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WEED SCIENCE

Tolerance of GlyTol® and GlyTol® + LibertyLink® Cotton to Glyphosate and Glufosinate in the Southeastern U. S.

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) has been genetically engineered with tolerance to both glyphosate and glufosinate. This new technology will give growers an additional tool to control weeds, including glyphosate-resistant (GR) Palmer amaranth (*Amaranthus palmeri* S. Wats). An experiment was conducted at eight locations in five southeastern states during 2008 to determine tolerance of an experimental line of GlyTol™ + LibertyLink® cotton to the ammonium salt of glyphosate (1.3 kg a.e. ha^{-1}) or the ammonium salt of glufosinate (0.6 kg a.e. ha^{-1}) applied four times alone or combined. Additional treatments included glyphosate and glufosinate in four alternating applications with glyphosate applied first or with glufosinate applied first. An experimental line of GlyTol™ cotton was included for comparison and received four glyphosate applications. Herbicides were applied topically to cotton with 1- to 3-, 6- to 8-, and 14- to 16-nodes and again at 50% cracked boll. Compared to non-treated checks, no visible injury was noted with glyphosate applied to GlyTol cotton or with glyphosate and glufosinate applied to GlyTol + LibertyLink cotton at any application timing. Herbicides had no effect on cotton height, boll morphology, lint yield, maturity, or fiber quality within cotton lines. This experiment demonstrates these new transgenic technologies have excellent tolerance of glyphosate and glufosinate.

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Cotton production is a critical component of farm sustainability in the southeastern U.S, with 5-year average plantings on 1 million ha in Alabama, Florida, Georgia, North Carolina, South Carolina, and Virginia combined (USDA-NASS, 2005, 2006, 2007, 2008, 2009). Weed control, and specifically control of herbicide-resistant Palmer amaranth, has become the greatest pest management challenge for cotton producers in each of these respective states (MacRae et al., 2008; Nichols et al., 2009; Sosnoskie et al., 2009; Webster, 2009). Depending upon the state, 97 to 100% of the cotton within the region is glyphosate tolerant (USDA-AMS, 2009). Glyphosate once controlled Palmer amaranth well (Corbett et al., 2004; Culpepper and York, 1998, 2000; Parker et al., 2005), however, Palmer amaranth resistant to glyphosate is now widespread across the region (Culpepper et al., 2008a; Nichols et al., 2009). Pyrithiobac, an acetolactate synthase (ALS)-inhibiting herbicide, applied postemergence (POST) will control small Palmer amaranth (Branson et al., 2005; Corbett et al., 2004). Unfortunately, Palmer amaranth resistant to ALS-inhibiting herbicides is also common across the southeastern U. S. and, in many cases the weed has resistance to both glyphosate and ALS-inhibiting herbicides (Sosnoskie et al., 2009; Whitaker, 2009; Wise et al., 2009).

Residual herbicides applied at planting can control Palmer amaranth during the early part of the season (Kichler et al., 2009; Marshall, 2009; Whitaker et al., 2009), however, control is unpredictable and often inadequate in production areas dependent on rainfall for herbicide activation (Culpepper et al., 2008b). Several herbicides applied POST-directed will control Palmer amaranth (York and Culpepper, 2009). However, Palmer amaranth grows very rapidly (Horak and Loughin, 2000), and if adequate control is not obtained with at-planting herbicides, the height differential between cotton and Palmer amaranth necessary for POST-directed application cannot be obtained. When biotypes of Palmer amaranth resistant to both glyphosate and ALS-inhibiting herbicides escape residual at-planting herbicides, growers have no option for POST topical application to glyphosate-tolerant cotton. Fluometuron and monosodium acid methanearsonate (MSMA) can be applied broadcast on cotton (York and Culpepper, 2009), but neither of these herbicides alone or in combination adequately control emerged Palmer amaranth (Whitaker, 2009). Additionally, these herbicides applied topically can adversely affect yield and maturity of cotton (Byrd and York, 1987; Guthrie and York, 1989).

