The *Journal of Cotton Science 17:131–139 (2013)*
http://journal.cotton.org, © The Cotton Foundation 2013


# WEED SCIENCE

## Evaluation of GlyTol™ and GlyTol™ + LibertyLink® Cotton in the Mid-South

J. Trenton Irby, Darrin M. Dodds, Daniel B. Reynolds*, Christopher L. Main, L. Thomas Barber, Kenneth L. Smith, and Alexander M. Stewart

### ABSTRACT

**Historically, weed control in cotton (*Gossypium hirsutum* L.) relied upon a combination of tillage, soil-applied herbicides, postemergence-directed herbicides, and hand weeding. More recently, weed control in cotton has become heavily reliant on transgenic technologies. Glyphosate-resistant and enhanced glyphosate-resistant cotton were commercialized in 1997 and 2006, respectively. Glufosinate-resistant cotton was commercialized in 2004. Although Monsanto Company has been the traditional provider of glyphosate-resistant technology, Bayer CropScience identified a novel glyphosate-resistant gene and released this technology to the market in 2011. In addition, Bayer CropScience introduced glyphosate/glufosinate-resistant cotton containing this new glyphosate-resistant trait plus the existing glufosinate-resistance trait. The new glyphosate-resistant technology is known as GlyTol™, whereas the glyphosate/glufosinate-resistant technology is known as GlyTol™ + LibertyLink®. Field experiments were conducted at 14 locations across Arkansas, Louisiana, Mississippi, and Tennessee from 2007 through 2009 to determine cotton response to multiple glyphosate and/or glufosinate applications. Glyphosate-resistant cotton was not visually injured by sequential glyphosate applications. Glyphosate/glufosinate-resistant cotton visual injury was 2% or less when treated with glyphosate, glufosinate, or glyphosate plus glufosinate. A reduction in plant height of up to 4 cm was observed only with the glyphosate/glufosinate-resistant cotton after two or three glufosinate applications were made; heights were not reduced by late season. Herbicide applications did not affect boll development or cotton yield. These data indicate GlyTol cotton has excellent tolerance to glyphosate applied topically and GlyTol + LibertyLink cotton has excellent tolerance to topical applications of glyphosate, glufosinate, and glyphosate plus glufosinate.**

Glyphosate-resistant (RR) cotton was developed by Monsanto Company using *cp4 epsps* trait genes designated as MON 1445 with an FMV 35S promoter (Green, 2009). Commercial introduction of RR cotton in 1997 dramatically altered weed control methodology in cotton. Weed control in cotton historically has been achieved through a combination of cultivation, soil-applied herbicides, and postemergence-directed (PD) herbicides (Culpepper and York, 1998; York et al., 2004). Herbicide-resistant cropping systems, specifically RR cropping systems, have allowed producers to rely less on tillage and more on herbicides for weed control. For example, tillage was utilized to manage pests on 62% of U.S. cotton acres in 1997 (USDA-NASS, 1998). By 2007, only 38% of the total cotton acreage in the U.S. utilized tillage for pest management (USDA-NASS, 2008). Growers utilizing RR cotton could apply glyphosate postemergence over-the-top (POST) of cotton through the 4-leaf stage of growth. Glyphosate applications made after the 4-leaf stage to RR cotton must be PD to prevent reduced boll retention, square abscission, delayed maturity, and yield reductions (Ferreira et al., 1998; Kalaher and Coble, 1998; Kalaher et al., 1997; Lemon et al. 2005; Martens et al., 2003; Matthews et al., 1998; Pline-Srnic et al., 2004). Adoption of RR cotton allowed producers to decrease the number of soil-applied herbicide applications (Culpepper and York, 1998, 1999), obtain broad spectrum weed control

J.T. Irby, D. M. Dodds, and D.B. Reynolds*, Plant and Soil Sciences, Mississippi State University, 117 Dorman Hall, Box 9555, Mississippi State, MS 39762; C.L. Main, (formerly) West Tennessee Research and Education Center, University of Tennessee, 605 Airways Blvd., Jackson, TN 38301; L.T. Barber, University of Arkansas, Division of Agriculture, 2301 S. University Ave., Little Rock, AR 72203; K.L. Smith, (formerly) Dept. of Crop, Soil, and Environmental Science, University of Arkansas—Monticello, Monticello, AR 71656; and A.M. Stewart, (formerly) LSU AgCenter, 8208 Tom Bowman Drive, Alexandria, LA 71302
*Corresponding author: dreynolds@pss.msstate.edu

(Faircloth et al., 2001; Green, 2009), and adopt conservation tillage practices (Bradley, 2000; York et al., 2004). RR cotton also allowed producers greater flexibility with respect to crop rotation due to lack of soil activity of glyphosate.

Enhanced glyphosate-resistant (RR Flex) cotton was commercially released in 2006. Roundup Ready Flex cotton was developed by Monsanto Company utilizing two *cp4 epsps* trait genes designated as MON 88913 with FMV/TSF1 and 35S/ACT 8 promoters (Green, 2009). RR Flex cotton offers similar advantages as RR cotton; however, glyphosate can be applied POST from emergence to within 1 wk of harvest in RR Flex cotton. Rapid adoption of RR and RR Flex technologies has occurred. Roundup Ready cotton was planted on 3.6% of total U.S. cotton acreage in 1997 (USDA-AMS, 1997) and by 2009, 92% of U.S. cotton was planted to RR or RR Flex cotton (USDA-AMS, 2009). A drawback of the RR Flex technology is that having the option for reliance on glyphosate as a sole means of weed control might facilitate the development of glyphosate-resistant weeds (Main et al., 2007).

