbicides is ideal due to increased herbicide costs associated with controlling GR Palmer amaranth. Previous studies of tank-mixture combinations determined that glufosinate plus *S*-metolachlor did not increase crop injury and were important for effective weed control and maintaining yields in GR and glufosinate-resistant cotton varieties (Clewis et al., 2008; Everman et al., 2009). Combining an insecticide with these herbicides is also becoming more common. Miller et al. (2008) found that some insecticides tank mixed with glyphosate and mepiquat chloride could increase cotton injury, but these treatments had no effect on yield. Additionally, pyrithiobac combined with various insecticide treatments (including dimethoate) did not reduce cotton leaf area, height, main stem node count, main stem nodes to first square, days to first square or flower, main stem nodes above white flower, or seed cotton yield (Costello et al., 2005). However, the stress of multiple herbicides that are injurious when applied alone combined with insecticide applications might increase crop injury and ultimately affect crop yield. Therefore, the objectives of this experiment were to determine cotton injury and effects on maturity and yield from glyphosate, glufosinate, *S*-metolachlor, and dimethoate applied alone or in tank mixtures.

MATERIAL AND METHODS

An experiment to determine tolerance of WideStrike cotton to glufosinate and glufosinate tank mixtures applied post-emergence was conducted at three locations across the midsouthern U.S. during 2010. Locations, soil descriptions, planting dates, and harvest dates are listed in Table 1. Cultivars planted included PHY 375 WRF (Dow Co., Indianapolis, IN) in Tennessee and Mississippi and PHY 485 WRF (Dow Co., Indianapolis, IN) in Louisiana. Conventional tillage production systems were utilized at all locations except in Tennessee where a no-tillage system planting into cotton stubble was utilized. All other production practices other than weed control followed university recommendations standard for each area.

The experimental design was a factorially arranged randomized complete block with treatments replicated four times. Plots were four rows by 9.1 m, with row spacing of 97 cm in all three locations. Pesticides evaluated included the herbicides glufosinate (Bayer CropScience, Research Triangle Park, NC), glyphosate (Monsanto Co., St. Louis, MO), and *S*-metolachlor (Syngenta Crop Protection, Greensboro, NC) and the insecticide dimethoate (Cheminova, Wayne, NJ). Pesticides were applied alone and in all possible combinations. Treatment and application rates are presented in Table 2. Pesticides were applied in a spray volume of 140 L ha^{-1} to two-leaf cotton with a CO_2-pressurized backpack sprayer at all locations. Experiments were maintained weed-free by application of fluometuron at 280 g ai ha^{-1} pre-emergence and applying glyphosate (Monsanto Co., St. Louis, MO) at 0.8 kg ae ha^{-1} applied topically when cotton was at the five-leaf stage of growth followed by hand-weeding as needed.

Table 1. Trial location and agronomic information.

Location	Planting date	Harvest date	Soil series	Soil texture	Soil pH	Soil OM (%)
Stoneville, MS	24 April	5 September	Dundee[z]	Very Fine Sandy Loam	6.1	1.2
Alexandria, LA	19 May	31 October	Coushatta[y]	Silt Loam	5.3	0.7
Jackson, TN	5 May	27 October	Lexington[w]	Silt Loam	6.0	1.5

[z] Fine-silty, mixed, active, thermic Typic Endoaqualfs.

[y] Fine-silty, mixed, superactive, thermic Fluventic Entrudepts.

[w] Fine-silty, mixed, active, thermic Ultic Hapludalfs.

Table 2. Cotton injury, height, node above cracked boll and lint yield for tank-mixture treatments.

Tank mixture	Rate g ha^{-1}	Injury[z] %	Height Cm	NACB	Yield kg ha^{-1}
Control	---	0	147	3.7	1470
s-metalochlor	1060	6	151	3.8	1340
dimethoate	280	3	150	3.6	1360
s-metalochlor + dimethoate	1060 + 280	8	150	3.1	1430
glyphosate	860	3	152	3.3	1480
glyphosate + s-metalochlor	860 + 1060	8	154	4.0	1430
glyphosate + dimethoate	860 + 280	3	151	3.4	1310
glyphosate + s-metalochlor + dimethoate	860 + 1060 +280	9	152	3.1	1330
glufosinate	590	18	149	3.9	1410
glufosinate+ s-metalochlor	590 + 1060	23	142	5.3	1250
glufosinate + dimethoate	590 + 280	20	148	4.0	1370
glufosinate+ s-metalochlor + dimethoate	590 + 1060 +280	27	140	3.7	1290
Glufosinate+ glyphosate	590 + 860	25	146	4.5	1200
Glufosinate+ glyphosate+ s-metalochlor	590 + 860 + 1060	33	142	3.5	1170
Glufosinate+ glyphosate+ dimethoate	590 + 860 + 280	25	137	4.7	1240
Glufosinate+ glyphosate+ s-metalochlor+ dimethoate	590 + 860 + 1060 + 280	38	137	4.1	1170
LSD (0.05)	10	6	3	1.2	130