Transgenic cotton cultivars tolerant to glyphosate (Roundup Ready® Flex cotton; event MON 88913) or glufosinate (LibertyLink® cotton; event LLCotton25), but not both, have been commercially available for several years. Second generation glyphosate-tolerant cotton technology from Monsanto (Roundup Ready Flex), was developed by jointly introducing two CP4 *epsps* gene expression cassettes into cotton along with a meristem-active promoter (Chen et al., 2006). LibertyLink cotton was developed through insertion of the bialaphos resistance (*bar*) gene isolated from the soil bacterium *Streptomycyes hygroscopius* (Agbios, 2009). Cotton transformed with the *bar* gene expresses the enzyme phosphinothricin-acetyl-transferase (PAT). The PAT enzyme acetylates L-phosphinothricin, the herbicidally active moiety of glufosinate, into non-phytotoxic *N*-acetyl-L-phosphinothricin (Mullner et al., 1993). Roundup Ready Flex cotton and LibertyLink cotton are highly tolerant to glyphosate and glufosinate, respectively (Blair-Kerth et al., 2001; Main et al., 2007).

Weed control efficacy of glyphosate and glufosinate varies by weed species. In general, glyphosate is more effective on annual grass species, especially goosegrass [*Eleusine indica* (L.) Gaertn.] and johnsongrass [*Sorghum halepense* (L.) Pers.]. Adequate control of broadleaves such as *Amaranthus* spp., velvetleaf (*Abutilon theophrasti* Medicus), and common lambsquarters (*Chenopodium album* L.) can also be achieved. Glufosinate is generally more effective on *Ipomoea* spp., hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. Ex. A.W. Hill], and prickly sida (*Sida spinosa* L.) (Corbett et al., 2004; Culpepper et al., 2000; Nelson et al., 2002; Price et al., 2008; Wilson et al., 2002). Additionally, glufosinate-based management systems are more effective than glyphosate-based systems on glyphosate-resistant Palmer amaranth (Culpepper et al., 2008b; Kichler et al., 2009; Whitaker et al., 2009). Cotton cultivars tolerant to both glyphosate and glufosinate would give growers an additional option to control glyphosate-resistant Palmer amaranth and also to improve overall weed control. Additionally, use of two modes of action could delay development of further weed resistance.

Cotton cultivars designated as WideStrike™ (DAS-21023-5 × DAS-24236-5) contain two transformation events that confer resistance to lepidopteran pests. Both of those events include the phosphinothricin acetyltransferase (*pat*) gene, which was inserted for use as a selectable marker during plant transformation. The *pat* gene confers tolerance to glufosinate, but glufosinate is not commercially promoted for use on WideStrike cultivars (Kichler, 2007). Several cultivars are now available that contain both the WideStrike and Roundup Ready Flex traits (Anonymous, 2010). Successful control of glyphosate-resistant Palmer amaranth has been obtained with glufosinate-based systems used in WideStrike cotton (Culpepper et al., 2009; Kichler et al., 2009; Whitaker et al., 2009). These cultivars have excellent tolerance to glyphosate, but injury as high as 30% from glufosinate may occur (Culpepper et al., 2009; Whitaker, 2009). Neither the company producing glufosinate (Bayer CropScience) nor the company producing Widestrike cotton (PhytoGen Seed Company, a subsidiary of Dow AgroSciences) recommends applying glufosinate to Widestrike cotton because of crop injury concerns.

GlyTol® cotton (event GHB614) is a new technology from Bayer CropScience. Resistance to glyphosate in GlyTol® cotton was achieved by insertion of the *2mepsps* gene, using *Agrobacterium*-mediated gene transfer (Bayer CropScience, 2006). This gene codes for a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme with greatly decreased binding affinity for glyphosate. GlyTol® + LibertyLink® cotton was developed by introgressing both the GlyTol® and the LibertyLink® traits into a commercial cultivar (Holloway et al., 2009).

