Evaluation of Post Applied S-metolachlor in Combination with Glufosinate in Cotton (*Gossypium hirsutum*)

by

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Abstract

Studies were conducted in Alabama in 2016 and 2017 to determine the effect of postemergence applications of glufosinate alone and glufosinate applied with S-metolachlor, using two different nozzle types, on LibertyLink®, Xtend®, and WideStrike® cotton. Field trials consisted of two applications of glufosinate at 0.6 kg ha^{-1}, and glufosinate with S-metolachlor at 1.39 kg ha^{-1} applied to each cotton cultivar at the four-leaf and eight-leaf growth stages using a flatfan and Turbo TeeJet Induction® nozzle. Visual estimates of cotton injury were evaluated after each application, as well as yield. No differences in yield, within each cotton cultivar were observed for either year. Visual injury was higher for WideStrike cotton than LibertyLink or Xtend cultivars. On average, glufosinate applied with S-metolachlor resulted in higher injury than glufosinate applied alone. In some instances, applications made with TTI nozzles resulted in greater injury than flatfan nozzles. Greenhouse studies were conducted to determine the effects of applications on gas exchange measurements such as CO_2 assimilation and stomatal conductance. There were no differences between gas exchange measurements in LibertyLink, and Xtend cotton following herbicide treatments. CO_2 assimilation and stomatal conductance in WideStrike cotton significantly decreased following herbicide application, regardless of herbicide treatment or nozzle type. Gas exchange measurements of WideStrike cotton fully recovered to levels equal to or greater than the nontreated cotton by seven days after treatment. These data indicate that applications of

glufosinate and glufosinate applied with S-metolachlor, at 0.6 kg ha^{-1} and 1.39 kg ha^{-1}, respectively, with either a flatfan or TTI nozzle, made under certain conditions can have no detrimental effect on cotton growth, yield, or the plant processes of photosynthesis or leaf conductance past seven days.

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Cotton (*Gossypium hirsutum*) Cultivar Response to Glufosinate Plus S-metolachlor Applied POST Using Two Nozzle Types

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Field studies were conducted in Alabama in 2016 and 2017 to determine the effect of postemergence applications of glufosinate alone and glufosinate applied with S-metolachlor, using two different nozzle types, on LibertyLink®, Xtend®, and WideStrike® cotton growth and yield. Two applications of glufosinate at 0.6 kg ha^{-1}, and glufosinate with S-metolachlor at 1.39 kg ha^{-1} were applied to each cotton cultivar at the four-leaf and eight-leaf growth stages using a flatfan and Turbo TeeJet Induction® nozzle. Visual estimates of cotton injury were evaluated after each application, as well as yield. No differences in yield, within each cotton cultivar were observed for either year. Visual injury was higher for WideStrike cotton than LibertyLink or Xtend cultivars. On average, glufosinate applied with S-metolachlor resulted in higher injury than glufosinate applied alone. In some instances, applications made with TTI nozzles resulted in greater injury than flatfan nozzles. However, cotton injury was transient and did not affect cotton yields. These data indicate that applications of glufosinate and glufosinate applied with S-metolachlor, at 0.6 kg ha^{-1} and 1.39 kg ha^{-1}, respectively, with either a flatfan or TTI

nozzle, made under certain conditions can have no detrimental effect on cotton growth or yield.

Introduction

Glyphosate and Glyphosate-Resistant Cotton

Glyphosate-resistant cotton (GR), introduced in 1997, was widely adopted due to the herbicide efficacy on a diverse spectrum of weed species postemergence (POST), as well as the economic benefits of the herbicide system (Askew et al. 2002; Culpepper and York 1998, 1999a). However, the extensive use of GR cotton cultivars has placed intense herbicide selection pressure on certain weeds, developing herbicide resistant weed species, ultimately limiting the utility of glyphosate for POST weed control in cotton (Culpepper et al. 2006; Steckel et al. 2008). The most notable GR weed species in cotton systems, Palmer amaranth (*Amaranthus palmeri*), was first documented in 2006, and has since become one of the most troublesome weed species and is widespread across the cotton producing regions of the United States (Heap 2017; Webster 2013).

Glufosinate and Glufosinate-Resistant Cotton

Glufosinate is a nonselective, contact herbicide used in agronomic crops in the United States since 1994. A member of the organophosphorus herbicide family, glufosinate inhibits the enzyme glutamine synthetase, leading to an accumulation of ammonia within the plant (CERA 2015; Senseman 2007; Vencill 2002; Zimdahl 2013). Accumulation of ammonia within plants leads to necrosis of plant tissue and ultimately plant death (Coetzer and Al-Khatib 2001; Devine et al. 1993; Everman et al. 2007; Larsen et al. 1981; Wendler et al. 1990).

Being a nonselective contact herbicide, historically the primary use of glufosinate in agronomic crops was either as a burn down application prior to crop emergence, or a postemergence - directed spray (PDS) application during the growing season (Coetzer et al. 2002). Glufosinate-resistant cotton was granted regulatory approval in 2003 and became commercially available in 2004 as LibertyLink® (Bayer CropScience, Research Triangle Park, NC) cultivars (CERA 2015; Gardner et al. 2006). The development of glufosinate-resistant cotton was achieved by inserting the *bar* gene from the soil bacterium *Streptomyces hygroscopicus* using *Agrobacterium tumefaciens* (CERA 2015). The *bar* gene is responsible for expressing resistance to glufosinate through the enzyme phosphinothricin-acetyl-transferase (*pat*) enzyme which acetylates glufosinate ammonium, transforming it to the inactive acetylated form , N-acetyl-L-phosphinothricin (CERA 2015; Devine et al. 1993; Hérouet et al. 2005; OECD 2002). Cotton that has the *bar* gene transformation has been shown to possess excellent tolerance to POST applications of glufosinate (Blair-Kerth et al. 2001). According to the manufacturer, glufosinate can be applied in broadcast, over-the-top applications to glufosinate-resistant cotton from emergence until early bloom (Anonymous 2017).

Glufosinate alone has been shown to be effective in controlling numerous weed species that are common in cotton cropping systems such as velvetleaf (*Abutilon theophrasti* Medik.), common ragweed (*Ambrosia artemisiifolia* L.), and morningglory species (*Ipomoea* spp.) (Beyers et al. 2002, Culpepper and York 1999b, Everman et al. 2007). However, glufosinate is typically less effective in controlling grass species than broadleaves (Corbett et al. 2004). Since the widespread occurrence of GR, weed control systems utilizing glufosinate based systems have shown to be an effective alternative to glyphosate based systems for POST weed control in cotton (Barnett et al. 2013, Culpepper et al. 2009, Gardner et al. 2006, Whitaker et al. 2011a). However, these cultivars were not widely adopted by growers, likely due to hesitation to abandon glyphosate use entirely, and poor agronomic performance across LibertyLink® cultivars. The ability to apply glufosinate in combination with glyphosate over the top in cotton, a key benefit for many producers, was not available until 2012 with the release of GlyTol® LibertyLink® (Bayer CropScience) cotton cultivars.

Glyphosate and Glufosinate-Resistant Cotton

Widestrike™ cotton (DowDuPont, Wilmington, Delaware) was developed to confer resistance to lepidopteran pests (CERA 2015; Culpepper et al. 2009). During the breeding process the *bar* gene was inserted as a marker to determine the presence of the insecticidal proteins, thereby conferring some resistance to glufosinate. Because the *pat* gene was used only as a marker in the breeding process, the level of activity of *pat* is lower in Widestrike cultivars compared to LibertyLink cultivars (OECD 2002; Tan et al. 2006). Crossing of Widestrike cultivars with glyphosate resistant cotton cultivars have produced cultivars that are both glyphosate resistant as well as glufosinate resistant, giving producers more flexibility in POST weed management strategies (Steckel et al. 2012). Unlike LibertyLink cultivars, Widestrike cultivars have been widely adopted by producers due to their agronomic traits as well as the combination of herbicide tolerance that they possess. In 2016, 29.98% of all cotton planted in Alabama was WideStrike cultivars, which only six years earlier in 2010 comprised only 13.9% of the cotton planted in the state (USDA-AMS 2010).

One to two POST- applications of glufosinate to Widestrike cotton has been documented to cause visual plant injury ranging from 5-25% without having a negative effect on yield (Culpepper et al. 2009; Dodds et al. 2015; Whitaker et al. 2011a). Three applications of glufosinate to WideStrike cotton however, has been shown to reduce yield up to 7 percent (Barnett et al. 2015). Glufosinate applications in combination with other herbicides has also been shown to delay cotton maturity and reduce yield (Steckel et al. 2012). The applied glufosinate rate influences the degree of visible cotton injury as well as yield, as rates higher than the standard 0.6 kg ha^{-1} rate, tending to cause more injury. Glufosinate applied at 1.2 kg ha^{-1} to Widestrike cotton has shown to delay plant maturity as well as reduce cotton yield (Dodds et al. 2015).

Dicamba and Dicamba-Resistant Cotton

Dicamba herbicide is classified as an auxin-mimicking MOA that has long been used for burndown applications prior to planting, as well within season for dicotyledenous weed control in grain crops such as corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], and wheat (*Triticum aestivum* L.) (Mithila et al. 2011). Auxin-mimicking herbicides act by increasing auxin levels within the plant (Grossmann 2010, Mithila et al. 2011). Symptomology of sensitive plants following dicamba exposure include leaf cupping and epinasty, and eventually death of terminal plant tissue, ultimately leading to plant death (Grabińska-Sota et al. 2003; Senseman 2007).