Glufosinate-tolerant (LL) cotton was commercially released in 2004. Glufosinate-tolerant cotton was developed by Bayer CropScience utilizing a bialaphos acetyltransferase (*bar)* gene designated as LLCotton25 with a CaMV35S promoter (Green, 2009). Growers utilizing LL cotton can apply glufosinate POST from emergence through early bloom (Anonymous, 2013). Glufosinate is a non-selective herbicide that has activity on many grass and broadleaf weed species. For maximum efficacy, glufosinate application should be based on the size of the target weeds, not a particular crop growth stage (Lemon et al., 2004). Corbett et al. (2004) observed greater control of Palmer amaranth (*Amaranthus palmeri* S. Wats.) following glufosinate applications when weed size ranged from 2 to 5 cm when compared to applications made when Palmer amaranth was 8 to 10 cm in height. Although adoption of LL cotton has been slow, glufosinate can be used effectively to manage weeds (Culpepper et al., 2009; Everman et al., 2007). Liberty Link cotton was planted on 1.1% and 1.7% of U.S. cotton acres in 2004 and 2009, respectively (USDA-AMS, 2004, 2009). However, 5.9% of U.S. cotton acres were planted to LL varieties in 2012 (USDA-AMS, 2012). Historically, low adoption rate of LL cotton varieties has been due to poor agronomic performance of available varieties (Culpepper et al., 2009).

Historically, glyphosate has been used successfully to control Palmer amaranth (Culpepper and York, 1998; Grichar et al., 2004; Parker et al., 2005). However, glyphosate-resistant Palmer amaranth has been documented in Arizona, Arkansas, Alabama, California, Delaware, Georgia, Illinois, Kansas, Louisiana, Michigan, Mississippi, Missouri, Ohio, New Mexico, North Carolina, Tennessee, and Virginia (Heap, 2013). Although not as effective as glyphosate on non-glyphosate–resistant Palmer amaranth (Koger et al., 2007), glufosinate can be used to effectively control Palmer amaranth if applied in a timely manner (Culpepper et al., 2009, 2000; Gardner et al., 2006; Norsworthy et al., 2008; Wilson, Jr. et al., 2007). Due to the lack of cotton varieties containing multiple herbicide-resistance traits, growers are beginning to plant cotton varieties containing Widestrike™ technology and applying glufosinate POST (L. Steckel, Personal Communication). The Widestrike technology utilizes the phosphinothricin acetyltransferase (*pat*) gene as a selectable marker and therefore imparts a natural mechanism of resistance to glufosinate. This practice is not supported by Bayer CropScience (supplier of glufosinate) or Dow AgroSciences (supplier of Widestrike cotton varieties).

Cultivars with tolerance to both glyphosate and glufosinate will provide growers with a needed tool to control glyphosate-resistant weeds and aid in resistance management (Culpepper et al., 2009). Bayer CropScience is currently marketing cotton varieties that contain resistance to glyphosate under the name GlyTol™. Bayer CropScience is also marketing glyphosate- plus glufosinate-resistant cultivars under the name of GlyTol™ + LibertyLink®. GlyTol cultivars utilize a new glyphosate-resistant event, GHB 614, to confer resistance to glyphosate. GHB 614 uses a modified-maize *epsps* gene and a Ph4a748At promoter (Green 2009). GlyTol + LibertyLink cultivars utilize GHB 614 to confer glyphosate resistance and LLCotton25 to confer resistance to glufosinate. Little previous research is available with regards to GlyTol or GlyTol + LibertyLink cotton tolerance to topical applications of glyphosate or glufosinate. Therefore, experiments were conducted to determine GlyTol cotton tolerance to glyphosate applied topically and GlyTol + LibertyLink tolerance to glyphosate, glufosinate, or glyphosate plus glufosinate applied topically.

## MATERIALS AND METHODS

Field studies were conducted from 2007 to 2009 at 14 locations across the Mid-South. In 2007, experimental locations included the Black Belt Branch Experiment Station near Brooksville, MS; the West Tennessee Research and Extension Center in Jackson, TN; and the Rohwer Research Station near Rohwer, AR. Locations in 2008 and 2009 included the Black Belt Branch Experiment Station; the West Tennessee Research and Extension Center; the Rohwer Research Station; the R.R. Foil Plant Science Research Center near Starkville, MS; and the Lonn Mann Cotton Research Station near Marianna, AR. In addition, a study was conducted at the Macon Ridge Research Station near Winnsboro, LA in 2008. Agronomic and herbicide application information is given for all locations in Tables 1 and 2. Herbicide treatments were arranged in a randomized complete block design with four replications. Insecticides, plant growth regulators, and defoliants were applied uniformly at each individual location according to standard management practices for each state.