Cotton that could be treated with both glyphosate and glufosinate, without crop injury, would allow growers to achieve the weed control benefits of both herbicides without the risk of injury that has been observed when glufosinate is applied to cotton with the WideStrike trait. The objective of our research was to determine resistance of GlyTol® cotton to glyphosate and GlyTol® + LibertyLink® cotton to both glyphosate and glufosinate applied multiple times during the season. This information will be essential in the development of recommendations for sustainable cotton production systems for the southeastern U.S.

MATERIALS AND METHODS

An experiment to determine resistance of GlyTol® cotton to glyphosate and resistance of GlyTol® + LibertyLink® cotton to glyphosate and glufosinate was conducted at eight locations across five states in the southeastern U.S. during 2008. Locations and soil descriptions are listed in Table 1. The crop was kept weed-free by use of hand weeding plus at-planting residual herbicides including pendimethalin (Prowl H_2O; BASF Ag Products; Research Triangle Park, NC), fluometuron (Cotoran 4L; Griffin LLC; Valdosta, GA), and pyrithiobac (Staple LX; DuPont Crop Protection Co., Inc), with herbicide rates determined by University extension recommendations for each soil type.

An experimental line derived from the cultivar Coker 312 with resistance to glyphosate (GlyTol® cotton) and an experimental line derived from the cultivar FM 958 with resistance to glyphosate and glufosinate (GlyTol® + LibertyLink® cotton) were planted on the dates listed in Table 1. Cotton was planted at eight to eleven seed m^{-1} of row across locations into conventionally prepared seedbeds. The experimental design was a randomized complete block with treatments replicated four times. Border rows were included between technologies to eliminate any herbicide drift potential. Plot size was four rows by 9 to 12 m and row spacing was 76 to 91 cm, depending on location. Other than weed control, production practices were standard for the regions where the experiment was conducted.

Table 1. Locations, soil types, herbicide application dates, and harvest dates.

	Locations							
	Attapulgus, GA	Elko, SC	Laurel Hill, NC	Lewiston-Woodville, NC	Quitman, GA	Sellers, SC	Tallahassee, FL	Shorter, AL
Soil series	Lucy[z]	Varina[y]	Noboco[x]	Goldsboro[w]	Tifton[v]	Claredon[u]	Notcher[t]	Norfolk[s]
Soil texture	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Fine sandy loam	Fine sandy loam
Planting date	12 -May-08	13-May-08	14-May-08	15-May-08	30-Apr-08	13-May-08	29-May-08	1-May-08
Application dates								
1- to 3-node cotton	28-May-08	30-May-08	9-June-08	2-June-08	23-May-08	2-June-08	19-June-08	27-May-08
6- to 8-node cotton	13-June-08	16-June-08	19-June-08	18-June-08	4-June-08	16-June-08	2-July-08	9-June-08
14- to 16-node cotton	2-July-08	2-July-08	25-July-08	7-July-08	9-July-08	14-July-08	29-July-08	24-June-08
50% cracked boll	2-Sept-08	23-Sept-08	N/A[r]	22-Sept-08	4-Sept-08	N/A[r]	19-Sept-08	3-Sept-08
Harvest date	23-Sept-08	22-Oct-08	26-Oct-08	20-Oct-08	14-Oct-08	17-Oct-08	24-Sept-08	29-Sept-08

[z] Loamy, kaolinitic, thermic Arenic Kandiudults.
[y] Fine, kaolinitic, thermic Plinthic Paleudults.
[x] Fine-loamy, siliceous, subactive, thermic Oxyaquic Paleudults.
[w] Fine-loamy, siliceous, subactive, thermic Aquic Paleudults.
[v] Fine-loamy, kaolinitic, thermic Plinthic Kandiudults.
[u] Fine-loamy, siliceous, semiactive, thermic Plinthaquic Paleudults.
[t] Fine-loamy, siliceous, subactive, thermic Plinthic Paleudults.
[s] Fine-loamy, kaolinitic, thermic Typic Kandiudults.
[r] N/A – Final application was not applied due to lodging of cotton stalks.