Cotton cultivars resistant to the auxin herbicide dicamba (DR) were brought to market in 2015 (USDA-APHIS 2015). DR cotton was attained through the insertion of the dicamba monooxygenase (*dmo*) gene, isolated from *Stenotrophomonas maltophilia*, which transfers dicamba from the active form to the inactive 3,6-dichlorosalicylic acid and formaldehyde compounds (Behrens et al. 2007). In addition to DR, cultivars resistant to dicamba are also stacked with resistance traits conferring resistance to glufosinate, through the insertion of the *bar* gene, expressing the *pat* enzyme (event MON88701). This cotton cultivar was further crossed with a cotton cultivar resistant to the herbicide glyphosate, resulting in a cultivar resistant to three different modes of action, (MON88701 by MON88913; known as Bollgard II® XtendFlex™ (Xtend); ([ISAAA] 2015).

Dicamba is effective at controlling numerous broad leaf weed species, including troublesome species and species resistant to other herbicides including GR horseweed (*Conyza Canadensis* (L.) Cronq.), GR Palmer amaranth, ALS herbicide resistant Palmer amaranth and Ipomoea spp. (Cahoon et al. 2015, Eubank et al. 2008, Joseph et al. 2012, Montgomery et al. 2017, Steckel et al. 2006). Tank mixing dicamba with glyphosate or glufosinate has resulted in increased control of GR weed species compared to single herbicide applications (Byker et al. 2013; Cahoon et al. 2015; Vann et al. 2017).

Certain environmental conditions, as well as application factors can influence the behavior of dicamba. Conditions such as high wind speed can cause dicamba droplets to drift off target, possibly causing damage to nearby sensitive crops (Everitt and Keeling 2009). Emphasis must be placed on correct nozzle selection in order to prevent off target movement of dicamba with DR cotton.

S-metolachlor

Overlapping residual, soil-applied herbicides applied throughout the growing season are recommended for effective weed control in cotton (Tredaway 2017). S-metolachlor is a herbicide in the chloroacetamide family (Senseman 2007). S-metolachlor's MOA works through the interference of shoot elongation (Fuerst and Gronwald 1986). S-metolachlor has been shown to effectively control many troublesome weeds, such as Palmer amaranth when applied preemergence (PRE) (Geier et al. 2006; Steele et al. 2005). In addition to PRE applications, S-metolachlor may be applied POST over the top in cotton (Tredaway 2017). Although POST applications of S-metolachlor does not provide any control of emerged weeds, the residual control provided is beneficial in cotton production systems (Clewis et al. 2006; Whitaker et al. 2011a, 2011b). However, POST applications of S-metolachlor to cotton typically results in minor crop injury such as necrotic speckling on leaf tissue, although cotton injury can be more severe if cotton is environmentally stressed (Clewis et al. 2006; Tredaway 2017). Cotton yields have not been shown to be adversely affected by POST applications of S-metolachlor (Clewis et al. 2006; Culpepper et al. 2009; Dodds et al. 2010; Stephenson IV et al. 2013).

Herbicide Sprayer Nozzle Selection

With the introduction of crop resistant to auxin mimicking herbicides, there has been a large focus on reducing off-target movement of herbicides. Spray droplet drift is one way that herbicides may move off target, and possibly cause damage to surrounding crops (Ellis and Griffin 2002, Everitt and Keeling 2009, Hurst 1982, Snipes et al. 1991, 1992). Droplet size has been shown to have a major impact on the drift potential of herbicide applications (Mueller and Womac 1997; Whisenant et al. 1993; Yates et al. 1985). There are numerous types of agricultural spray nozzles, which differ primarily based on the spectrum of droplets produced. Other than environmental factors such as wind speed and direction, as well as application speed and terrain type, nozzle type has a great effect on the overall droplet spectrum as it influences spray deposition and pesticide drift (Taylor et al. 2004). Generally, droplet size ranges from 10 to greater than 1,000 μm (Bouse et al. 1990). Droplets with a diameter of 200 μm or less are considered ‘driftable fines’ (Etheridge et al. 1999; Yates et al. 1985). In addition to off-target movement, droplet size may influence the performance of herbicides. Herbicide efficacy has been shown to increase with decreasing droplet size, more so in grass species than broadleaves (Etheridge et al. 2001; Knoche 1994). A standard flatfan nozzle, standard for most agricultural operations produced droplets in the rage of 200-300 μm, whereas newer nozzles designed to produced larger, and therefore less driftable droplets produce droplets in the range of 300 – 500 μm.

Little is known however about the effect that nozzle type, and therefore droplet size, has on foliar injury symptoms such as leaf necrosis caused by POST applications of glufosinate and glufosinate applied with S-metolachlor on the new Xtend cultivar compared to WideStrike and LibertyLink systems. The objective of this research is to evaluate cotton response to glufosinate applied with and without S-metolachlor, across LibertyLink, WideStrike, and Xtend cotton cultivars using a traditional flatfan nozzle, and a low drift nozzle.

Materials and Methods

Field experiments were conducted in 2016 and 2017 at the Alabama Agricultural Experiment Station Prattville Agricultural Research Unit in Prattville, AL (32.43° N, -86.44° W). The soil type is a Lucedale fine sandy loam (fine-loamy, siliceous, subactive, thermic Rhodic Paleudult) with a pH of 6.2 and 1.5 % organic matter. Three cotton (*Gossipium hirsutum* L.) cultivars were planted on May 9, 2016 and May 15, 2017 at a rate of 7-10 seed per m row. Plot size was four 91-cm rows by 7.7 m in long. Agronomic practices such as fertilization, insect management, growth regulators, and harvest aides were followed using Auburn University and Alabama Cooperative Extension Service recommendations. Herbicide treatments were applied using a CO_2- pressurized backpack sprayer with four nozzles calibrated to deliver 140 L ha^{-1} at 262 kPA and 4.83 km h^{-1}. Boom width was 1.9 m with 48 cm nozzle spacing. Treatments were applied over the two center rows, leaving a non-treated check on each side of the plot.

Three cotton cultivars with varying herbicide resistance traits were planted in both years. Cultivars planted were Stoneville ‘ST 4848’ (Bayer CropScience), a LibertyLink cultivar; ‘Phytogen 333’ (Dow Agrosciences, Indianapolis, IN), a Widestrike cultivar; and ‘Americot NG3406 B2XF’ (Americot, Inc., Lubbock, TX), an Xtend cultivar. Herbicide treatments consisted of either glufosinate (Liberty® 280 SL herbicide, Bayer CropScience) applied alone at 0.59 kg ha^{-1} or glufosinate applied at 0.59 kg ha^{-1} in combination with S-metolachlor (Dual Magnum®, Syngenta, Greensboro, NC) applied at 1.39 kg ha^{-1} applied with two nozzles types. A 110015 Turbo TeeJet® (TeeJet Technologies, Glendale Heights, IL) Induction nozzle or an 110015 XR TeeJet (TeeJet Technologies) extended range flat spray tips were used to apply to 4-leaf and 8-leaf cotton. The trial was maintained weed free through methods similar to Dodds et al. (2015). The center two rows of each four-row plot were harvested using a spindle picker modified for small-plot harvesting. Dates of cotton planting, herbicide applications and cotton harvest are presented in Table 1.

Visual evaluations were made on a scale of 0-100% with 0 = no crop injury, 100 = crop death (Frans et al. 1986). Cotton injury was assessed 7 and 14 d after herbicide treatments (DAT). A randomized complete block design with a factorial treatment arrangement was utilized with four replications. Treatment factors included a main treatment effect of cotton cultivar, a secondary effect of herbicide regime, and a third effect of nozzle type. Treatment effects were determined using ANOVA (R Core Team 2015) to test for main effects and all interactions. Statistical analysis was conducted using the methodology described by Crawley (2013). Cotton cultivar, herbicide treatment, and nozzle type were considered fixed effects. Years, replications (nested within years) and all interactions of these effects were considered random (Blouin et al. 2011). Considering year as an environmental, or random effect, allows inferences about treatments to be made over a range of environments (Blouin et al. 2011, Carmer et al. 1989). A similar statistical approach has been used by several researchers using a factorial arrangement of treatments in a randomized complete-block design (Bond et al. 2008; Dodds et al. 2010; Ottis et al. 2004; Walker et al. 2006; Zhang et al. 2005). Multiple pairwise comparisons between treatments were performed using the "multcompView" package in R (Graves 2015). Each visual injury rating, as well as yield were evaluated separately, and means were separated using Fisher's Protected LSD at $P \leq 0.05$.

End of season cotton mapping was performed in 2016 using the methods described by Jenkins (1995). However, no differences were observed.

Results and Discussion

Cotton response and yield differed by year, therefore cotton injury as well as yield were analyzed separately for 2016 and 2017. Interactions between cultivar, herbicide combination, and nozzle type were present, therefore interactions of main effects are presented.

Visual Injury Estimates – 2016. Table 2. All herbicide treatments caused significant injury to DT cotton, compared to the non-treated control at 7 DAT. Glufosinate applied in combination with S-metolachlor using a TTI nozzle caused 25% injury, the highest injury for any DT treatment. There were no differences between the same herbicide treatments applied with different nozzle types. Similar to DT, all herbicide treatments resulted in higher injury compared to the non-treated control in LibertyLink cotton. Glufosinate applied in combination with S-metolachlor using a TTI nozzle resulted in 25% injury, the highest for any treatment in LibertyLink cotton. There was no difference in visual injury between LibertyLink cotton treated with glufosinate and S-metolachlor using a flatfan or TTI nozzle. With WideStrike cotton, all herbicide treatments caused injury compared to the nontreated control, ranging from 33 – 39%, however there were no differences between herbicide treatments.

