The *Journal of Cotton Science 20:271–279 (2016)*
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WEED SCIENCE

Effect of Shading, Cultivar, and Application Timing on Cotton Tolerance to Glufosinate

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ABSTRACT

The increasing presence of glyphosate-resistant (GR) weeds in the Midsouth, and inconsistent crop injury and moisture dependence of residual herbicides has created a need for effective post-emergence options. Cotton cultivars with tolerance to glufosinate have been widely adopted by growers throughout the Midsouth because glufosinate provides an effective option for controlling GR weeds like Palmer amaranth (*Amaranthus palmeri* (S.) Wats.]. The objective of this study was to determine if differences exist in tolerance of PhytoGen® and Liberty Link® cultivars to glufosinate applied at different growth stages in the presence and absence of low-light conditions. At two weeks after cotton emergence (WAE), tolerance to glufosinate differed by cultivar, although some injury was observed on Liberty Link cotton. Injury was often greatest when applied at the one-leaf stage to PhytoGen® cultivars, but by four to five weeks after treatment, all cultivars showed similar potential to recover. In general, cotton plants that were shaded three days prior to applying glufosinate were injured to a greater extent than non-shaded plants. Similarly, seed cotton yields were reduced in shaded plots by 72 and 76 g m^{-1} of row in 2012 and 2013, respectively. This research indicates that there is greater risk for early-season injury from glufosinate if applied to young cotton experiencing prolonged cloudy conditions prior to application; albeit, this injury does not translate into seed cotton yield loss for the three cultivars evaluated, compared to an untreated control. Hence, it is recommended that growers make timely applications of glufosinate to optimize weed control, even when conditions have been less than ideal for cotton growth prior to application.

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Since the 1950s, synthetic herbicides have become an increasingly critical tool in the improvement of cotton yields through the control of problematic weed species that compete for light, nutrients, and moisture (Duke and Powles, 2008; McWhorter and Bryson, 1992). Improvements in weed control are directly attributable to increased cotton yield and quality throughout the southern United States (US), while also reducing labor costs and time requirements. Arguably, the most influential achievement in weed control over the past 50 years was the increased availability of post-emergence (POST) herbicides.

Glyphosate was first registered in 1974 for burn-down purposes and the control of perennial weeds in non-crop areas. Increased utility of glyphosate was recognized in 1996 with the release of glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.], which was subsequently followed by the release of GR cotton, corn (*Zea mays* L.), and canola (*Brassica napus* L.). Midsouth cotton producers widely accepted GR technology due to cost-savings, improved weed management, and simplicity of use (Duke and Powles, 2009; Norsworthy et al., 2016). In 2000, after the loss of patent rights to glyphosate, the price of glyphosate decreased by 40% in the United States (Duke and Powles, 2009; USDA-NASS, 2016). The low price of glyphosate and ability to control a broad spectrum of weed species resulted in extensive in-crop use of this herbicide. This over-reliance on a single mechanism of action increased the selection pressure on weed populations for potential resistance. The eventual occurrence of GR weeds was inevitable considering the vast acreage treated and the fact that other herbicides with differing mechanisms of action were seldom employed in glyphosate-resistant crops.

The presence of GR weeds has encouraged many to evaluate the efficacy of the glutamine synthetase inhibitor glufosinate (Bellinder et al., 1987). Glufosinate-resistant varieties of cotton were developed using either the *pat* or *bar* gene which expresses different enzymes that confer varying levels of tolerance to glufosinate (Steckel et al., 2012). The *pat* gene is generally used as a selective marker to confirm successful insertion of an insect resistance trait,

such as WideStrike®. A higher level of tolerance to foliar applications of glufosinate is conferred by the *bar* gene, which is found in glufosinate-resistant cotton cultivars, such as Liberty Link® (Anonymous, 2015). Although glufosinate provides no soil activity, it can serve as an effective management tool as no weed biotypes in cotton are currently resistant to glufosinate (Heap, 2016). Many scientists believe the answer to acceptable control and prevention of resistance should involve the integration of soil-applied residual herbicides with a glyphosate/glufosinate rotation program (Norsworthy et al., 2012). Exploring the efficacy of glufosinate in various environmental and cultural conditions could potentially reduce the number of applications and selection for herbicide resistance (Wilcut et al., 2002; Norsworthy et al., 2012; UADOA, 2012).