Treatments consisted of two herbicide options in the GlyTol® cotton and six herbicide options in the GlyTol® + LibertyLink® cotton applied POST (Table 2). In GlyTol® cotton, the first treatment had no POST herbicide applied, the second treatment had isopropylamine salt of glyphosate (Glyfos® X-tra Herbicide, Cheminova, Inc., Research Triangle Park, NC) at 1.3 kg a.e. ha^{-1} applied four times. Treatments in GlyTol® + LibertyLink® cotton included the following: 1) no POST herbicide; 2) four applications of glyphosate (Glyfos® X-tra Herbicide) at 1.3 kg ha^{-1}; 3) four applications of the ammonium salt of glufosinate (Ignite® 280 SL Herbicide, Bayer CropScience LP, Research Triangle Park, NC) at 0.6 kg a.e. ha^{-1}; 4) alternating applications of glufosinate, glyphosate, glufosinate, and glyphosate; 5) alternating applications of glyphosate, glufosinate, glyphosate, and glufosinate; and 6) four applications of glyphosate and glufosinate simultaneously. Herbicides were applied topically to cotton in the 1- to 3-, 6- to 8-, and 14- to 16-node stages and again at the 50% cracked boll stage (half of the bolls either open or cracked) on the dates listed in Table 1. Herbicides were applied using CO_2-pressurized backpack sprayers equipped with flat-fan nozzles (TeeJet XR® 11002 nozzles; Spraying Systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} at 160 kPa.

Cotton was evaluated for stand, injury, height, maturity, boll morphology, lint yield, and fiber quality. Cotton stand was determined by counting all plants in the center two rows of each plot 14 days after planting, before the first POST application. Visible crop injury was estimated at 7 to 14 days after each application using a scale of 0 = no injury and 100 = crop death (Frans et al., 1986).

Table 2. GlyTol® and GlyTol® + LibertyLink® cotton height, maturity measured as percentage open bolls, and lint yield as affected by glyphosate and glufosinate applied topically.[z]

Treatment number	Cotton technology	Herbicide application (cotton stage of growth)[y]				Height 7 to 9 WAP (cm)	Open Boll (%)	Lint yield (kg ha^{-1})
		1-to 3-node	6- to 8-node	14- to 16-node	50% cracked boll			
1	GlyTol	None	None	None	None	73.8	48.4	1040
2	GlyTol	Glyphosate	Glyphosate	Glyphosate	Glyphosate	73.4	48.1	1060
3	GlyTol + LibertyLink	None	None	None	None	77.6	49.7	1170
4	GlyTol + LibertyLink	Glyphosate	Glyphosate	Glyphosate	Glyphosate	76.1	49.1	1130
5	GlyTol + LibertyLink	Glufosinate	Glufosinate	Glufosinate	Glufosinate	76.7	48.4	1130
6	GlyTol + LibertyLink	Glufosinate	Glyphosate	Glufosinate	Glyphosate	77.5	47.5	1150
7	GlyTol + LibertyLink	Glyphosate	Glufosinate	Glyphosate	Glufosinate	77.2	47.8	1150
8	GlyTol + LibertyLink	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	77.0	46.6	1120
Contrasts[w]						Pr > F	Pr > F	Pr > F
GlyTol vs. GlyTol + LibertyLink						<0.0001	0.9133	<0.01
Within GlyTol								
Treatments 1 vs. 2						0.8107	0.8504	0.6412
Within GlyTol + LibertyLink								
Treatments 3 vs. 4						0.2990	0.7061	0.3024
Treatments 3 vs. 5						0.4938	0.4511	0.3091
Treatments 3 vs. 6						0.9298	0.1884	0.5198
Treatments 3 vs. 7						0.7657	0.2591	0.4678
Treatments 3 vs. 8						0.6583	0.0612	0.1510

[z] Data averaged over eight locations in five states in the southeastern U.S.

[y] Glyphosate applied at 1.3 kg a.e. ha^{-1}; glufosinate applied at 0.6 kg a.e. ha^{-1}.

[x] WAP = weeks after planting.

[w] Orthogonal contrasts for cotton technologies and treatments.