Other than the treatment of Xtend cotton treated with glufosinate and S-metolachlor using a TTI nozzle, and LibertyLink cotton treated with glufosinate and S-metolachlor using a TTI nozzle, injury was higher with all treatments made to WideStrike cotton. Across all individual treatments, injury was higher on WideStrike cotton compared to Xtend and LibertyLink.

At 14 DAT, cotton injury was less across all cotton cultivars. Within Xtend cotton, glufosinate applied with S-metolachlor using a TTI (14%) injured cotton more than Glufosinate applied alone with a XR flatfan nozzle (6%). Glufosinate applied alone with a flatfan nozzle did not cause significant injury compared to the nontreated control. The other three herbicide treatments, glufosinate applied alone with a TTI nozzle (9%), glufosinate applied with S-metolachlor using a flatfan nozzle (9%), and glufosinate applied with S-metolachlor using a TTI nozzle (14%) did injure cotton compared to the nontreated control. With LibertyLink cotton, both treatments applied with a flatfan nozzle, glufosinate alone (5%) and glufosinate applied with S-metolachlor (6%), did not cause injury compared to the nontreated control. Glufosinate applied alone with the TTI nozzle was not different than the previous treatments (9%). Glufosinate applied with S-metolachlor with a TTI nozzle injured cotton 14% which was more than any other treatment. All herbicide treatments applied to WideStrike cotton resulted in higher injury compared to the nontreated control, with injury ranging from 14 – 23% Glufosinate applied alone with a flatfan nozzle resulted in less cotton injury (14%) than any other treatment.

Seven DAT to 8 leaf cotton resulted in less overall injury than the 4 leaf application in all cotton cultivars. Glufosinate applied with S-metolachlor using a TTI nozzle resulted in 13% injury, the only herbicide treatment causing injury in the Xtend cotton. Similar to Xtend cotton, the only herbicide treatments to cause injury in LibertyLink cotton was glufosinate applied with S-metolachlor using a TTI nozzle (15%) and a flat fan nozzle (9%). All herbicide treatments resulted in injury higher than the nontreated control in WideStrike cotton (19-29%), however there were no differences between herbicide treatments.

Across all three cotton cultivars, visual injury ratings were lower 14 DAT to 8 leaf cotton than 7 DAT. Similar to the 7 DAT evaluation, the only herbicide treatment resulting in injury to Xtend cotton was glufosinate applied with S-metolachlor using a TTI nozzle, causing 9% cotton injury. Results were similar with LibertyLink cotton with the only herbicide treatment to cause injury was glufosinate applied with S-metolachlor using a TTI nozzle, resulting in 13% injury. All herbicide treatments caused injury to WideStrike cotton compared to the nontreated control. However, glufosinate applied alone with a flatfan nozzle resulted in 9% injury, which was lower compared to the other three herbicide treatments (16-19%).

Visual Injury Estimates – 2017. Table 2. All herbicide treatments injured the Xtend cotton 7 DAT to 4-leaf cotton. Glufosinate applied with S-metolachlor using a TTI nozzle resulted in the highest cotton injury, 35%. Glufosinate applied alone with a flatfan nozzle caused 13% injury, the lowest for all herbicide treatments in Xtend cotton and Glufosinate applied alone with a TTI or Glufosinate applied with S-metolachlor using a flatfan injuring cotton 26%. As in the Xtend cotton, there was no difference between the Glufosinate applied alone with the TTI nozzle (16%) and Glufosinate applied with S-metolachlor with the flatfan nozzle (18%). Glufosinate applied with S-metolachlor using a TTI nozzle injured cotton 35% which was the highest for all herbicide treatments in LibertyLink cotton. Glufosinate applied alone with a flatfan nozzle resulted in 5% injury, the lowest for all herbicide treatments to LibertyLink cotton, which was the same as the nontreated LibertyLink cotton. All herbicide treatments made to WideStrike cotton resulted in injury compared to the nontreated control. There were no differences between glufosinate applied alone, either with a flatfan (29%) or TTI (34%) nozzle, and glufosinate applied with S-metolachlor using a flatfan nozzle (36%). Glufosinate applied with S-metolachlor using a TTI nozzle resulted in 44% injury, the highest injury to WideStrike cotton.

At 14 DAT to 4-leaf cotton, overall injury decreased for all treatments, across all cotton cultivars. In Xtend cotton, glufosinate applied alone with a flatfan nozzle caused the lowest cotton injury, 10%. Glufosinate applied alone with a TTI nozzle, as well as glufosinate applied with S-metolachlor using a flatfan or a TTI nozzle resulted in 21, 24, and 30% injury, respectively. Similar to Xtend, glufosinate applied alone with a flatfan nozzle injured LibertyLink cotton, 5% which was similar to glufosinate applied alone with a TTI nozzle (14%), and glufosinate applied with S-metolachlor using a flatfan (13%). The most LibertyLink cotton injury resulted from Glufosinate plus S-metolachlor applied using a TTI nozzle (29%). All herbicide treatments made to WideStrike cotton resulted in visual injury. The most injury to WideStrike cotton occurred following applications of glufosinate with S-metolachlor using a flatfan or TTI nozzle which resulted in 29% and 38% injury, respectively. Glufosinate applied alone with a flatfan and TTI nozzle resulted in 21% and 28% injury, respectively.

At 7 days following the 8-leaf application to Xtend cotton, glufosinate applied with S-metolachlor using a flatfan or a TTI nozzle resulted in 24% and 30% injury, respectively. Injury from glufosinate applied alone, either with a flatfan or TTI nozzle was 11% and 15%, respectively. In LibertyLink cotton, glufosinate applied with S-metolachlor using a TTI nozzle resulted in 30% injury, higher than the 19% injury from glufosinate applied with S-metolachlor using a flatfan nozzle. Glufosinate applied alone to LibertyLink cotton, with either a flatfan or TTI nozzle resulted in 5% and 10% injury, the lowest injury for treatments made to LibertyLink cotton. The highest injury from herbicide treatments made to WideStrike cotton was from glufosinate alone using a TTI nozzle, and glufosinate with S-metolachlor using a TTI nozzle, which resulted in 43% and 36% injury, respectively. Glufosinate applied with S-metolachlor using a flatfan nozzle resulted in lower injury (34%) as did glufosinate applied using a flatfan nozzle to WideStrike cotton resulting in 23% injury, the lowest for all herbicide treatments made to WideStrike cotton.

At 14 DAT to 8-leaf cotton, injury was less across all cultivars and treatments. Within Xtend cotton, glufosinate applied with S-metolachlor using a flatfan and TTI nozzle resulted in 20% and 24% injury, respectively. Glufosinate applied alone with a flatfan and TTI nozzle resulted in 11% and 10% injury to Xtend cotton, respectively. Glufosinate applied with S-metolachlor using a TTI nozzle resulted in 21% injury to LibertyLink cotton which was equivalent to glufosinate applied with S-metolachlor using a flatfan nozzle (15%). Glufosinate applied alone, either with a flatfan or TTI nozzle, resulted in 5% and 8% injury, respectively. The highest injury to WideStrike cotton at 14 DAT to 8-leaf cotton was observed following the application of glufosinate alone using a TTI nozzle, which resulted in 39% injury. Glufosinate applied with S-metolachlor using a flatfan or a TTI nozzle resulted in 26% and 29% injury, respectively. Glufosinate applied alone with a flatfan nozzle to WideStrike cotton resulted in 19% injury, the lowest for all herbicide treatments made to WideStrike cotton.

Seed Cotton Yield – 2016. Table 4. There were no differences in yield within each cotton cultivar in 2016. When all herbicide treatments, across all three cotton cultivars were compared there were few differences. WideStrike cotton treated with glufosinate and S-metolachlor using a TTI nozzle had higher yields than nontreated LibertyLink cotton, as well as nontreated Xtend cotton. WideStrike cotton treated with Glufosinate with TTI nozzles yielded higher than the LibertyLink nontreated.

Seed Cotton Yield – 2017. In 2017 there were no differences in seed cotton yield within cultivars, as well as across cotton cultivars.

The results of this study support previous research that WideStrike cotton is more sensitive to POST applications of glufosinate, compared to other cotton cultivars such as LibertyLink (Dodds et al. 2015). Additionally, this study supports previous research that tank mixtures of S-metolachlor with glufosinate can result in higher injury than glufosinate applied alone (Steckel et al. 2012). According to this research, nozzle type does not have a significant effect on visual crop injury when comparing identical treatments within cultivars in most comparisons. However, a trend of higher injury is observed when glufosinate is applied with S-metolachlor using a TTI nozzle, suggesting that although it does not affect yields, this particular treatment leads to greater crop injury. This suggests that although decreasing droplet size leads to decreased overall herbicide coverage (Knoche 1994), concentration of herbicide per droplet is increased with larger droplets, leading to the larger necrotic speckling and higher visible injury trend with TTI nozzles. Cotton yield was not affected by herbicide treatment in this study, supporting previous research which suggests that applications of glufosinate can be applied to WideStrike cotton without reducing yield (Dodds et al. 2015). This research supports research suggesting that glufosinate can be applied with S-metolachlor to WideStrike cotton without reducing yield (Culpepper et al. 2009), which is different from the results of others who reported a yield reduction (Steckel et al. 2012).