In croplands with high populations of GR weeds like Palmer amaranth, glufosinate can provide adequate control when applied at appropriate times and rates (Culpepper et al., 2009; Everman et al., 2007; Steckel et al., 1997). Because glufosinate is a contact herbicide, efficacy is dependent on several factors including coverage, relative humidity (RH), and weed size (Coetzer et al., 2001; Hoss et al., 2003; Riar et al., 2011). Properly evaluating the environmental and agronomic factors that influence the efficacy of glufosinate could improve the utility of this herbicide as a tool for the management of GR weeds.

The efficacy of POST herbicides is influenced by environmental conditions before, during, or after the time of application as plant foliar and root uptake is impacted (Cole, 1983). Factors such as temperature, sunlight, time of day, relative humidity, and soil water content have been well documented to affect the foliar activity of POST herbicides such as glufosinate (Garcia et al., 2002; Stevens and Baker, 1987; Sellers et al., 2004). However, the impact of shade on glufosinate activity has not been explored. This is important since the activity of carfentrazone-ethyl, which is also a post-emergence contact herbicide, has been shown to increase across many crop species when shade is present (Hammerton, 1967; Kolattukudy, 1970; Kunst and Samuels, 2003; Thompson and Nissen, 2002).

Little research has been conducted evaluating the impact of glufosinate on vegetative cotton injury under low-light or cloudy conditions. Soybean crop injury from herbicides has been associated with reduced herbicide metabolism, herbicide sequestration, chlorophyll biosynthesis, proto-degradation, or free radical detoxification under low-light conditions (Dayan and Duke, 1997). In cotton, the existence of lowered *pat* gene activity in WideStrike (Phytogen) cultivars translates into incomplete glufosinate tolerance compared to the *bar* gene found in LibertyLink® cultivars (Herouet et al., 2005; Steckel et al., 2012). Furthermore, this reduced tolerance has been documented to result in 11 to 25% crop injury with single applications of glufosinate (Culpepper et al., 2009; Whitaker et al., 2011; Sweeney and Jones, 2015). Therefore, the objective of this research was to assess the response of PhytoGen® and LibertyLink® cotton to glufosinate applied at different growth stages when low-light conditions precede the application.

MATERIALS AND METHODS

In 2012 and 2013, a split-split-strip plot field experiment with four replications was conducted at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR on a Leaf silt loam soil (Fine, mixed, active, thermic Typic Albaquults; with 34% sand, 53% silt, 13% clay, 1.5% organic matter, and a pH of 6.9) (USDA-NRCS 2015). These cultivars were chosen based on their use in the midsouthern US. Additionally, two cultivars of PhytoGen® cotton were used because these cultivars do not express the *bar* gene sufficiently to warrant complete tolerance to glufosinate. The main plot consisted of three cotton cultivars (PHY 375 WRF, PHY 499 WRF, and ST 4145LLB2). PhytoGen® seed was obtained from Dow AgroSciences in Indianapolis, IN, and Stoneville® seed was acquired from Bayer Crop Science in Research Triangle Park, NC. Cotton was planted at a rate of 125,000 seed ha^{-1} at a two-cm depth in mid-May. The strip-plot dimensions included two bedded rows spaced 91 cm apart, 3.8 m long. The subplot consisted of three cotton growth stages (one-, four-, and six-leaf stage) at application and the sub-subplot consisted of three herbicide treatments (glufosinate at 0.88 and 1.76 kg ai ha^{-1} and an untreated control). The strip of this experiment consisted of light intensity (shade and non-shaded) organized horizontally across replications. The shaded plots (front three m of sub-subplot) were covered with neutral shade cloth allowing for 50% light penetration without spectrum limitation three d prior to herbicide treatment, and the shade cloth was removed less than one hour prior to applying the herbicide treatments. Shade cloth (Gempler's,

Madison, WI) was supported by a polyvinyl chloride pipe frame 40 cm above the plant canopy. Weed control was supplemented by a pre-emergence (PRE) application of fluometuron (Cotoran®4L, MANA Inc., Raleigh, NC) and *S*-metolachlor (Dual Magnum, Syngenta, Greensboro, NC) at 1.12 and 1.07 kg ai ha^{-1}, respectively, along with hand weeding.