Herbicide applications were made at the following growth stages each year: 1 to 2 leaf, 7 to 9 leaf, 13 to 16 leaf, and at 10 to 50% open boll. In 2007, cultivars containing only the GHB614 trait were available and were screened for tolerance to multiple glyphosate formulations. Glyphosate formulations, application rates, and suppliers for the 2007 experiment are listed in Table 3. A non-glyphosate control was included at all locations for comparison purposes. Utilizing results from 2007, only the Glyphos X-TRA (Cheminova, Inc., Research Triangle Park, NC) glyphosate formulation was utilized in 2008 and 2009. Utilizing a cultivar containing both the GHB614 and LLCotton25 traits, herbicide applications were made at four cotton growth stages including 1 to 2 leaf, 7 to 9 leaf, 13 to 16 leaf and 50% boll open. Herbicide options and rates included glyphosate at 1.12 kg ae $ha^{-1}$ and/or glufosinate (Ignite 280 SL, Bayer CropScience, Research Triangle Park, NC) at 0.60 kg ai $ha^{-1}$ applied as follows: 1) glyphosate applied sequentially; 2) glufosinate applied sequentially; 3) glyphosate followed by (fb) glufosinate fb glyphosate fb glufosinate; 4) glufosinate fb glyphosate fb glufosinate fb glyphosate; and 5) glyphosate plus glufosinate applied sequentially. A non-glyphosate and/or glufosinate control was included for comparison purposes; therefore, various herbicide or mechanical tillage practices were used as methods to maintain this system as weed free. Visual estimates of cotton injury were collected 7 and 14 d after each treatment and were

**Table 1. Seeding rates and planting, application, and harvest dates for all locations.**

| Location | Year | Planting Date | Seeding Rate | Harvest Date | Date of 1-2 Leaf Application[z] | Date of 7-9 Leaf Application[y] | Date of 13-16 Leaf Application[x] | Date of 50% Open Boll Application[x] |
|---|---|---|---|---|---|---|---|---|
| | | | Seed $ha^{-1}$ | | | | | |
| Rowher, AR | 2007 | 12 June | 136,000 | No Harvest | 06 July | 19 July | 16 August | -- |
| Brooksville, MS | 2007 | 08 June | 136,000 | 30 November | 29 June | 24 July | 07 September | 03 October |
| Jackson, TN | 2007 | 12 June | 136,000 | 23 October | 28 June | 11 July | 01 August | 24 September |
| Marianna, AR | 2008 | 26 May | 136,000 | 28 October | 16 June | 26 June | 16 July | 23 September |
| Rowher, AR | 2008 | 28 May | 136,000 | No Harvest | 12 June | 01 July | 30 July | 09 October |
| Winnsboro, LA | 2008 | 28 May | 145,000 | 10 October | 13 June | 27 June | 22 July | -- |
| Brooksville, MS | 2008 | 06 June | 128,000 | 18 November | 23 June | 02 July | 15 August | 10 October |
| Starkville, MS | 2008 | 04 June | 128,000 | 18 November | 19 June | 05 July | 04 August | 17 October |
| Jackson, TN | 2008 | 19 May | 136,000 | 03 November | 30 May | 19 June | 10 July | 04 September |
| Marianna, AR | 2009 | 18 June | 136,000 | No Harvest | 08 July | 17 July | 12 August | 12 October |
| Rowher, AR | 2009 | 15 June | 136,000 | No Harvest | 07 July | 20 July | 26 August | 11 November |
| Brooksville, MS | 2009 | 16 June | 136,000 | No Harvest | 09 July | 23 July | 17 August | 02 November |
| Starkville, MS | 2009 | 18 June | 120,000 | No Harvest | 10 July | 30 July | 14 August | 16 November |
| Jackson, TN | 2009 | 01 June | 136,000 | 09 November | 22 June | 06 July | 21 July | 17 August |

based on a scale of 0 to 100, where 0 denoted no cotton injury and 100 denoted complete cotton death (Frans et al., 1986). Visual injury estimates were based upon observed chlorosis and necrosis of treated cotton plants. In 2007, plant heights from five plants per plot were collected 14 d after the final application to assess cotton height response to multiple applications of glyphosate. In 2008 and 2009, plant heights from five plants per plot were collected prior to and 14 d after each application to assess cotton height response to herbicide applications. Uppermost fruiting nodes were marked immediately prior to the 13 to 16 leaf application. Sixty days after the 13 to 16 leaf application, a visual, non-destructive boll sample was conducted to assess the impact of herbicide application on cotton pollination and subsequent boll development. Twenty-five cotton bolls from each plot were examined. All normal and misshapen cotton fruiting were counted for each plot. Seed cotton yields were collected from the two center rows of each plot utilizing a spindle harvester modified for small plot research. With the exception of the Jackson, TN, location, no seed cotton yields were collected in 2009 due to extreme rainfall throughout the harvest season.