Table 1.1. Cotton planting, herbicide application and cotton harvest dates.

Year	Cotton Planting	Herbicide applications		Cotton Harvest
		Early POST	Mid POST	
2016	May 6	June 8	June 22	November 8
2017	May 15	June 19	July 20	November 27

Table 1.2. 2016 Cotton cultivar response to applications of glufosinate and glufosinate applied with S-metolachlor using two nozzle types to 4-leaf and 8-leaf cotton.

Cotton Cultivar[a]	Herbicide Treatment[b]	Nozzle Type[c]	Injury[d]			
			7 DAT 4-leaf	14 DAT 4-leaf	7 DAT 8-leaf	14 DAT 8-leaf
			%			
Xtend	nontreated		0 e	0 e	0 f	0 d
Xtend	glufosinate	flatfan	14 d	6 de	0 f	0 d
Xtend	glufosinate	TTI	15 cd	9 cd	4 def	0 d
Xtend	glufosinate + S-metolachlor	flatfan	16 cd	9 cd	6 def	3 cd
Xtend	glufosinate + S-metolachlor	TTI	25 bc	14 bc	13 cde	9 bc
LibertyLink	nontreated		0 e	0 e	0 f	0 d
LibertyLink	glufosinate	flatfan	11 d	5 de	4 def	0 d
LibertyLink	glufosinate	TTI	16 cd	9 cd	3 ef	0 d
LibertyLink	glufosinate + S-metolachlor	flatfan	11 c	6 de	9 cdef	5 cd
LibertyLink	glufosinate + S-metolachlor	TTI	25 bc	14 bc	15 bcd	13 ab
WideStrike	nontreated		0 e	0 e	0 f	0 d
WideStrike	glufosinate	flatfan	33 ab	14 bc	20 abc	9 bc
WideStrike	glufosinate	TTI	34 ab	16 ab	19 abc	16 a
WideStrike	glufosinate + S-metolachlor	flatfan	33 ab	19 ab	25 ab	18 a
WideStrike	glufosinate + S-metolachlor	TTI	39 a	23 a	29 a	19 a

[a] Xtend cultivar used was ‘Americot NG 3406B2XF’; LibertyLink cultivar used was ‘Stoneville 4848 GLT’; WideStrike cultivar used was ‘Phytogen 333 WRF’.

[b] All treatments containing glufosinate used the product Liberty, treatments containing S-metolachlor used the product Dual Magnum.

[c] flatfan indicates TeeJet XR 110015 nozzle; TTI, Turbo TeeJet Induction nozzle.

[d] Means followed by the same letter within a column are not statistically different according to Fisher’s protected LSD test at $P \leq 0.05$.

[e] Abbreviations: DAT, days after treatment.

Table 1.3. 2017 Cotton cultivar response to applications of glufosinate and glufosinate applied with S-metolachlor using two nozzle types to 4-leaf and 8-leaf cotton.

Cotton Cultivar[a]	Herbicide Treatment[b]	Nozzle Type[c]	Injury[d] 7 DAT[e] 4-leaf	14 DAT 4-leaf	7 DAT 8-leaf	14 DAT 8-leaf
			%			
Xtend	nontreated		0 g	0 e	0 i	0 i
Xtend	glufosinate	flatfan	13 ef	10 d	11 fgh	11 efgh
Xtend	glufosinate	TTI	26 cd	21 bc	15 efg	10 fgh
Xtend	glufosinate + S-metolachlor	flatfan	26 cd	24 b	24 cd	20 bcde
Xtend	glufosinate + S-metolachlor	TTI	35 abc	30 ab	30 bc	24 bcd
LibertyLink	nontreated		0 g	0 e	0 i	0 i
LibertyLink	glufosinate	flatfan	5 fg	5 de	5 hi	5 hi
LibertyLink	glufosinate	TTI	16 e	14 cd	10 gh	8 ghi
LibertyLink	glufosinate + S-metolachlor	flatfan	18 de	13 cd	19 def	15 defg
LibertyLink	glufosinate + S-metolachlor	TTI	35 abc	29 ab	30 bc	21 bcd
WideStrike	nontreated		0 g	0 e	0 i	0 i
WideStrike	glufosinate	flatfan	28 bc	21 bc	23 cde	19 cdef
WideStrike	glufosinate	TTI	34 bc	28 b	43 a	39 a
WideStrike	glufosinate + S-metolachlor	flatfan	36 ab	29 ab	34 b	26 bc
WideStrike	glufosinate + S-metolachlor	TTI	44 a	38 a	36 ab	29 b

[a] Xtend cultivar used was ‘Americot NG 3406B2XF’; LibertyLink cultivar used was ‘Stoneville 4848 GLT’; WideStrike cultivar used was ‘Phytogen 333 WRF’.

[b] All treatments containing glufosinate used the product Liberty, treatments containing S-metolachlor used the product Dual Magnum.

[c] flatfan indicates TeeJet XR 110015 nozzle; TTI, Turbo TeeJet Induction nozzle.

[d] Means followed by the same letter within a column are not statistically different according to Fisher’s protected LSD test at $P \leq 0.05$.

[e] Abbreviations: DAT, days after treatment.

Table 1.4. Seed cotton yield for 2016 and 2017 following applications of glufosinate and glufosinate applied with S-metolachlor using two nozzle types.

Cotton Cultivar[a]	Herbicide Treatment[b]	Nozzle Type[c]	Seed Cotton Yield[d] 2016	Seed Cotton Yield[d] 2017
			kg ha^{-1}	
Xtend	nontreated		1383 bc	1497 a
Xtend	glufosinate	flatfan	1596 abc	1358 a
Xtend	glufosinate	TTI	1647 abc	1627 a
Xtend	glufosinate + S-metolachlor	flatfan	1922 abc	1236 a
Xtend	glufosinate + S-metolachlor	TTI	1667 abc	1594 a
LibertyLink	nontreated		1261 c	1407 a
LibertyLink	glufosinate	flatfan	1796 abc	1350 a
LibertyLink	glufosinate	TTI	1952 abc	1179 a
LibertyLink	glufosinate + S-metolachlor	flatfan	1840 abc	1122 a
LibertyLink	glufosinate + S-metolachlor	TTI	1596 abc	1318 a
WideStrike	nontreated		1576 abc	1334 a
WideStrike	glufosinate	flatfan	1891 abc	854 a
WideStrike	glufosinate	TTI	2084 ab	903 a
WideStrike	glufosinate + S-metolachlor	flatfan	1861 abc	862 a
WideStrike	glufosinate + S-metolachlor	TTI	2267 a	1000 a

[a] Xtend cultivar used was ‘Americot NG 3406B2XF’; LibertyLink cultivar used was ‘Stoneville 4848 GLT’; WideStrike cultivar used was ‘Phytogen 333 WRF’.

[b] All treatments containing glufosinate used the product Liberty, treatments containing S-metolachlor used the product Dual Magnum.

[c] flatfan indicates TeeJet XR 110015 nozzle; TTI, Turbo TeeJet Induction nozzle.

[d] Means followed by the same letter within a column are not statistically different according to Fisher’s protected LSD test at $P \leq 0.05$.

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The Effects of Post Applied S-metolachlor in Combination with Glufosinate on Gas Exchange Measurements of Cotton (*Gossypium hirsutum*)

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Greenhouse studies were conducted in 2017 to determine the effects of postemergence (POST) applications of glufosinate and glufosinate applied with S-metolachlor, on gas exchange measurements such as CO_2 assimilation and stomatal conductance, across three cultivars of cotton using two nozzle types. Glufosinate at 0.6 kg $ha^{-1}$and glufosinate combined with S-metolachlor at 1.39 kg ha^{-1} was applied to LibertyLink®, Xtend®, and WideStrike® cotton cultivars using a 110015 XR flatfan and 11005 TTI nozzle (TeeJet Technologies, Glendale Heights, IL). CO_2 assimilation and leaf stomatal conductance were measured one day before treatment (DBT) and 1, 2, 3, and 7 days after treatment (DAT). There were no differences between gas exchange measurements in LibertyLink, and Xtend cotton following herbicide treatments. CO_2 assimilation and stomatal conductance in WideStrike cotton significantly decreased following herbicide application, regardless of herbicide treatment or nozzle type. Gas exchange measurements of WideStrike cotton fully recovered to levels equal to or greater than the nontreated cotton by 7 DAT. These data indicate that injury of WideStrike cotton following glufosinate and S-metolachlor plus glufosinate applications is aesthetic only,

not detrimentally affecting the plant processes of photosynthesis or leaf conductance past 7 DAT.