All herbicide applications were made midday using a CO_2-pressurized backpack sprayer calibrated to deliver 187 L ha^{-1}. Cotton injury was assessed at two and four to five weeks after treatment (WAT) on a 0 to 100 scale, with 0 being no injury and 100 being complete death. The test site was routinely scouted and sprayed as needed to manage insects and diseases.

In 2013, the youngest most fully expanded leaf of cotton was sampled to determine cuticle quantity on the day of herbicide application by harvesting three leaves per plot from both the shade and non-shade portions of each plot. Three three-cm^2 sections from each sampled leaf were submersed in chloroform and shaken for 10 min. After removing the cuticle, the chloroform was permitted to evaporate and the vials were weighed to determine the amount of epicuticular wax (ECW). In both years, aboveground cotton biomass was harvested from two m of row and the number of harvested plants counted at 12 WAE. The harvested biomass was oven-dried for seven d at 60 C and weighed. Upon maturity, seed cotton was hand harvested from two m of row from all plots and weighed.

Data were subjected to a fixed effects test in JMP Pro 11 (SAS Institute Inc., Cary, NC). The variability of irrigation and precipitation events led to years being analyzed separately as a by-variable using GLM Mixed under an effect leverage personality. Under this mixed model and residual maximum likelihood (REML), p-values were generated and means associated with significant interactions were separated using Tukey's HSD at the alpha level of 0.05 (SAS Institute Inc., 2014).

RESULTS AND DISCUSSION

In 2012, malfunction of irrigation equipment accompanied by extended periods of hot, dry weather contributed to an overall reduction in cotton growth compared to 2013. The irrigation and environmental differences between years in Fayetteville, AR prompted the separation of trial years as a by-variable during statistical analyses (Figure 1).

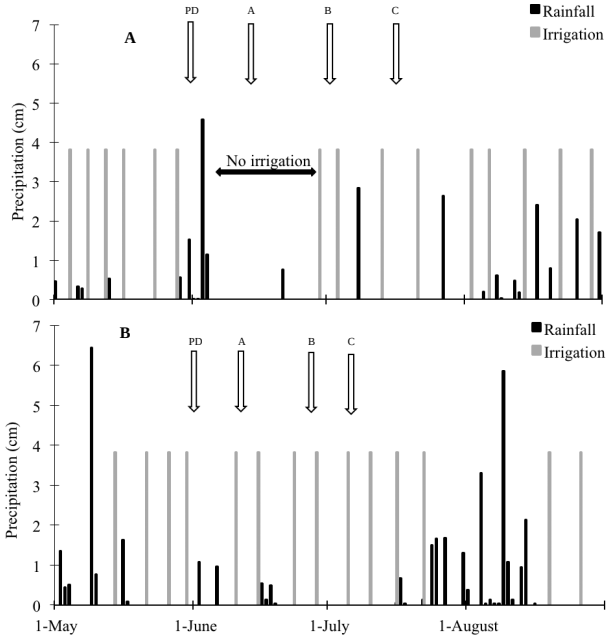

Figure 1. Rainfall and irrigation distribution at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR in 2012 (a) and 2013 (b) displaying planting dates (PD) and application timings (A,B,C).

2012. At two WAT, there was a significant (P=0.0146) interaction between stage and shade (Table 1); however by four to five WAT, this interaction was no longer significant, likely as a result of the ability of cotton to recover from the initial stresses from the herbicide and shading. Late-season assessment of cotton aboveground biomass was impacted only by the main effects of shading and herbicide, but not cultivar.