**Table 2. Soil characteristics, plot dimensions, and herbicide application information for all locations.**

| Location | Year | Soil Series | Soil Texture | Soil Taxonomic Classification | Irrigation | Row Spacing | Plot Dimensions | Application Pressure | Spray Tip | App. Volume | Speed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | cm | # rows * length (m) | kPa | | L ha$^{-1}$ | Km hr$^{-1}$ |
| Rowher, AR[Z] | 2007 | Herbert | Silt Loam | Fine-silty, mixed, superactive, mesic Udollic Epiaqualfs | Furrow | 97 | 4 * 8.5 | 345/379 | Air Induction | 112 | 4.8 |
| Brooksville, MS | 2007 | Okolona | Silty Clay | Fine, smectitic, thermic Oxyaquic Hapluderts | Furrow | 97 | 4 * 12.2 | 220 | Flat Fan | 140 | 4.8 |
| Jackson, TN | 2007 | Collins | Silt Loam | Coarse-silty, mixed, active, acid, thermic Aquic Udifluvents | None | 97 | 4 * 9.1 | 207 | Flat Fan | 114 | 6.4 |
| Marianna, AR | 2008 | Calloway | Silt Loam | Fine-silty, mixed, active, thermic Aquic Fraglossudalfs | Furrow | 97 | 4 * 12.2 | 262 | Flat Fan | 140 | 5.6 |
| Rowher, AR[Z] | 2008 | Herbert | Silt Loam | Fine-silty, mixed, superactive, mesic Udollic Epiaqualfs | Furrow | 97 | 4 * 9.1 | 379/310 | Air Induction | 112 | 6.1 |
| Winnsboro, LA | 2008 | Jigger | Silt Loam | Fine silty, mixed, thermic, Typic Fragiudalf | None | 102 | 4 * 12.2 | 276 | Flat Fan | 140 | 5.1 |
| Brooksville, MS | 2008 | Okolona | Silty Clay | Fine, smectitic, thermic Oxyaquic Hapluderts | Furrow | 97 | 4 * 12.2 | 220 | Flat Fan | 140 | 4.8 |
| Starkville, MS[Z] | 2008 | Leeper | Sandy Loam | Fine, smectitic, nonacid, thermic Vertic Epiaquepts | None | 97 | 4 * 12.2 | 290/222 | Flat Fan | 140 | 4.8 |
| Jackson, TN | 2008 | Collins | Silt Loam | Coarse-silty, mixed, active, acid, thermic Aquic Udifluvents | None | 97 | 4 * 9.1 | 207 | Flan Fan | 114 | 6.4 |
| Marianna, AR | 2009 | Calloway | Silt Loam | Fine-silty, mixed, active, thermic Aquic Fraglossudalfs | Furrow | 97 | 4 * 12.2 | 283 | Flat Fan | 140 | 5.6 |
| Rowher, AR | 2009 | Herbert | Silt Loam | Fine-silty, mixed, superactive, mesic Udollic Epiaqualfs | Furrow | 97 | 4 * 8.5 | 310 | Air Induction | 112 | 5.6 |
| Brooksville, MS[Z] | 2009 | Okolona | Silty Clay | Fine, smectitic, thermic Oxyaquic Hapluderts | Furrow | 97 | 4 * 12.2 | 207/220 | Flat Fan | 140 | 4.8 |
| Starkville, MS[Z] | 2009 | Leeper | Sandy Loam | Fine, smectitic, nonacid, thermic Vertic Epiaquepts | None | 97 | 4 * 12.2 | 290/207/414 | Flat Fan / Hollow Cone | 140/93 | 4.8/5.6 |
| Jackson, TN | 2009 | Collins | Silt Loam | Coarse-silty, mixed, active, acid, thermic Aquic Udifluvents | None | 97 | 4 * 9.1 | 207 | Flan Fan | 114 | 6.4 |

[Z] Varying application pressure and/or spray tips is due to the use of multiple sprayers at these locations.

**Table 3. Glyphosate formulations applied to GlyTol cotton during the 2007 growing season.**

| Common Name | Trade Name | Formulation | Supplier | Application Rate |
|---|---|---|---|---|
| | | kg ae/L | | kg ae/ha |
| Glyphosate | Roundup OriginalMax | 7.74 | Monsanto Company[z] | 1.12 |
| Glyphosate | Roundup WeatherMax | 7.74 | Monsanto Company[z] | 1.12 |
| Glyphosate | Glyfos X-TRA | 5.16 | Cheminova[y] | 1.12 |
| Glyphosate | Credit Extra | 5.16 | Nufarm Agricultural Products[x] | 1.12 |
| Glyphosate | Touchdown Total | 7.17 | Syngenta Crop Protection[w] | 0.84 |
| Glyphosate | Honcho Plus | 5.16 | Monsanto Company[z] | 1.12 |

[z] Monsanto Company, St. Louis, MO 63167

[y] Cheminova, Inc., Research Triangle Park, NC 27709

[x] Nufarm Agricultural Products, Burr Ridge, IL 60527

[w] Syngenta Crop Protection, Greensboro, NC 19810

Data were analyzed using the PROC MIXED procedure of the Statistical Analysis System (SAS® version 9.1; SAS Institute Inc., Cary, NC). To determine the effect of glyphosate formulation on cotton varieties containing the GHB614 trait, data collected in 2007 were combined over environments, analyzing environment as a random effect, and subjected to an ANOVA, and the means were separated by Fisher's Protected LSD at the 0.05 level of significance. In addition, data were pooled over all locations in 2008 and 2009 to determine effects of glyphosate and/or glufosinate on cotton cultivars containing both the GHB614 and LLCotton25 traits.