Plant height was collected from 10 plants per plot and was recorded at 7 to 9 weeks, occurring after all applications excluding the 50% cracked boll application were made. Cotton maturity, measured as the percentage of open bolls, was determined by counting the number of opened and unopened bolls when the GlyTol® + LibertyLink® untreated check visually appeared to have 50% open bolls. At the same time, 25 bolls per plot, from the upper 50% of unopened bolls, were examined for abnormalities in shape. The center two rows of each plot were mechanically harvested. A 200-g sample of harvested seed cotton was collected from each plot and used for lint percentage and fiber quality determinations. Seed cotton was ginned by Bayer CropScience on a 10-saw laboratory gin and fiber length, fiber length uniformity, fiber strength, and micronaire were determined by high volume instrumentation testing (Sasser, 1981).

Data were analyzed using the ProcMixed procedure in SAS (version 9.1, SAS Institute, Cary, NC). Treatments were considered fixed effects while replication and site were considered random effects. Orthogonal contrast statements were used to make comparisons between and within cotton lines.

RESULTS AND DISCUSSION

Plant stands of GlyTol® and GlyTol® + LibertyLink® cotton, recorded 14 days after planting and prior to first POST herbicide application, averaged 7.7 and 9.4 plants m^{-1} of row, respectively (data not shown). Within locations, planting of both lines of cotton was conducted with the same equipment operated in the same manner. Differences in stands were due to higher seed quality with the GlyTol® + LibertyLink® cotton cultivar.

Herbicide treatments did not visibly injure cotton at any time during the season (data not shown). GlyTol® + LibertyLink® cotton was taller than GlyTol® cotton at 7 to 9 weeks after planting (Table 2). Compared to the non-treated checks, however, glyphosate and/or glufosinate application did not affect cotton height.

In the first generation of glyphosate-resistance cotton (Roundup Ready®, event 1445), boll abortion was often observed when glyphosate was applied topically to cotton in the reproductive stage (Jones and Snipes, 1999; Pline-Srnic et al., 2004). Glyphosate applied to Roundup Ready cotton in the reproductive stage interfered with anther development, reduced pollen deposition on stigmas, and reduced pollen viability (Pline et al., 2002, 2003). This was attributed to reduced expression of the EPSPS enzyme in the male reproductive tissues (Pline et al., 2002). “Beaked” or “moon-shaped” bolls, resulting from incomplete pollination, were also commonly observed (Yasuor et al., 2007). Yields were often not reduced because cotton was able to compensate for reduced boll retention on the lower fruiting branches by setting more fruit higher on the plant. However, this compensatory response could lead to delayed crop maturity (Jones and Snipes, 1999). In our experiment, we examined the morphology of green bolls at the 50% cracked boll stage as an indicator of herbicide effects on pollination. No misshapen bolls were observed late in the season following multiple applications of glyphosate to GlyTol® or multiple applications of glyphosate or glufosinate to GlyTol® + LibertyLink® cotton (data not shown).

Percentage of open bolls was used as an indicator of crop maturity. No differences in the percentage of open bolls were observed between GlyTol® and GlyTol® + LibertyLink® cotton (Table 2). Additionally, glyphosate and glufosinate had no effect on maturity of either cotton line.

Compared to the non-treated checks, neither glyphosate applied to GlyTol® cotton nor glyphosate or glufosinate applied to GlyTol® + LibertyLink® cotton affected yield. Herbicide application had no effect on fiber length, micronaire, strength, or length uniformity of either GlyTol® or GlyTol® + LibertyLink® cotton (Table 3). However, differences in fiber quality parameters were observed between the two cotton lines. These fiber quality parameters commonly vary among cultivars (May, 1999), but herbicides seldom affect cotton fiber quality, especially when yield is not impacted (Culpepper and York, 1998, 2000; Jordan et al., 1993; Pline-Srnic et al., 2004).