Initial Injury. Cotton that had been shaded prior to treatment at the one-leaf stage exhibited more damage at two WAT when compared to other growth stages at application, averaged over cultivar and glufosinate rate (Figure 2). No difference was observed between cultivars or shading when cotton was treated with glufosinate at 0.88 kg ai ha^{-1} although trends for injury to increase were present when all cultivars were subjected to simulated cloud cover three d prior to application (Figure 3).

Recovery from Injury. At four to five WAT, all cotton cultivars had shown potential to recover from earlier injury (Table 1). Tukey's HSD as well as a Student's t-test failed to observe statistical separation of means for crop injury at four to five WAT in 2012, prompting a more detailed contrast for two-way components within the two-way interaction of shading and cultivar. Further data analysis indicated that there was a significant (P=0.0240) interaction of cultivar and shade on cotton injury four to five WAT in 2012 (Table 1).

Table 1. Fixed effects test for three cotton cultivars treated with various rates of glufosinate at three leaf stages in the presence and absence of preceding shade at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR

Source	2012			2013		
	2 WAT[z]	4 to 5 WAT	Biomass[y]	2 WAE	4 to 5 WAT	Biomass
	---------- Prob > F[x] ----------					
Cultivar	0.0700	0.2373	0.5673	0.0008	0.0893	0.9597
Stage	0.0001	0.7544	0.0188	0.0001	0.0613	0.6139
Herbicide	0.0008	0.1198	0.0005	0.0001	0.7346	0.6588
Shade	0.0247	0.2702	0.0533	0.0031	0.0582	0.1063
Cultivar*Stage	0.0190	0.6366	0.3044	0.0006	0.7346	0.5490
Cultivar*Herbicide	0.8487	0.3525	0.6473	0.0780	0.0531	0.6368
Cultivar*Shade	0.2349	0.0240	0.9181	0.0131	0.0966	0.2143
Stage*Herbicide	0.0688	0.1052	0.4099	0.0068	0.7094	0.6366
Stage*Shade	0.0146	0.1863	0.0385	0.0066	0.0832	0.7982
Herbicide*Shade	0.3045	0.9696	0.0068	0.2861	0.7160	0.7014
Cultivar*Stage*Herbicide	0.3504	0.3372	0.0689	0.1517	0.0393	0.2845
Cultivar*Stage*Shade	0.6772	0.7815	0.3884	0.0360	0.0817	0.7748
Cultivar*Herbicide*Shade	0.0413	0.1666	0.7116	0.3747	0.5445	0.8540
Stage*Herbicide*Shade	0.1399	0.5397	0.2997	0.5985	0.7439	0.1847
Cultivar*Stage*Herbicide*Shade	0.2175	0.1103	0.0957	0.6164	0.5310	0.5045

[z] 2 WAT refers to early-season cotton injury observed 2 weeks after treatment with glufosinate.

[y] Biomass includes all above ground plant tissue harvested, dried, and weighed 12 WAP.

[x] Source values lower than the alpha level of 0.05 are statistically significant.

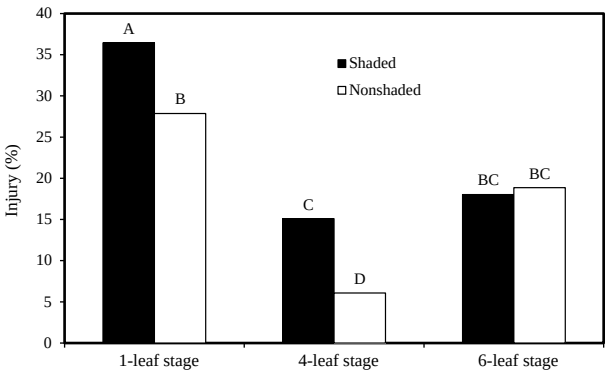

Figure 2. Cotton injury observed 2 weeks after treatment in 2012 on cotton of three growth stages applied with glufosinate in the presence and absence of shade 3 days prior to application. Letters represent significant differences according to Tukey's HSD.