## RESULTS AND DISCUSSION

**Cotton Injury.** In 2007, no cotton injury was observed for any treatment after any application timing (Table 4). These results are similar to those from GlyTol cotton tolerance experiments conducted in other locations across the cotton belt (Humphries et al., 2009). During 2008 and 2009, less than 2% injury was noted following 1 to 2 leaf or 7 to 9 leaf applications (Table 5). No crop injury was observed after any other application timing. Similar results were observed by Henniger et al. (2009).

**Plant Height Assessment.** Glyphosate did not influence plant height in 2007 (Table 4). In 2008 and 2009, plant heights ranged from 24 to 25 cm, 57 to 61 cm, 91 to 94 cm, and 106 to 112 cm, 14 d after the 1 to 2 leaf, 7 to 9 leaf, 13 to 16 leaf, and 50% open boll applications, respectively (Table 6). Plant height reductions were not observed following any herbicide application at the 1- to 2-leaf crop stage. A reduction in plant height of up to 4 cm was observed 14 d after application of glufosinate alone compared to glufosinate fb glyphosate at the 7 to 9 and 13 to 16 leaf stages of growth (Table 6). These results are similar to those found by Holloway et al. (2008). Herbicide systems did not reduce plant heights late in the season (Table 6).

**Table 4. Visual GlyTol cotton injury 14 d after glyphosate applications and late-season plant heights in 2007.**

| Treatment[z] | Application Rate | Application Timing | | | | Plant Height |
|---|---|---|---|---|---|---|
| | | 1-2 leaf[y] | 7-9 leaf[y] | 13-16 leaf[y] | 50% open[y] | |
| | kg ae/ha | % | | | | cm |
| Non-treated Check | | 0 | 0 | 0 | 0 | 84 |
| Roundup Original Max | 1.12 | 0 | 0 | 0 | 0 | 81 |
| Roundup Weather Max | 1.12 | 0 | 0 | 0 | 0 | 80 |
| Touchdown Total | 0.84 | 0 | 0 | 0 | 0 | 78 |
| Glyfos X-TRA | 1.12 | 0 | 0 | 0 | 0 | 81 |
| Credit Extra | 1.12 | 0 | 0 | 0 | 0 | 81 |
| Honcho Plus | 1.12 | 0 | 0 | 0 | 0 | 80 |
| LSD (0.05)[x] | | NSD | NSD | NSD | NSD | NSD |

[z] All treatments received four sequential applications at the 1-2 leaf fb 7-9 leaf fb 13-16 leaf fb 50% open growth stages.

[y] Cotton growth stage at time of application.

[x] Means separated according to Fisher's Protected LSD at $P = 0.05$.

**Table 5. Visual injury of GlyTol + LibertyLink cotton following applications of glyphosate and/or glufosinatein 2008 and 2009.**

| Treatment | Application Rate[y] | Application Timing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1-2 leaf[z] | | 7-9 leaf[z] | | 13-16 leaf[z] | | 50% open[z] | |
| | | Days After Treatment | | | | | | | |
| | | 7 | 14 | 7 | 14 | 7 | 14 | 7 | 14 |
| | kg ae ha$^{-1}$[x] | | | | | | | | |
| Non-treated Check | -- | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glyphosate[w] | 1.12 | 1.1 | 0.6 | 1.3 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glufosinate[v] | 0.60 | 0.0 | 0.6 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glufosinate[v] fb glyphosate[w] | 0.60 fb 1.12 | 0.8 | 0.9 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glyphosate[w] fb glufosinate[v] | 1.12 fb 0.60 | 0.0 | 0.7 | 1.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Glufosinate[v] + glyphosate[w] | 0.60 + 1.12 | 0.0 | 0.8 | 1.4 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| LSD (0.05)[u] | | NSD | 0.3 | 0.5 | 0.1 | NSD | NSD | NSD | NSD |

[z] Cotton growth stage at time of application.

[y] All treatments received four sequential applications at the 1-2 leaf fb 7-9 leaf fb 13-16-leaf fb 50% open growth stages. Where glufosinate was followed by glyphosate, glufosinate was applied at 1-2 leaf fb glyphosate at 7-9 leaf glufosinate at 13-16 leaf fb glyphosate at 50% open. Where glyphosate was followed by glufosinate, glyphosate was applied at 1-2 leaf fb glufosinate at 7-9 leaf fb glyphosate at 13-16 leaf fb glufosinate at 50% open.

[x] Glufosinate application rates given as kg ai ha$^{-1}$, whereas glyphosate application rates given as kg ae ha$^{-1}$.

[w] Glyphos X-TRA, Cheminova, Inc. Research Triangle Park, NC 27709 ·

[v] Ignite 280 SL, Bayer CropScience, Research Triangle Park, NC 27709.

[u] Means separated according to Fisher's Protected LSD at $P = 0.05$.