Introduction

Glufosinate is a nonselective, contact herbicide which has been used in agronomic crops in the United States since 1994. A member of the organophosphorus herbicide family, glufosinate inhibits the enzyme glutamine synthetase, the enzyme responsible for incorporating nitrogen in the plant, from the form of ammonia into glutamate, producing glutamine (CERA 2015; Senseman 2007; Zimdahl 2013). Without the ability to safely incorporate nitrogen into the plant, ammonia is accumulated. Accumulation of ammonia within plants has been associated with decreased photosynthetic rates (Larsen et al. 1981). Excess ammonia can destroy plant tissue necessary for photosynthesis such as the chloroplasts leading to necrosis of tissue and plant death (Coetzer and Al-Khatib 2001; Devine et al. 1993; Everman et al. 2007; Wendler et al. 1990). Ammonia, being acidic, diminishes the pH gradients across membranes within the plant, causing the uncoupling of photophosphorylation (Senseman 2007). Glufosinate application has also been shown to cause stomatal closure in treated plants by increasing internal CO_2 concentration and therefore reducing overall transpiration in the plant, although this is considered a secondary effect that occurs after the reduction of CO_2 assimilation (Lacuesta et al. 1993). There is contradictory evidence as to whether the decrease in photosynthetic activity is due to accumulation of deleterious amounts of ammonia within the plant, or due to another effect such as decreased stomatal conductance. Accumulation of ammonia at levels that are known to be detrimental to photosynthesis, by causing an uncoupling of photophosphorylation, (Krogmann et al. 1959) has occurred at greater levels and a faster rate than the inhibition of photosynthesis (Coetzer and Al-Khatib 2001a). Specifically, inhibition of photosynthesis is caused by the creation of radicals within the plant which inhibit the photorespiration cycle by inhibiting the RUBISCO enzyme necessary for the light reactions of photosynthesis which in turn leads to membrane destruction in a manner very similar to that of the Photosystem II inhibitors (Dan Hess 2000).

Being a nonselective contact herbicide, historically the primary use of glufosinate in agronomic crops was either as a burn down application prior to crop emergence, or a postemergence directed spray (PDS) application during the growing season. Glufosinate is a racemic mixture of L-phosphinothricin, the herbicidal active enantiomer and D-phosphinothricin enantiomer. The introduction of glufosinate resistant cotton, was granted regulatory approval in 2003 and became commercially available in 2004 (CERA 2015; Gardner et al. 2006). The development of glufosinate resistant cotton was achieved by inserting the bar gene from the soil bacterium *Streptomyces hygroscopicus* using *Agrobacterium tumefaciens* (CERA 2015). *Streptomyces hygroscopicus* is a gram-positive soil bacterium that is ubiquitous in the soil. The bar gene is responsible for expressing resistance to glufosinate through the enzyme phosphinothricin-acetyl-transferase (*pat*) gene which acetylates glufosinate ammonium, transforming it to the inactive acetylated form, N-acetyl-L-phosphinothricin (CERA 2015; Devine 1993; Hérouet et al. 2005; OECD 2002). Cotton that has the bar gene through the transformation has been shown to possess excellent tolerance to postemergence (POST) applications of glufosinate (Blair-Kerth et al. 2001). According to the manufacturer, glufosinate can be applied broadcast, over the top application to glufosinate resistant cotton from emergence until early bloom. (Anonymous 2017).

For years, glyphosate-resistant cotton (GR), first introduced in 1997, has been cultivated on the majority of cotton acreage throughout the cotton producing regions of the United States. The high adoption rates of GR cotton can be attributed to the efficacy of glyphosate on a broad spectrum of grasses as well as broadleaf weeds, and the convenience of being able to make POST applications of an effective herbicide (Culpepper and York 1998). Economically, GR cotton systems are favored by growers for how their net profit compares to that of conventional cotton systems (Askew et al. 2002; Culpepper and York 1999). GR cotton systems have also been shown to require less herbicide applications throughout the growing season as well as less overall herbicide applied (Culpepper and York 1998). However, the increase in glyphosate use placed intense selection pressure on certain species, leading to the development of herbicide resistant weed species, which in turn decreases the options for POST weed control in cotton (Culpepper et al. 2006; Steckel et al. 2008). The most notable GR species in cotton, Palmer amaranth, was first documented in 2006, and has since then become one of the most troublesome weed species and is widespread across the cotton producing regions of the United States (Heap 2017; Webster 2013).

Studies have shown glufosinate, applied in a timely manner, to be an effective herbicide option in the management of GR Palmer amaranth (Culpepper et al. 2009; Everman et al. 2009; Gardner et al. 2006; Whitaker et al. 2011a). However, although shown to be an effective weed management tool, glufosinate-resistant cotton systems were not widely adopted until the introduction of GlyTol LibertyLink cotton cultivars in 2012, which were resistant to both glyphosate and glufosinate.

Widestrike

Widestrike® cotton (Dow Agrosciences, Indianapolis, IN) was bred to contain the insecticidal proteins *Cry1Ac* and *Cry1F* which confer resistance to lepidopteran pests (CERA 2015; Culpepper et al. 2009). During the breeding process, the *pat* gene was used as a marker to determine the presence of the insecticidal proteins. Because of the use of the *pat* gene in the breeding process, Widestrike cultivars conferred a level of tolerance to glufosinate. Because the *pat* gene was used only as a marker in the breeding process, the level of activity of *pat* is lower in Widestrike cultivars compared to LibertyLink cultivars. Crossing of Widestrike lines with glyphosate resistant cotton cultivars have produced cultivars that are stacked with both glyphosate tolerance as well as glufosinate tolerance. Unlike LibertyLink cultivars, Widestrike cultivars have been widely adopted by producers due to their agronomic traits as well as the combination of herbicide tolerance that they possess. In 2016, 29.98% of all cotton planted in Alabama was WideStrike, which only six years earlier in 2010 comprised only 13.9% of the cotton planted in the state (USDA-AMS 2010, 2016).

POST applications of glufosinate to Widestrike cotton have resulted in visible crop injury, and in some cases yield reduction, depending on application timing and glufosinate rate. Widestrike cotton sprayed with one to two applications of glufosinate has resulted in visual injury ratings from 10 -25% without decreasing cotton yields (Barnett et al. 2013), however, yields have been reduced up to 7% when glufosinate was applied three times (Barnett et al. 2015). Rate of glufosinate influences the degree of visible cotton injury as well as yield. Glufosinate applied at 1.2 kg ha^{-1} to Widestrike cotton has shown to delay plant maturity as well as reduce cotton yield (Dodds et al. 2015).

Xtend Cotton (Dicamba Resistant)

Cotton cultivars resistant to the auxin-mimicking herbicide dicamba were brought to market in 2015 after several years of regulation following their development and testing (USDA-APHIS 2015). Dicamba has shown to be effective in controlling many species of broadleaf weeds, including herbicide resistant species such as GR Palmer amaranth (Merchant et al. 2013). Tolerance to dicamba was achieved through the use of the dicamba monooxygenase (*dmo*) gene, isolated from *Stenotrophomonas maltophilia*, which transfers dicamba from the active form to the inactive 3,6-dichlorosalicylic acid and formaldehyde compounds (Behrens et al. 2007). In addition to dicamba tolerance, herbicides resistant to dicamba are also stacked with tolerance to glufosinate through the insertion of the *bar* gene, expressing the *pat* enzyme (event MON88701). This is significant because dicamba has been shown to be more effective in managing resistant weed species when combined with other modes of action (Sanders and Marshall 2014; York et al. 2012, 2015). This cotton cultivar was further crossed with a cotton cultivar resistant to the herbicide glyphosate, resulting in a cultivar resistant to three different modes of action, (MON88701 by MON88913; known as Bollgard II® XtendFlex™ (B2XF); ([ISAAA] 2015)]. In addition to being an effective management tool for the control of weed species, dicamba resistant cotton introduces a new mode of action into cotton weed control systems, which can delay the development of resistance to herbicides already in use (Vann et al. 2017; York et al. 2012). However utilizing sites of action, other than dicamba, is necessary to minimize the development of weed species resistant to dicamba as Palmer amaranth has been shown to develop enhanced tolerance to dicamba in as few as three generations (Norsworthy et al. 2012; Tehranchian et al. 2017).

S-metolachlor

Overlapping residual, soil-applied herbicides applied throughout the growing season are recommended for effective weed control in cotton (Tredaway 2017). S-metolachlor is a herbicide in the chloroacetamide family that inhibits the formation of very long chain fatty acids through the inhibition of the enzymes responsible for elongating fatty acids, very long chain fatty acid elongases (Böger 2003; Böger et al. 2000; Tanetani et al. 2009). S-metolachlor interferes with shoot elongation of weeds that have already germinated, but has little to no herbicidal activity on plant roots or plant tissue that has already emerged (Fuerst and Gronwald 1986). S-metolachlor has been shown to effectively control many troublesome weeds, such as Palmer amaranth when applied preemergence (PRE) (Geier et al. 2006; Steele et al. 2005). In addition to PRE applications, S-metolachlor may be applied POST in cotton (Tredaway 2017). Although POST applications of S-metolachlor does not provide any control of emerged weeds, the residual control provided is beneficial in cotton production systems (Clewis et al. 2006; Whitaker et al. 2011a, 2011b). However, POST applications of S-metolachlor to cotton typically results in minor crop injury such as necrotic speckling on leaf tissue, although cotton injury can be more severe if cotton is environmentally stressed (Clewis et al. 2006; Tredaway 2017). Cotton yields have not been shown to be adversely affected by POST applications of S-metolachlor (Clewis et al. 2006; Culpepper et al. 2009; Dodds et al. 2010; Stephenson IV et al. 2013).