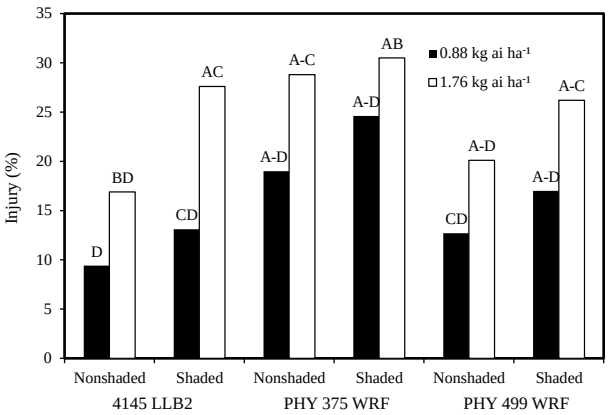

Figure 3. Cotton injury at 2 weeks after treatment in 2012 on three cotton cultivars following applications of glufosinate that had been shaded or not shaded for 3 days prior to application. Means were averaged over growth stage at application. Letters represent significant differences according to Tukey's HSD.

Late-Season Biomass. The biomass collected 12 weeks after planting (WAP) displayed shade by growth stage and shade by herbicide interactions in 2012 (Table 1). Cotton treated at the four-leaf stage in absence of shade produced 135 g m^{-1} of row (38%) more biomass than shaded plants (Figure 4). This result may be partially attributed to the greater injury caused by the glufosinate application to shaded cotton as well as the reduced photosynthesis and growth associated with the three-day period of shaded conditions. When cotton was treated with glufosinate at 1.76 kg ha^{-1}, shaded plants produced less biomass, averaged over cultivars (Figure 5). It is also interesting that cotton biomass under non-shaded conditions increased 42 to 44% when treated with glufosinate. Escaped weeds were hand removed periodically throughout the growing season; albeit, the lower biomass production in the absence of the herbicide would indicate that presence of some weeds impacted cotton growth slightly (although statistically not significantly). There was one or two escaped Palmer amaranth plants in each plot, but these were considered to be late emerging and were often small. These escapes often emerged from four to six weeks after planting. It is implausible to believe that glufosinate in the absence of weeds increased cotton growth (Sweeney and Jones, 2015).

Seed cotton Yield. A significant (P=0.0123**)** herbicide by shade interaction was observed for seed cotton yield (Table 2). There was 45% greater seed cotton yield for non-shaded compared to shaded plants when treated with glufosinate at 1.76 kg ai ha^{-1}, averaged over cultivars and growth stages (Figure 6). Although seed cotton yield differences between shaded and non-shaded cotton treated with glufosinate at 0.88 kg ai ha^{-1} were not significant, the trend was similar to that observed for the higher rate of glufosinate.

Cotton treated with glufosinate at the one- or four-leaf stage produced higher yields than applications to six-leaf cotton (data not shown). The fact that the irrigation system was not functional during a portion of the 2012 growing season and that some yield impacting weed interference may have occurred early in the season prior to applying glufosinate likely contributed to these observed differences. However, the following results are due primarily to a treatment effect and not to the presence of some weeds. Interestingly, there was no cultivar main effect or interaction with cultivar, indicating that the cultivars had similar yields and were not impacted

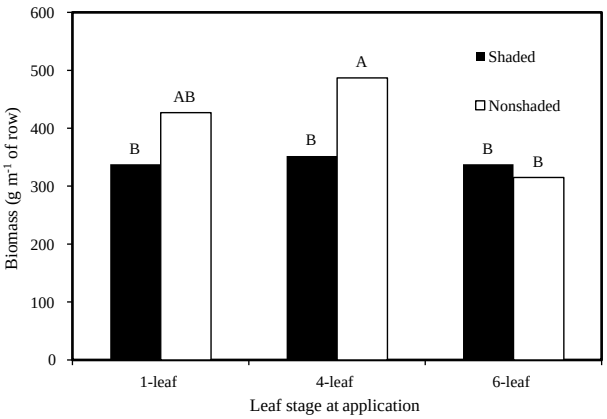

Figure 4. Cotton biomass at 12 weeks after planting from plots of various growth stages treated with various rates of glufosinate in the presence and absence of shade 3 days prior to the glufosinate application in 2012. Means are averaged over application rate and cultivar. Letters represent significant differences according to Tukey's HSD test.