**Table 6. Cotton plant height and abnormal boll number following herbicide application(s) to GlyTol + LibertyLink cotton in 2008 and 2009.**

| Treatment | Application Rate[y] | Plant Height[z] | | | | Abnormal Bolls |
|---|---|---|---|---|---|---|
| | | Application Timing | | | | |
| | | 1-2 leaf[x] | 7-9 leaf[x] | 13-16 leaf[x] | 50% open[x] | |
| | kg ae/ha[w] | cm | | | | number |
| Non-treated Check | --- | 25 | 61 | 93 | 106 | 1 |
| Glyphosate[v] | 1.12 | 25 | 59 | 92 | 109 | 1 |
| Glufosinate[u] | 0.60 | 24 | 57 | 91 | 109 | 1 |
| Glufosinate[u] fb glyphosate[v] | 0.60 fb 1.12 | 25 | 61 | 94 | 112 | 1 |
| Glyphosate[v] fb glufosinate[u] | 1.12 fb 0.60 | 25 | 60 | 93 | 109 | 1 |
| Glufosinate[u] + glyphosate[v] | 0.60 + 1.12 | 24 | 60 | 92 | 110 | 1 |
| LSD (0.05)[t] | | NSD | 3 | 2 | 3 | NSD |

[z] Plant heights were collected from five plants per plot 14 d after each application timing.

[y] All treatments received four sequential applications at the 1-2 leaf fb 7-9 leaf fb 13-16 leaf fb 50% open growth stages. Where glufosinate was followed by glyphosate, glufosinate was applied at 1-2 leaf fb glyphosate at 7-9 leaf fb glufosinate at 13-16 leaf fb glyphosate at 50% open. Where glyphosate was followed by glufosinate, glyphosate was applied at 1-2 leaf fb glufosinate at 7-9 leaf fb glyphosate at 13-16 leaf fb glufosinate at 50% open.

[x] Cotton growth stage at time of application.

[w] Glufosinate application rates given as kg ai ha$^{-1}$, whereas glyphosate application rates given as kg ae ha$^{-1}$.

[v] Glyphos X-TRA, Cheminova, Inc. Research Triangle Park, NC 27709.

[u] Ignite 280 SL, Bayer CropScience, Research Triangle Park, NC 27709.

[t] Means separated according to Fisher's Protected LSD at $P = 0.05$.

**Abnormal or Misshapen Boll Assessment.** No differences in the number of misshapen or abnormal bolls were observed for any treatment (Table 6). All treatments, including the non-treated check, had an average of one misshapen boll.

**Cotton Yield.** Machine-harvested yields in 2007 indicated no difference in seed cotton yield of the GlyTol cultivar due to glyphosate formulation (Table 7). Reduced yield in the non-treated check was likely due to weed competition that might have occurred. Additionally, machine-harvested yields in 2008 and 2009 indicate no adverse effects from glyphosate and/or glufosinate application on yield of the GlyTol + LibertyLink cultivar with yields ranging from 2,806 to 2,902 kg seed cotton per hectare (Table 8). Cultivars provided for these experiments were for proof of concept only. These cultivars were not selected for yield potential, which might account for lower than expected yields.

**Table 7. Machine harvested seed cotton yields in 2007 following four applications of glyphosate to GlyTol cotton.**

| Treatment[z] | Application Rate | Cotton Yield[y] |
|---|---|---|
| | kg ae ha$^{-1}$ | kg ha$^{-1}$ |
| Non-treated Check | -- | 947 |
| Roundup OriginalMax | 1.12 | 1540 |
| Roundup WeatherMax | 1.12 | 1347 |
| Touchdown Total | 0.84 | 1557 |
| Glyfos X-TRA | 1.12 | 1576 |
| Credit Extra | 1.12 | 1485 |
| Honcho Plus | 1.12 | 1410 |
| LSD (0.05)[x] | | 246 |

[z] All treatments received four sequential applications at the 1-2 leaf fb 7-9 leaf fb 13-16 leaf fb 50% open growth stages.

[y] Cotton yield collected from Brooksville, MS in 2007. Other locations were not taken to harvest.

[x] Means separated according to Fisher's Protected LSD at $P = 0.05$.

These data indicate the GlyTol trait technology provides excellent tolerance to multiple POST applications of glyphosate. No adverse effects on plant height, visual injury, or maturity following multiple applications of six different formulations of glyphosate were observed. In addition, GlyTol + LibertyLink technology also provided excellent tolerance to multiple applications of both glyphosate and/or glufosinate with the maximum observed injury being no greater than 2%. Differences in plant height after any application, although occasionally significant, did not exceed 6 cm. Abnormal and/or misshapen boll assessments indicated no adverse effects on boll development due to glyphosate and/or glufosinate application. Development and proliferation of glyphosate-resistant weeds is of major concern, especially in cropping systems that have relied heavily on glyphosate for total POST weed control. GlyTol + LibertyLink technology will allow broad spectrum weed control from two different herbicide chemistries. Although this technology will provide benefits for management of glyphosate-resistant weed species, it is vital to utilize additional modes of action in conjunction with this technology to delay the further development of herbicide-resistant weeds. Proper weed management strategies are necessary to maintain the utility of herbicide-resistant technology.

**Table 8. Machine-harvested seed cotton yields in 2008 and 2009 following multiple applications of glyphosate and/or glufosinate to GlyTol + LibertyLink cotton.**

| Treatment[z] | Application Rate | Cotton Yield[y] |
|---|---|---|
| | kg ae ha$^{-1}$[x] | kg ha$^{-1}$ |
| Non-treated Check | --- | 2806 |
| Glyphosate[w] | 1.12 | 2832 |
| Glufosinate[v] | 0.60 | 2839 |
| Glufosinate[v] fb glyphosate[w] | 0.60 fb 1.12 | 2881 |
| Glyphosate[w] fb glufosinate[v] | 1.12 fb 0.60 | 2896 |
| Glufosinate[v] + glyphosate[w] | 0.60 + 1.12 | 2902 |
| LSD (0.05)[u] | | NSD |

[z] All treatments received four sequential applications at the 1-2 leaf fb 7-9 leaf fb 13-16 leaf fb 50% open growth stages. Where glufosinate was followed by glyphosate, glufosinate was applied at 1-2 leaf fb glyphosate at 7-9 leaf fb glufosinate at 13-16 leaf fb glyphosate at 50% open. Where glyphosate was followed by glufosinate, glyphosate was applied at 1-2 leaf fb glufosinate at 7-9 leaf fb glyphosate at 13-16 leaf fb glufosinate at 50% open.