Nozzle Selection

Spray droplet drift is one way that herbicides may move off-target, and possibly cause damage to surrounding crops. Droplet size has been shown to have a major impact on the drift potential of herbicide applications (Whisenant et al. 1993; Yates et al. 1985). Other than environmental factors such as wind speed and direction, and application speed and terrain type, the primary factor affecting droplet spectrum is nozzle selection. Generally, droplet size ranges from 10 to greater than 1,000 μm (Bouse et al. 1990). Droplets with a diameter of 200 μm or less are considered driftable (Etheridge et al. 1999; Yates et al. 1985). In addition to off target movement, droplet size may influence the performance of herbicides. Herbicide performance has been shown to increase with decreasing droplet size (Knoche 1994). However, little is known about the effect that nozzle type, and therefore droplet size, has on foliar injury symptoms such as leaf necrosis, and to what extent these symptoms have on physiological processes within the plant such as photosynthesis and leaf conductance.

Photosynthesis and Leaf Conductance

Plant physiological measurements such as CO_2 assimilation, representing photosynthetic activity, and leaf conductance can be useful in order to quantify the activity of a herbicide on a given species, rather than solely relying on subjective visual injury data (Cutts et al. 2011; Ferrel et al. 2004; Ferrell et al. 2003). Glufosinate has been shown to cause rapid decreases in photosynthetic activity following application in species such as Palmer amaranth (Coetzer and Al-Khatib 2001b). However, the effect of glufosinate application has not been photosynthetically quantified on different cultivars, specifically WideStrike cultivars. Additionally, there is a lack of data regarding the effect of S-metolachlor, and S-metolachlor applied with glufosinate, on physiological processes in cotton. Field observations are consistent with WideStrike cotton cultivars recovering following glufosinate application at 0.6 kg ha^{-1} (Dodds et al. 2015), however it would be useful to see how visual injury symptomology associated with glufosinate applications correlated with measurements of photosynthesis and leaf conductance, as well as knowing the duration of impairment.

Materials and Methods

Three cotton cultivars, Phytogen 333 WRF(Dow Agrosciences, Indianapolis, IN) a WideStrike cultivar; Stoneville 4848 GLT (Bayer Cropscience, Research Triangle Park, NC), a LibertyLink cultivar; and Americot ng3406 B2FX (Americot, Inc., Lubbock, TX), an Xtend cultivar, were planted in 3.8L pots filled with standard potting soil mix. Plants were grown for approximately two months in the Plant Science Research Center in Auburn, Alabama. Supplemental light was provided from LumiGrow® (LumiGrow Inc, Emoryville, CA) Pro 325 lamps emitting a PPFD of approximately 122 μmol m^{-2} s^{-1}. Greenhouse temperature ranged from 18 – 28 °C, with an average daily temperature of 22 °C.

Treatments were arranged in a randomized complete block with factorial treatment arrangement with four replications consisting of glufosinate applied alone at 0.59 kg ai ha^{-1}, as well as in combination with S-metolachlor applied at 1.39 kg ai ha^{-1}, using TeeJet TTI 11002 and TeeJet XR11002 nozzle tips across the three cultivars of cotton, Xtend, LibertyLink, and WideStrike. Herbicide treatments were applied using a DeVries Manufacturing® spray chamber calibrated to deliver 140 L ha^{-1}. The experiment was repeated three times in time.

To determine the effects of herbicide treatment on cotton growth, CO_2 assimilation measurements were recorded using an LI-6400 (LI-COR, Inc.). The LI-6400 is an open gas exchange system that measures photosynthesis based on differences in CO_2 concentration within the leaf chamber. Measurements were taken according to the methods described by Ferrell (Ferrell et al. 2003). One leaf per plant was placed in the chamber to determine CO_2 exchange between the leaf and the measurement chamber.

Constants within the chamber consisted of photosynthetically active radiation of 1,000 μmol m^{-2} s^{-1} from red and blue light, and a CO_2 concentration of 450 μmol mol^{-1} delivered by the systems CO_2 injector. Time was allowed to reach approximately steady photosynthesis and leaf conductance levels before being measured. Photosynthesis and leaf conductance were rated 24 hours before treatment, and 1, 2, 3, and 7 days after treatments (DAT).

Treatment effects were determined using ANOVA (R Core Team 2015) to test for main effects and all interactions. Cotton cultivar, herbicide regime, and nozzle type were considered fixed effects. Experimental run, replications (nested within run) and all interactions of these effects were considered random (Blouin et al. 2011). Considering year as an environmental, or random effect, allows inferences about treatments to be made over a range of environments (Blouin et al. 2011, Carmer et al. 1989). A similar statistical approach has been used by several researchers using a factorial arrangement of treatments in a randomized complete-block design (Bond et al. 2008; Dodds et al. 2010; Ottis et al. 2004; Walker et al. 2006; Zhang et al. 2005). Means were separated using Fisher's Protected LSD at $P \leq 0.05$. Additionally, gas exchange measurements were expressed as a percentage of the nontreated control and fit to an order 2 polynomial equation in order to model plant response over time.

Results and Discussion

Comparing the residual variance to the variance caused by experimental run, determined that experimental run by treatment was not significant, therefore data were pooled over experimental run. Data is presented individually for 1,2,3 and 7 DAT due to differences detected. (is this right?)

Comparison of Gas Exchange Measures – Stomatal Conductance. Pre-treatment stomatal conductance differences were detected (Figure 1). Treatments with Xtend had higher conductance (0.35 mmol m^{-2} s^{-1}) than treatments with LibertyLink and WideStrike (0.3 mmol m^{-2} s^{-1}) (Figure 1). There were no differences in leaf stomatal conductance with any treatment and nozzle combination between Xtend and LibertyLink cotton cultivars at 1 DAT (Figure 2). With WideStrike cotton, all herbicide treatments resulted in lower stomatal conductance, however glufosinate with S-metolachlor and glufosinate alone both using a flatfan nozzle, differed from the nontreated control.

At 2 DAT, there were no differences within LibertyLink cotton (Figure 3). Both treatments containing S-metolachlor regardless of the nozzle type used, resulted in lower conductance in Xtend cotton, however there were no differences between the two treatments. All herbicide treatments lowered stomatal conductance in WideStrike cotton, but there were no differences between treatments.

At 3 DAT no differences were detected between treatments in LibertyLink cotton (Figure 4). There were no differences between herbicide treatments within Xtend cotton cultivars, however S-metolachlor combined with glufosinate using a flatfan nozzle resulted in lower stomatal conductance compared to the nontreated control. There were no differences between herbicide treatments in WideStrike cotton, with all herbicide treatments resulting in lower stomatal conductance compared to the nontreated control.

There were no differences between any treatment with LibertyLink and Xtend cotton 7 DAT (Figure 5). In WideStrike cotton, no differences were detected between herbicide treatments but glufosinate applied alone with a flatfan nozzle resulted in higher stomatal conductance compared to the nontreated control.

Comparison of Gas Exchange Measures – CO_2 Assimilation. Gas exchange measures prior to treatments resulted in Xtend (18 mmol m^{-2} s^{-1}) having the highest CO2 assimilation, followed by WideStrike (17 mmol m^{-2} s^{-1}) (Figure 6). LibertyLink had the lowest amount of CO2 assimilation at 15 mmol m^{-2} s^{-1}. There were no differences in CO_2 assimilation among treatments for Xtend and LibertyLink cultivars 1 DAT (Figure 7). All herbicide treatments resulted in lower levels of CO_2 assimilation compared to the nontreated control in WideStrike cotton with no differences between any herbicide treatment.

At 2 DAT, S-metolachlor applied with glufosinate using a flatfan nozzle resulted in lower CO_2 assimilation compared to the nontreated Xtend cotton (Figure 8). There were no differences between herbicide treatments within Xtend cotton. There were no differences with any herbicide treatment within LibertyLink cotton. All herbicide treatments reduced CO_2 assimilation in WideStrike cotton, with no differences between any herbicide treatments.

There were no differences between any herbicide treatments in Xtend or LibertyLink cotton 3 DAT (Figure 9). Levels of CO_2 assimilation were reduced with all herbicide treatments on WideStrike cotton, however there were no differences between treatments.

Seven DAT, there were no differences between any herbicide treatments across all three cotton cultivars (Figure 10). These results indicate that gas exchange measures of Xtend and LibertyLink cotton cultivars are initially affected but quickly recover, thus not greatly affected by applications of glufosinate or glufosinate applied with S-metolachlor, indicating that these cultivars possess robust tolerance to both herbicides. WideStrike cotton was more sensitive to either herbicide application at 1, 2, and 3 DAT. By 7 DAT, levels of photosynthesis of treated WideStrike cotton was equal to, or even greater than that of the nontreated control, indicating full plant recovery.

Cotton Response Modeled Over Time. When gas exchange measures of each cultivar, pooled across herbicide treatments and nozzle types, was modeled over time as a percentage of the nontreated control (Figure 11) it was determined that levels of stomatal conductance of Xtend cotton was reduced to approximately 80% of the nontreated control 3.5 DAT. WideStrike was reduced as low as 45% of the nontreated control 2.5 DAT and LibertyLink was reduced to 85% of the nontreated control 2 DAT. According to the model, Xtend cotton reached full recovery approximately 6 DAT, WideStrike cotton reached full recovery approximately 5 DAT, and LibertyLink reached full recovery at 5.5 DAT.

When CO_2 assimilation measures of each cultivar, pooled across herbicide treatments and nozzle types, was modeled over time as a percentage of the nontreated control (Figure 12) it was determined that levels of photosynthesis of Xtend cotton was reduced to approximately 80% of the nontreated control 3.5 DAT. WideStrike was reduced as low as 40% of the nontreated control 3 DAT and LibertyLink was reduced to 95% of the control 3.5 DAT. According to the model, Xtend and WideStrike cotton reached full recovery approximately 6.5 DAT, and LibertyLink cotton reached full recovery approximately 5.5 DAT.