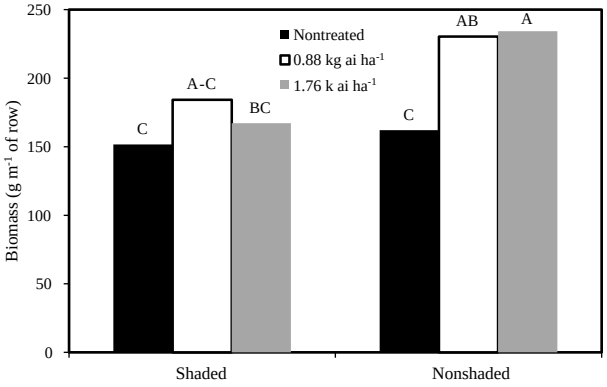

Figure 5. Cotton biomass at 12 weeks after planting from plots treated with various rates of glufosinate in the presence and absence of shade 3 days prior to the glufosinate application in 2012. Means are averaged over cotton stage at application and cultivar. Letters represent significant differences according to Tukey's HSD test.

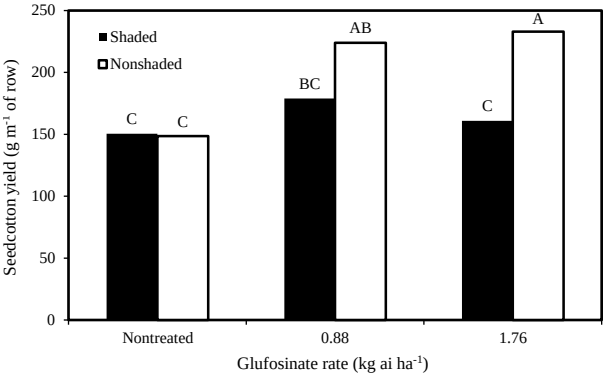

Figure 6. Seedcotton yield after early-season glufosinate applications made in the presence and absence of shade 3 days prior to application in 2012, averaged over cotton stage at application and cotton cultivar. Letters represent significant differences according to Tukey's HSD test.

by the factors evaluated. Hence, differences in early-season injury to cotton among cultivars caused by glufosinate did not translate into yield loss. Similar results were determined by Barnett et al. (2015) who showed no significant differences in yield loss when one or two glufosinate applications were made at the two- and seven-leaf stage to WideStrike cotton.

Table 2. Fixed effects tests for the seedcotton yield of three cotton cultivars applied with various rates of glufosinate at three leaf stages in the presence and absence of shade at the Arkansas Agricultural Research and Extension Center in Fayetteville, AR

Source	Seedcotton yield[z]	
	2012	2013
	------- Prob > F[y] -------	
Cultivar[x]	0.1172	0.9559
Stage[w]	0.0081	0.4295
Herbicide[v]	0.0001	0.5711
Shade[u]	0.0003	0.0001
Cultivar*Stage	0.6314	0.2324
Cultivar*Herbicide	0.9830	0.5214
Cultivar*Shade	0.6795	0.3314
Stage*Herbicide	0.7648	0.5399
Stage*Shade	0.1117	0.8849
Herbicide*Shade	0.0123	0.9065
Cultivar*Stage*Herbicide	0.0762	0.1603
Cultivar*Stage*Shade	0.5412	0.8949
Cultivar*Herbicide*Shade	0.9756	0.9507
Stage*Herbicide*Shade	0.6964	0.5177
Cultivar*Stage*Herbicide*Shade	0.4503	0.8974

[z] Seedcotton was collected upon reproductive maturity in the form of g m row^{-1}.

[y] Source values lower than the alpha level of 0.05 are statistically significant.

[x] Cotton cultivars tested include PHY 375 WRF, PHY 499 WRF, and 4145 LLB2.