[y] Cotton yield collected from Rower, AR in 2008; Marianna, AR in 2008; Brooksville, MS in 2008; Starkville, MS in 2008; and Jackson, TN in 2008 and 2009. Extreme rainfall prevented plot harvest at other locations in 2009.

[x] Glufosinate application rates given as kg ai ha$^{-1}$, whereas glyphosate application rates given as kg ae ha$^{-1}$.

[w] Glyphos X-TRA, Cheminova, Inc. Research Triangle Park, NC 27709.

[v] Ignite 280 SL, Bayer CropScience, Research Triangle Park, NC 27709.

[u] Means separated according to Fisher's Protected LSD at $P = 0.05$.

## ACKNOWLEDGEMENT

The authors would like to thank Bayer CropScience for partial support of this research. Approved for publication as Journal Article No. J-12331 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

## REFERENCES

Anonymous. 2013. Liberty 280 SL specimen label [Online]. Available at http://www.cdms.net/LDat/ldUA5000.pdf (verified 19 Mar. 2013).

Bradley, J.F. 2000. Economic comparison of weed control systems in conservation tillage systems. p. 1474–1476 *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX. 4-8 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN.

Corbett, J.L., S.D. Askew, W.E. Thomas, and J.W. Wilcut. 2004. Weed efficacy evaluations for bromoxynil, glufosinate, pyrithiobac, and sulfosate. Weed Technol. 18:443–453.

Culpepper, A.S., A.C. York, P. Roberts, and J.R. Whitaker. 2009. Weed control and crop response to glufosinate applied to 'PHY 485 WRF' cotton. Weed Technol. 23:356–362.

Culpepper, A.S., A.C. York, R.B. Batts, and K.M. Jennings. 2000. Weed management in glyphosate- and glufosinate-resistant soybean (*Glycine max*). Weed Technol. 14:77–88.

Culpepper, A.S., and A.C. York. 1998. Weed management in glyphosate-tolerant cotton. J. Cotton Sci. 2:174–185.

Culpepper, A.S., and A.C. York. 1999. Weed management and net returns with transgenic, herbicide-resistant, and non-transgenic cotton (*Gossypium hirsutum* L.). Weed Technol. 13:411–420.

Everman, W.J., I.C. Burke, J.R. Allen, J. Collins, and J.W. Wilcut. 2007. Weed control and yield with glufosinate-resistant cotton weed management systems. Weed Technol. 21:695–701.

Faircloth, W.H., M.G. Patterson, C.D. Monks, and W.R. Goodman. 2001. Weed management programs for glyphosate-tolerant cotton (*Gossypium hirsutum*). Weed Technol. 15:544–551.

Ferreira, K.L., D.J. Jost, G.A. Dixon, and D.W. Albers. 1998. Roundup Ready Cotton fruiting patterns response to over the top applications of Roundup Ultra after the 4 leaf stage. p. 848 *In* Proc. Beltwide Cotton Prod. Res. Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Frans, R.E., R. Talbert, D. Marx, and H. Crowley. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. p. 37–38 *In* N.D. Camper (ed.) Research Methods in Weed Science. 3rd ed. Southern Weed Science Society, Champaign, IL .

Gardner, A.P., A.C. York, D.L. Jordan, and D.W. Monks. 2006. Management of annual grasses and *Amaranthus* spp. in glufosinate-resistant cotton. J. Cotton Sci. 10:328–338.

Green, J.M. 2009. Evolution of glyphosate-resistant crop technology. Weed Sci. 57: 108–117.

Grichar, W.J., B.A. Besler, K.D. Brewer, and B.W. Minton. 2004. Using soil-applied herbicides in combination with glyphosate in a glyphosate-resistant cotton herbicide program. Crop Protect. 23:1007–1010.

Heap, I. 2013. The International Survey of Herbicide Resistant Weeds [Online]. Available at www.weedscience.org (verified 19 Mar. 2013).

Henniger, G., T. Baughman, P. Baumann, R. Boman, P. Dotray, W. Keeling, R. Lemon, D. Palrang, L. Trolinder, and J. Holloway. 2009. Performance of GlyTol™ and GlyTol™ + LibertyLink® cotton technologies in Texas. p. 1357 *In* Proc. Beltwide Cotton Prod. Res. Conf., San Antonio, TX. 5-8 Jan. 2009. Natl. Cotton Counc. Am., Memphis, TN.

Holloway, J., L. Trolinder, J.M. Ellis, and S. Baker. 2008. New Herbicide Tolerance Technology for Glyphosate Resistant Weed Management in Cotton. p. 1726 *In* Proc. Beltwide Cotton Prod. Res. Conf. Nashville, TN 8-11 Jan. 2008. Natl. Cotton Counc. Am., Memphis, TN.