[z] **Rating taken 5 d after application.**

Evaluations of cotton response at all locations included estimations of early-season injury from five to 15 d after application (DAA), plant height 80 DAA, node above cracked boll (NACB) 14 d before harvest, and lint cotton yield. Estimations of cotton injury were recorded on a scale of 0 to 100, where 0 indicates no cotton injury (chlorosis, necrosis, crop stunting) and 100 indicates cotton death (Frans et al., 1986). Plant height was measured by randomly selecting 10 plants per plot and measuring from soil level to the top of the plant. The center two rows of each plot were harvested using a spindle picker modified for small-plot harvesting. A sample of mechanically harvested seed cotton was collected from each plot and used to determine lint percentage and fiber quality. Seed cotton was ginned on a laboratory gin without lint cleaning.

Experiment was run as a randomized complete block design. Data were subjected to analysis of variance using the PROC MIXED procedure of SAS (ver. 9.2; SAS Institute, Cary, NC). Main effects and all possible interactions were tested using the appropriate expected mean square values as recommended by McIntosh (1983). Each location was considered an environment sampled at random from a population as suggested by Carmer et al. (1989). Designating environments random broadens the possible inference space the experimental results are applicable to. Environments, replications (nested within environments), and all interactions containing these effects were declared random effects in the model; pesticide treatments were designated fixed effects. Arcsine square root transformation of percentage data did not improve homogeneity of variance; therefore, non-transformed data were used in the analysis. Means were separated using Fishers Protected LSD test at the 0.05 significance level. Single degree of freedom contrasts were used to compare all glufosinate plus *S*-metalochlor treatments to all glyphosate plus *S*-metalochlor treatments as well as all glufosinate plus dimethoate treatments to all glyphosate plus dimethoate treatments.

RESULTS AND DISCUSSION

Cotton Injury and Height. Visual assessment of cotton injury 5 DAA were found to be significant ($P < 0.0001$). Glufosinate alone injured cotton 18%, which was higher than injury observed following *S*-metolachlor-, dimethoate-, and glyphosate-alone treatments (< 7%) (Table 2). This level of injury was consistent with other researchers (Culpepper et al., 2009; Whitaker et al., 2011) who found similar injury from glufosinate applications to WideStrike cotton. Glyphosate tank mixtures with dimethoate or *S*-metolachlor and dimethoate combined with *S*-metolachlor also resulted in little cotton injury (3 to 9%). This is in contrast to glufosinate tank mixtures, which all resulted in much higher cotton injury (20 to 33%). Of these tank mixtures, the glufosinate plus *S*-metolachlor treatment injured cotton 23%, which is similar to the range of injury (5 to 19%) reported by Whitaker et al. (2011) for the same treatment. Growers often desire to combine dimethoate or other insecticides for thrips control with an early-season herbicide application (Stewart et al. 2010). Our research showed that glufosinate plus dimethoate plus *S*-metolachlor injured cotton 27%. The highest numerical injury (38%) was observed following the four-way combination of glyphosate plus glufosinate plus dimethoate plus *S*-metolachlor.

Differences in plant height were detected 80 DAA ($P < 0.0001$). Cotton plant height was not affected with any single pesticide treatments (Table 2). Even the two-way tank mixtures of glyphosate plus *S*-metolachlor, glyphosate plus dimethoate, and glufosinate plus dimethoate were not different than the control. However, treatments that consisted of glufosinate plus *S*-metahlochlor or glufosinate plus glyphosate or three- and four-way combinations of those products did reduce plant height.

NACB, Fiber Quality, and Cotton Yield. Significant differences ($P = 0.0378$) in NACB were detected 14 d before harvest. NACB was not affected by any single pesticide treatment (Table 2). The tank mixture that most delayed maturity was the glufosinate plus *S*-metolachlor treatment. The treatments had no effect on percent lint turnout and fiber quality (Data not shown).