[w] Glufosinate was applied at the 1-, 4-, and 6-leaf stage.

[v] Herbicide treatments included glufosinate at 0.88 and 1.76 kg ai ha^{-1} and a nontreated control.

[u] Treatments included the presence and absence of simulated cloud cover (shade cloth) 3 days prior to treatment with glufosinate.

2013. Initial Injury. In 2013, there was a shade by cultivar by leaf stage interaction for injury at two WAT (Table 1). PHY 357 WRF treated at the one-leaf stage and PHY 499 WRF treated at the four-leaf stage with glufosinate had increased injury at two WAT when shading occurred prior to application (Figure 7). It may be plausible that low leaf photosynthesis of shaded cotton and decreased electron transport capacity (Zhao and Oosterhuis, 1998b), hindered the ability of cotton to detoxify ammonia. Glufosinate could then potentially lead to uncoupling of photophosphorylation, resulting in both membrane disruption and lipid peroxidation. Similar to that concluded by Culpepper et al. (2009) and Steckel et al. (2012), the lower tolerance of PhytoGen® WideStrike cultivars is likely to result in greater injury. It appears that the likelihood of observing glufosinate-induced injury to cotton is greatest when the herbicide is applied to small cotton (one- to four-leaf stage) during prolonged periods of cloud cover. It has long been established that cotton cultivars do not respond similarly to cloud events (Goodman, 1955).

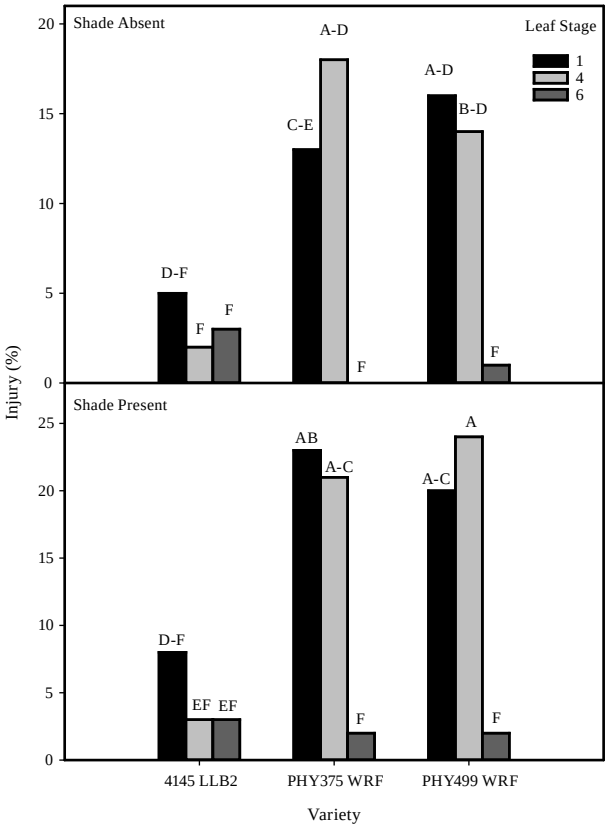

Figure 7. Cotton injury at 2 weeks after treatment in 2013 on three cotton cultivars following glufosinate applications at three different growth stages in the presence and absence of shade at 3 days prior to the glufosinate application. Means are averaged over glufosinate rates. Letters represent significant differences according Tukey's HSD test.

Recovery from Injury. The initial differences in injury from glufosinate among cultivars and ability of cotton to recover from this injury resulted in an interaction of cultivar, leaf stage, and glufosinate rate at four to five WAT in 2013 (Table 1). Even though ANOVA indicated the three-way interaction to be significant, the conservative nature of Tukey's HSD failed to observe significant separation among treatments. Numeric trends indicated that there may be greater risk for injury when glufosinate is applied at 1.76 kg ai ha^{-1} (2X rate) to PhytoGen® WideStrike cultivars compared to the Liberty Link® cultivar, and this risk for injury can escalate when applications are made at the one-leaf stage of cotton (data not shown). This result is further supported by Dodds et al. (2015), who showed Liberty Link cotton experiencing less injury (4%) than PhytoGen cotton (31%) at a 2X rate of glufosinate at seven and 14 d after application at both the three- and eight-leaf stage. Furthermore, Whitaker et al. (2011) observed 3 to 10% injury on PHY 485 WRF cotton five d after treating one- to two-leaf plants with glufosinate at 0.4 kg ha^{-1}. Culpepper et al. (2009) reported 15% injury to PhytoGen WideStrike cotton when sprayed at the same rate.