Humphries, S. Wright, L. Trolinder, H. Moser, and J. Holloway. 2009. Performance of GlyTol™ and GlyTol™ + LibertyLink® cotton in California and Arizona. p. 1337 *In* Proc. Beltwide Cotton Prod. Res. Conf. San Antonio, TX 5-8 Jan. 2009. Natl. Cotton Counc. Am., Memphis, TN.

Kalaher, C.J., and H.D. Coble. 1998. Fruit abscission and yield response of Roundup Ready cotton to topical applications of glyphosate. p. 849 *In* Proc. Beltwide Cotton Prod. Res. Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Kalaher, C.J., H.D. Coble, and A.C. York. 1997. Morphological effects of Roundup application timings on Roundup-Ready cotton. p. 780 *In* Proc. Beltwide Cotton Prod. Res. Conf., New Orleans, LA. 7-10 Jan. 1997. Natl. Cotton Counc. Am., Memphis, TN.

Koger, C.H., I.C. Burke, D.K. Miller, J.A. Kendig, K.N. Reddy, and J.W. Wilcut. 2007. MSMA antagonizes glyphosate and glufosinate efficacy on broadleaf and grass weeds. Weed Technol. 21:159–165.

Lemon, R., R. Boman, T. Baughman, P. Baumann, and P. Dotray. 2005. Roundup Ready Flex cotton system. Texas Cooperative Extension publication [Online]. Available at http://varietytesting.tamu.edu/cotton/files/scs-2005-18.pdf (verified 19 Mar. 2013).

Lemon, R., T. Baughman, R. Boman, P. Dotray, and P. Baumann. 2004. LibertyLink cotton system. Texas Cooperative Extension publication [Online]. Available at http://lubbock.tamu.edu/files/2011/10/liblinkcot.pdf (verified 25 February 2013).

Main, C.L., M.A. Jones, and E.C. Murdock. 2007. Weed response and tolerance of enhanced glyphosate-resistant cotton to glyphosate. J. Cotton Sci. 11: 104–109.

Martens, A.J. Hart, B. Salmons, E. Cerny, S.Huber, and M. Oppenhuizen. 2003. 2002 Results of Roundup Ready Flex Trial. p. 2245 *In* Proc. Beltwide Cotton Prod. Res. Conf., Nashville, TN. 6-10 Jan. 2003. Natl. Cotton Counc. Am., Memphis, TN.

Matthews, S.G., G. N. Rhodes, T.C. Mueller, and R.M. Hayes. 1998. Effects of Roundup Ultra on Roundup Ready cotton. p. 2245 *In* Proc. Beltwide Cotton Prod. Res. Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Norsworthy, J.K., G.M Griffith, R.C. Scott, K.L. Smith, and L.R. Oliver. 2008. Confirmation and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in Arkansas. Weed Technol. 22:108–113.

Parker, R.G., A.C. York, and D.L. Jordan. 2005. Comparison of glyphosate products in glyphosate-resistant cotton (*Gossypium hirsutum*) and corn (*Zea mays*). Weed Technol. 19:796–802.

Pline-Srnic, W.A., K.L. Edmisten, J.W. Wilcut, R.W. Wells, and J.L. Thomas. 2004. Effect of glyphosate on fruit retention, yield, and fiber quality of glyphosate resistant cotton. J. Cotton Sci. 8:24–32.

United States Department of Agriculture–Agriculture Marketing Service [USDA-AMS]. 1997. Cotton varieties planted–1997 crop. USDA AMS–Cotton Division, Memphis, TN.

United States Department of Agriculture–Agriculture Marketing Service [USDA-AMS]. 2004. Cotton varieties planted–2004 crop. USDA AMS–Cotton Division., Memphis, TN.

United States Department of Agriculture–Agriculture Marketing Service [USDA-AMS]. 2009. Cotton varieties planted–2009 crop. USDA AMS–Cotton Division, Memphis, TN.

United States Department of Agriculture–Agriculture Marketing Service [USDA-AMS]. 2012. Cotton varieties planted–2012 crop. USDA AMS–Cotton Division, Memphis, TN.

United States Department of Agriculture–National Agriculture Statistics Service [USDA-NASS]. 1998. Pest management practices. 1997 Summary [Online]. Available at http://usda.mannlib.cornell.edu/usda/nass/PestMana//1990s/1998/PestMana-08-25-1998.pdf (verified 19 Mar. 2013).

United States Department of Agriculture–National Agriculture Statistics Service [USDA-NASS]. 2008. Agricultural chemical usage 2007 field crops summary [Online]. Available at http://usda.mannlib.cornell.edu/usda/nass/AgriChemUsFC//2000s/2008/AgriChemUsFC-05-21-2008.pdf (verified 19 Mar. 2013).

Wilson, D.G, Jr., A.C. York, and D.L. Jordan. 2007. Effect of row spacing on weed management in glufosinate-resistant cotton. Weed Technol. 21:489–495.

York, A.C., A.S. Culpepper, D.T. Bowman, and O.L. May. 2004. Performance of glyphosate-tolerant cotton cultivars in official cultivar trials. J. Cotton Sci. 8:261–270.