Differences in cotton lint yield were also found to be significant ($P < 0.0001$). Cotton yields were highest following the glyphosate-alone treatment. Yields following *S*-metolachlor were lower than those after glyphosate alone. Yield for the glufosinate- and dimethoate-alone treatments were not different from the glyphosate-alone application. Glufosinate topically applied to WideStrike cotton did not delay cotton maturity or reduce yield. This was consistent with research conducted in the southeastern U.S. (Culpepper et al., 2009; Whitaker et al., 2011). The lowest cotton yields were following the four-way tank mixture of glyphosate plus glufosinate plus dimethoate plus *S*-metolachlor, the three-way tank mixtures of glufosinate plus glyphosate plus *S*-metolachlor, and glufosinate plus glyphosate plus *S*-metolachlor. The two-way tank mixtures of glufosinate plus *S*-metalochlor and glufosinate plus glyphosate also reduced yield.

Tank-Mixture Effect on Injury, Maturity, and Yield. It appeared that both dimethoate or *S*-metolachlor combined with glufosinate could increase injury and yield loss. Therefore, single degree of freedom contrasts were used to determine if adding either dimethoate or *S*-metolachlor to glufosinate would cause more cotton injury than when these were mixed with glyphosate (Table 3). When all the tank mixtures that contained both glufosinate and *S*-metolachlor were compared to all the tank mixtures that contained glyphosate and *S*-metolachlor, injury was increased (21 vs 8%), which resulted in delayed maturity (4.1 vs 3.5 NACB) and lower yield (1100 vs 1230 kg ha^{-1}).

When all treatments that contained both glufosinate and dimethoate were compared to those that contained both glyphosate and dimethoate, injury was higher (18 vs 5%), which resulted in delayed maturity (4.1 vs 3.3 NACB) but did not decrease yield.

Table 3. Single degree of freedom contrasts comparing *S*-metaloclor tank mixtures with glyphosate to *S*-metalochlor tank mixtures with glufosinate and dimethoate tank mixtures with glyphosate to dimethoate tank mixtures with gluphosanate for 5 DAA visual assessment, NACB, and lint yield.

Contrast	Injury[z] %	Maturity NACB	Lint Yield kg/ha
S-metalochlor in glufosinate tank mixtures	21	4.1	1100
S-metalochlor in glyphosate tank mixtures	8	3.5	1230
Pr > F	0.0001	0.0550	0.0008
Dimethoate in glufosinate tank mixtures	18	4.1	1160
Dimethoate in glyphosate tank mixtures	5	3.3	1210
Pr > F	0.0001	0.0226	0.1884

[z] Visual determination of cotton injury in treated plot compared with a non-treated control.

Our research documents that glufosinate will injure PHY 375 WRF and PHY 485 WRF when applied at the two-leaf cotton stage. However, this injury is transient and did not affect yield. These results agree with previous research in North Carolina (Whitaker et al., 2011) and Georgia (Culpepper et al., 2009) where similar results were found. Our research was conducted without weed pressure, which was different than the research conducted in North Carolina and Georgia. Our study measured only the effect of the pesticides and was not confounded by any weed effect on the cotton. Despite this difference, we were able to replicate some of their results. The results from this study also documented the effect some potential glufosinate tank mixtures will have on WideStrike cotton. This research is particularly important for Tennessee cotton producers as more than 63% of cotton acres were planted to PHY 375 WRF in 2010 (USDA-AMS, 2010). Tennessee growers in 2010 widely utilized glufosinate on PHY 375 WRF cotton to control GR weeds (authors' experience). They often combined *S*-metolachlor and/or an insecticide targeting thrips as they applyed glufosinate (authors' experience). This study along with other published and unpublished data indicates that the probability of glufosinate alone causing yield loss to WideStrike cotton is low. Growers will likely continue to adopt this practice to combat GR Palmer amaranth, particularly in states like Tennessee that has little irrigation (USDA-ERS, 2011) to ensure preplant herbicides are activated. However, our data would suggest that growers should be cautious combining glufosinate with other pesticides and applying to WideStrike cotton. There is no safety net for a grower who uses glufosinate over the top of WideStrike cotton. The universities represented in this manuscript, nor Bayer CropScience, the manufacturers of glufosinate, nor PhytoGen, who market PHY 375 WRF and PHY 485 WRF, recommend nor stand behind glufosinate applications to WideStrike cotton.

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