Late-Season Biomass. Glufosinate applications to shaded and non-shaded cotton did not translate into reduced cotton biomass in 2013. All tested effects for cotton biomass, including main effects and interactions, were nonsignficant at 12 WAP in 2013. Again, these results are evidence that cotton fully recovers from initial injury caused by glufosinate, even when the application and subsequent injury were preceded by a simulated period of cloud cover.

Epicuticular Wax Quantity. As the leaf cuticle plays a crucial role in defending leaves from chemical penetration, thicker cuticles can be expected to reduce the penetration of foliar-applied herbicides (Oosterhuis et al., 1991). Cotton leaves sampled just prior to the one-leaf application stage contained 0.6 to 0.15 g greater ECW per 3.14 cm^2 sample than four- and six-leaf samples; however, no differences in ECW occurred between shaded and non-shaded treatments (data not shown). Therefore, differences in injury between shaded and non-shaded treatments are not likely a result of the ECW content.

Seed cotton Yield. Similar to 2012, early-season injury to cotton from glufosinate did not result in seed cotton yield loss, regardless of herbicide rate, growth stage at application, or cultivar (Table 2). These results over two distinctly different years are promising in that growers can make timely applications of glufosinate for controlling Palmer amaranth and other weeds without fear of yield reduction.

By mid-August, all treatments had visually recovered from injury sustained by the application of glufosinate (data not shown). These visual assessments of phytotoxicity did not, however, illustrate the potential metabolic disruptions that took place resulting in shaded plots producing 820 kg ha^{-1} less seed cotton than non-shaded plots (data not shown). Dunlap (1943) reported that interruptions for two or three days in high sunlight intensities often causes a significant yield reduction. Cotton is extremely sensitive to low photosynthetic photon flux density (PPFD) stress and numerous studies have documented yield reductions reaching 67% within eight days of shading (Knight, 1935; Zhao and Oosterhuis, 2000). Most research, including the before mentioned, explored simulated cloud cover during the fruiting period rather than early vegetative growth. This research would suggest that shading cotton at younger growth stages (prior to seven-leaf) can have lasting effects, and in certain years like 2013, can negatively impact yield. Granted, it is unusual that young cotton would be detrimentally affected by a mere three days of simulated cloud cover, but cotton is very sensitive to early season interference or stress of any kind and any evidence suggesting a potential lag in vegetative and ultimately reproductive growth cannot be overlooked. Zhao and Oosterhuis (2000) hypothesized that "the effects of low PPFD at different developmental stages on cotton growth and yield may be quite different because cotton is perennial with an indeterminate growth habit, and it is very responsive to changes in environments, especially PPFD." This research compliments that hypothesis.

CONCLUSIONS

The impact of cloud cover, which was simulated by means of shade cloth in this research, has been documented as a challenge to global cotton production regarding variability in injury, biomass, ECW, and yield by various cultivars treated with glufosinate. The decrease in photosynthetic irradiance by shading can increase otherwise irrelevant injury and yield losses. It has become evident that the application of glufosinate, namely to control GR weed species such as Palmer amaranth, should be reserved for times of high photosynthetic activity by cotton (Zhao and Oosterhuis, 1998a). It is suggested that

cotton producers refrain from applying high rates of glufosinate on cotton that has been subjected to cloudy conditions for three d. Although no significant yield differences were observed, special caution is advised when the use of glufosinate is employed to control GR weeds in typically less tolerant PhytoGen® cotton systems compared to Liberty Link®.

ACKNOWLEDGEMENT

Approved for publication as Journal Article No. J-0000

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