Similar experiment have also been conducted in Arizona, Arkansas, California, Louisiana, Mississippi, Tennessee, and Texas (Henniger et al., 2009; Humphries et al., 2009; Irby et al., 2009). Similar to our results, no visible crop injury and no effects on cotton height, boll morphology, lint yield, maturity, or fiber quality were noted in those studies with glyphosate applied to GlyTol® cotton or glyphosate and glufosinate applied to GlyTol® + LibertyLink® cotton. Our results, along with results of these other researchers, demonstrate that GlyTol® + LibertyLink® cotton is highly resistant to glyphosate, glufosinate, or glyphosate plus glufosinate applied multiple times throughout the growing season. The level of resistance to each respective herbicide is similar to that previously observed with glufosinate applied to LibertyLink ®cotton (Blair-Kerth et al., 2001; Gardner et al., 2006) and glyphosate applied to Roundup Ready Flex cotton (Main et al., 2007). Resistnace of GlyTol® + LibertyLink® cotton to glufosinate is greater than resistance of Widestrike cotton to glufosinate (Culpepper et al., 2009). GlyTol® + LibertyLink® technology, once incorporated into elite germplasm, will offer cotton producers the opportunity to use both herbicides to control glyphosate-resistant weeds and to broaden the spectrum of weed control. Additionally, use of two modes of action will help delay further herbicide resistance in weeds. Although this technology will offer growers greater flexibility, successful weed management will require a systems approach using timely herbicide application and residual at-planting herbicides as glyphosate-resistant weeds and glufosinate-tolerant weeds are now common in many cotton producing areas (MacRae et al., 2007; Patterson et al., 2008; Whitaker, 2009).

Table 3. GlyTol® and GlyTol® + LibertyLink® cotton fiber quality parameters as affected by glyphosate and glufosinate applied topically.[z]

Treatment number	Cotton technology	Herbicide application (cotton stage of growth)[y]				Micronaire	Upper half mean length (cm)	Strength (k N m kg^{-1})	Length uniformity index (%)
		1-to 3-node	6- to 8-node	14- to 16-node	50% cracked boll				
1	GlyTol	None	None	None	None	5.08	3.10	336	85.4
2	GlyTol	Glyphosate	Glyphosate	Glyphosate	Glyphosate	5.12	3.10	337	85.2
3	GlyTol + LibertyLink	None	None	None	None	4.49	3.02	323	83.9
4	GlyTol + LibertyLink	Glyphosate	Glyphosate	Glyphosate	Glyphosate	4.50	3.00	329	83.7
5	GlyTol + LibertyLink	Glufosinate	Glufosinate	Glufosinate	Glufosinate	4.52	3.05	325	83.9
6	GlyTol + LIbertyLink	Glufosinate	Glyphosate	Glufosinate	Glyphosate	4.48	3.00	324	84.0
7	GlyTol + LibertyLink	Glyphosate	Glufosinate	Glyphosate	Glufosinate	4.48	3.02	324	83.7
8	GlyTol + LibertyLink	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	Glyphosate + glufosinate	4.51	3.02	330	84.1
Contrasts[x]						Pr > F	Pr > F	Pr > F	Pr > F
GlyTol vs. GlyTol + LibertyLink						<0.0001	<0.0001	<0.0001	<0.0001
Within GlyTol									
Treatments 1 vs. 2						0.5137	0.6464	0.7652	0.2576
Within GlyTol + LibertyLink									
Treatments 3 vs. 4						0.8853	0.3850	0.2060	0.2244
Treatments 3 vs. 5						0.6308	0.2130	0.6696	0.9876
Treatments 3 vs. 6						0.8853	0.5708	0.8634	0.7082
Treatments 3 vs. 7						0.8101	0.8798	0.9451	0.3339
Treatments 3 vs. 8						0.7365	0.6776	0.1067	0.3339

[z] **Data averaged over eight locations in five states in the southeastern U.S.**

[y] **Glyphosate applied at 1.3 kg a.e. ha^{-1}; glufosinate applied at 0.6 kg a.e. ha^{-1}.**

[x] **Orthogonal contrasts for cotton technologies and treatments.**

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