Research Implications. Glufosinate, as well as S-metolachlor, will continue to be a valuable weed control tool in cotton cropping systems. Our research suggests that glufosinate applied alone or glufosinate applied with S-metolachlor does not adversely affect gas exchange measures past 6 days in Xtend cotton, past 5 days in WideStrike cotton, or past 5.5 days in LibertyLink cotton. Additionally, our data suggests that nozzle type does not, on average, have an effect on levels of stomatal conductance or CO_2 assimilation. Visual injury symptomology, resulting from applications of glufosinate applied alone or in combination with S-metolachlor, does not typically become noticeable until 5-7 days following application. Our results suggest that by this time, the physiological processes of stomatal conductance as well as CO_2 assimilation are no longer inhibited by herbicide treatment. This research further supports the theory that cotton injury following herbicide application is merely aesthetic, and the plant is able to recovery within 7 days, therefore not significantly affecting plant growth and development.

Figure 2.1. Stomatal conductance 24 hours before treatment

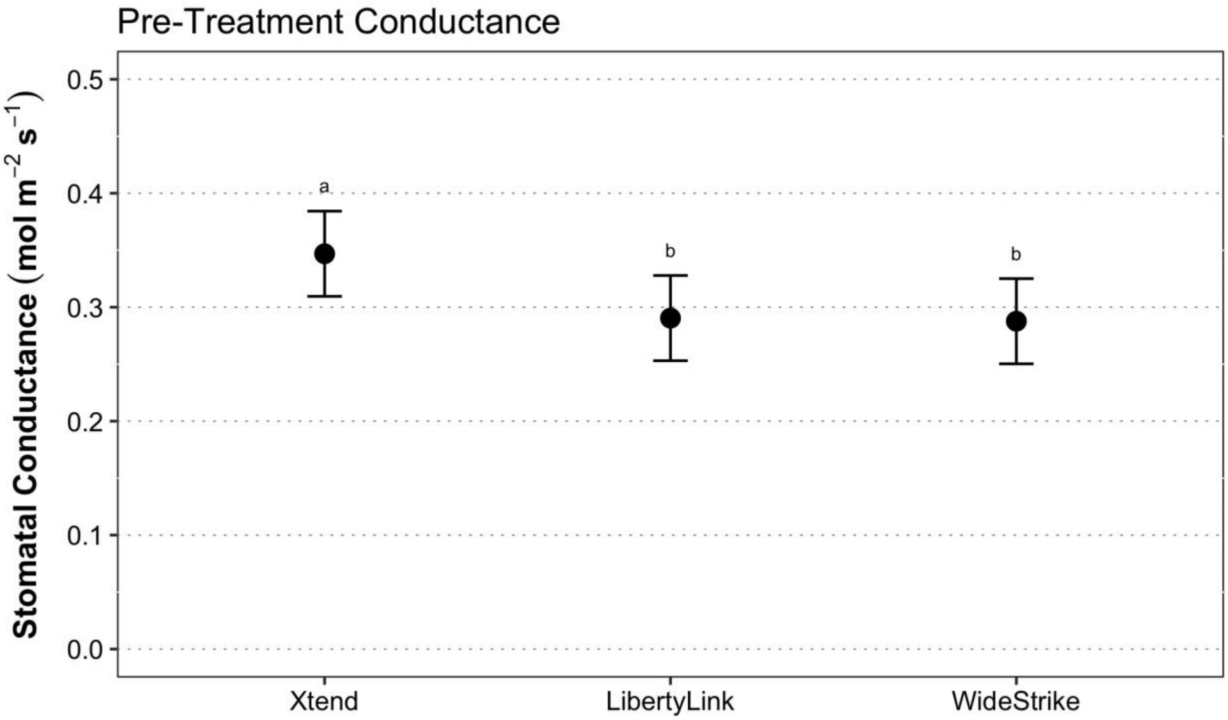


Figure 2. Stomatal conductance 1 DAT

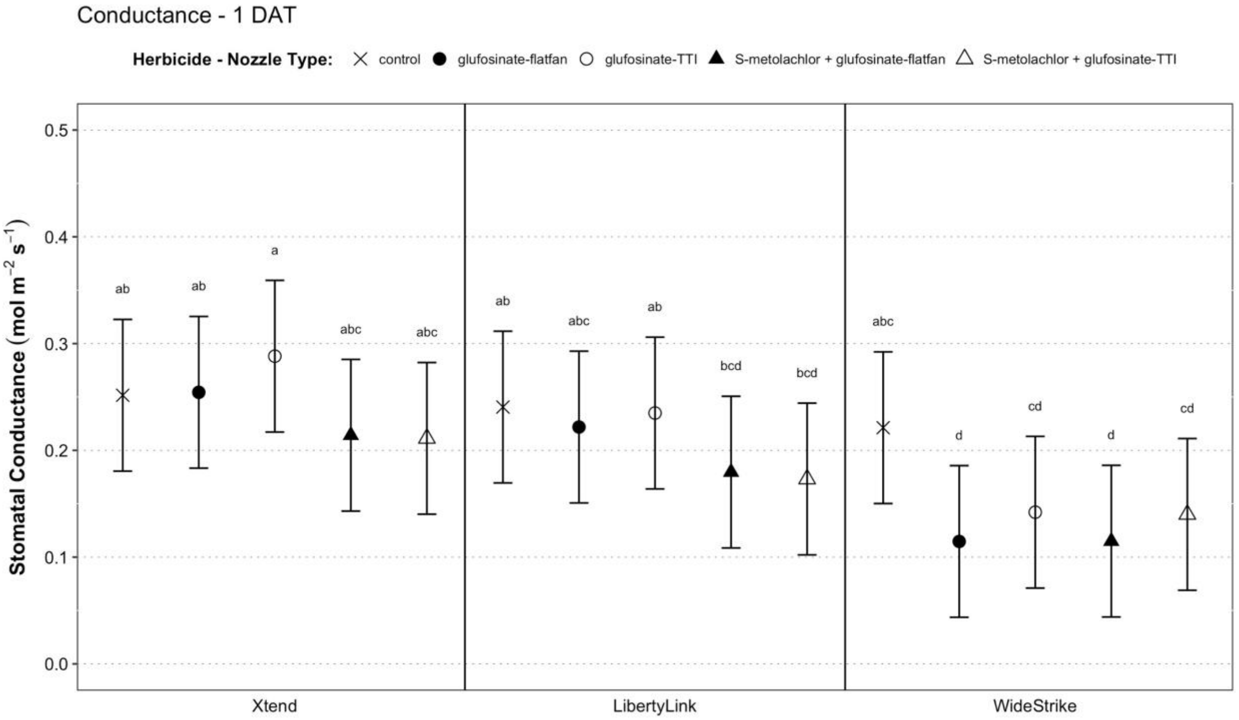


Figure 2.3. Stomatal conductance 2 DAT

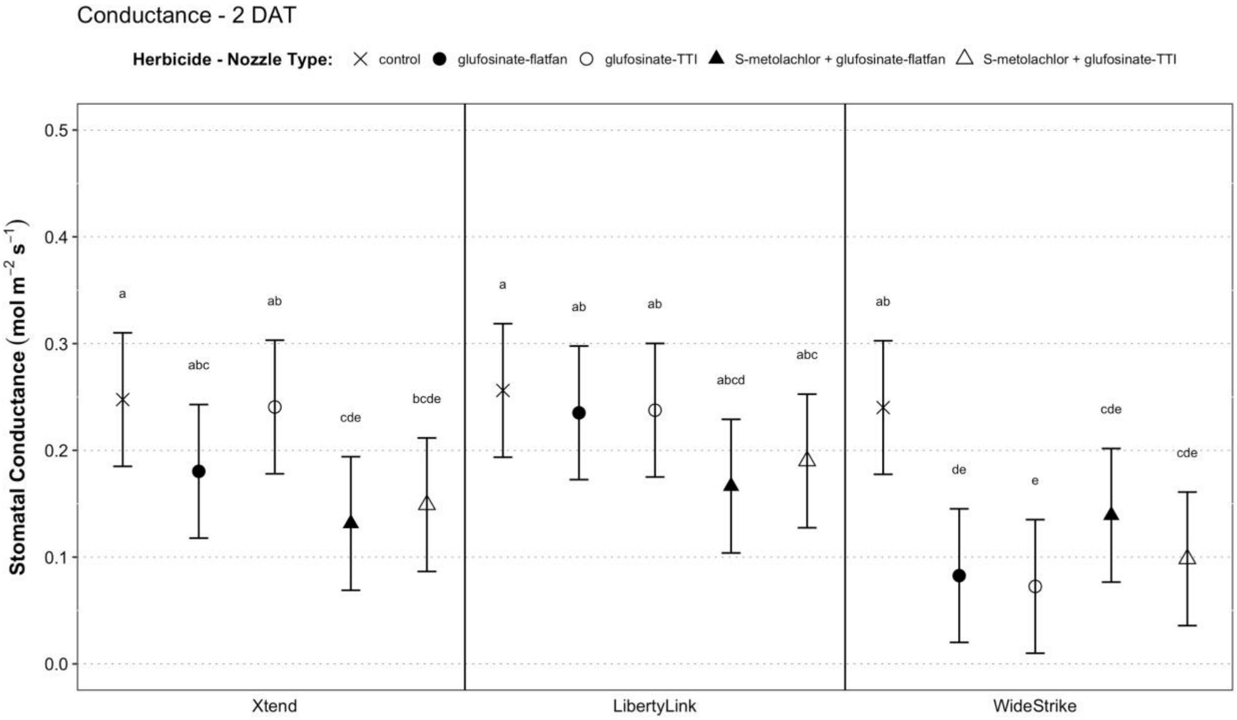


Figure 2.4. Stomatal conductance 3 DAT

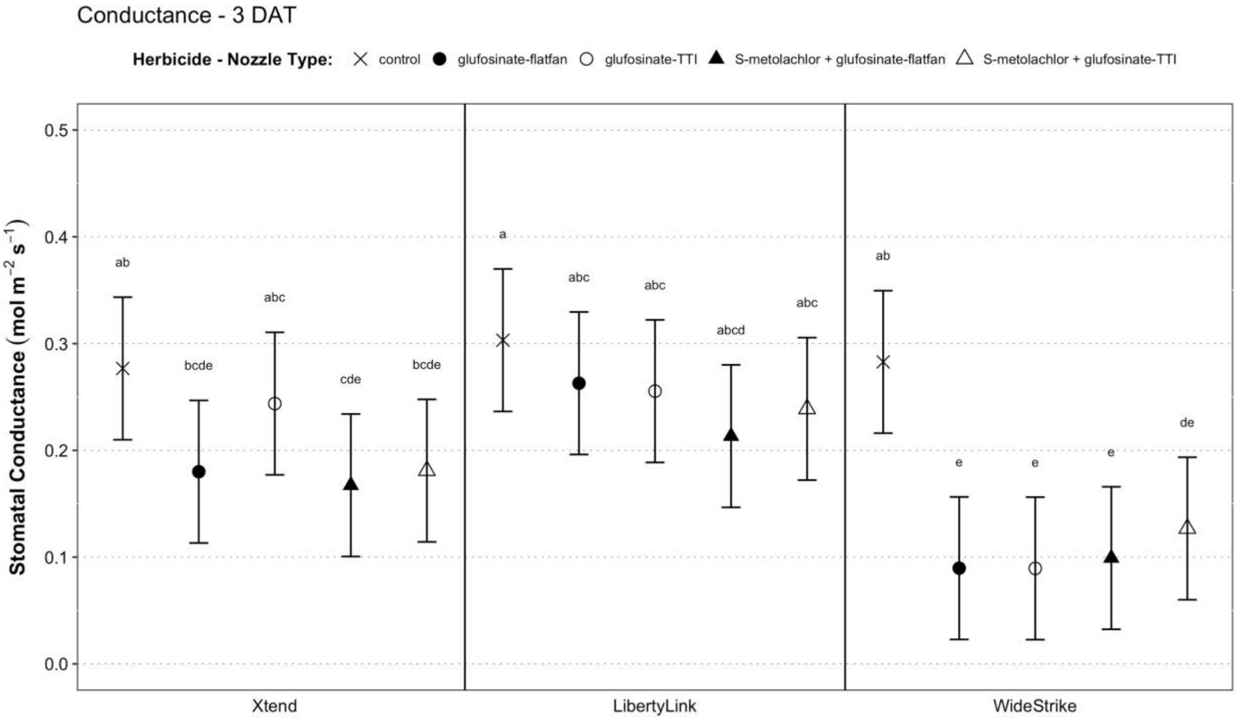


Figure 2.5. Stomatal conductance 7 DAT

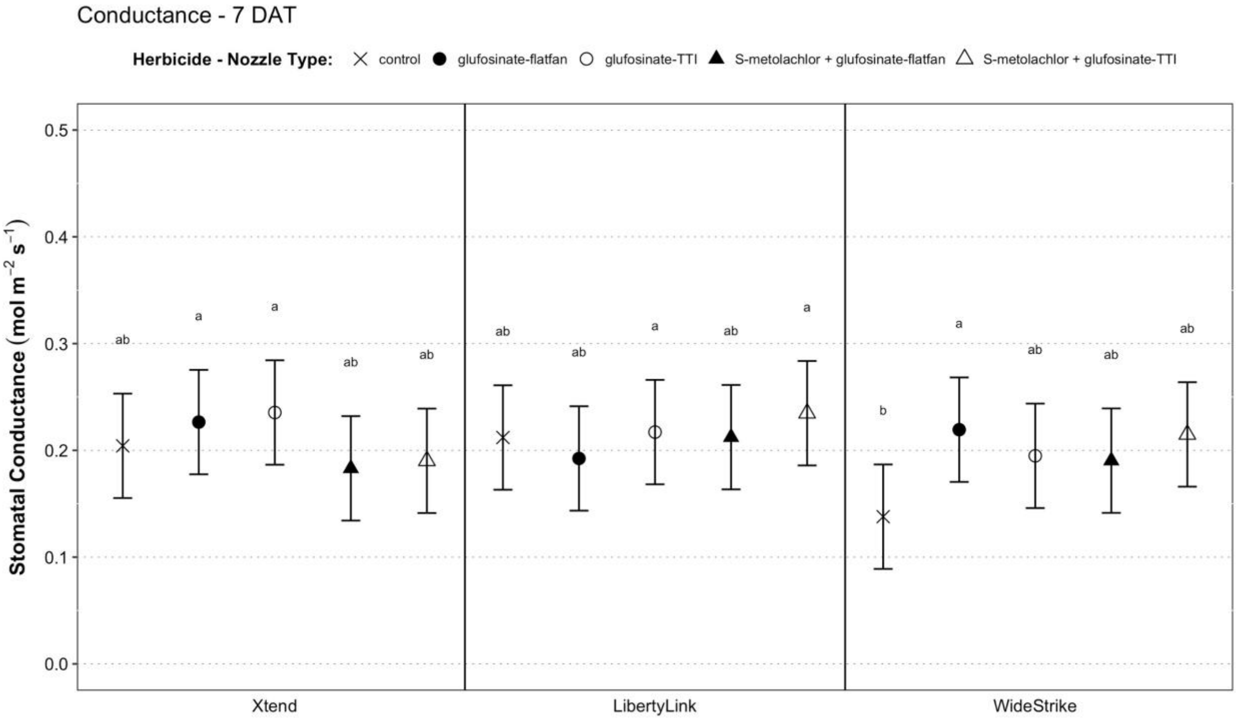


Figure 2.6. Leaf photosynthesis 24 hours before treatment

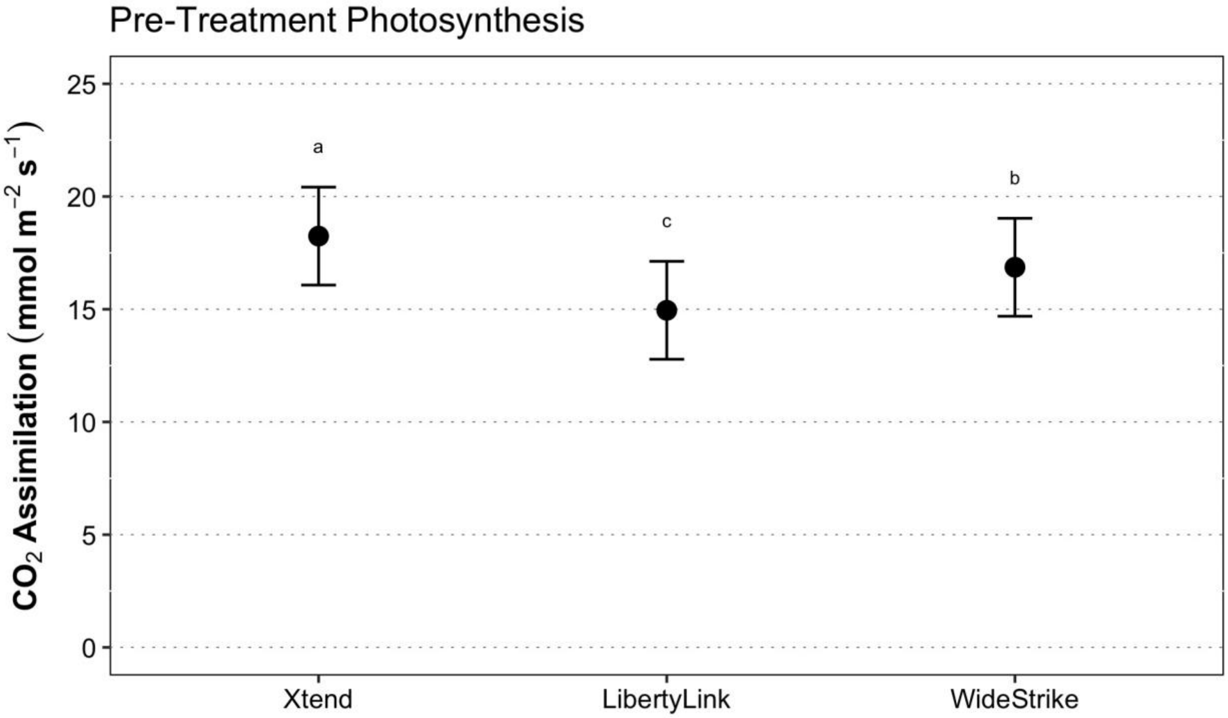


Figure 2.7. Leaf photosynthesis 1 DAT

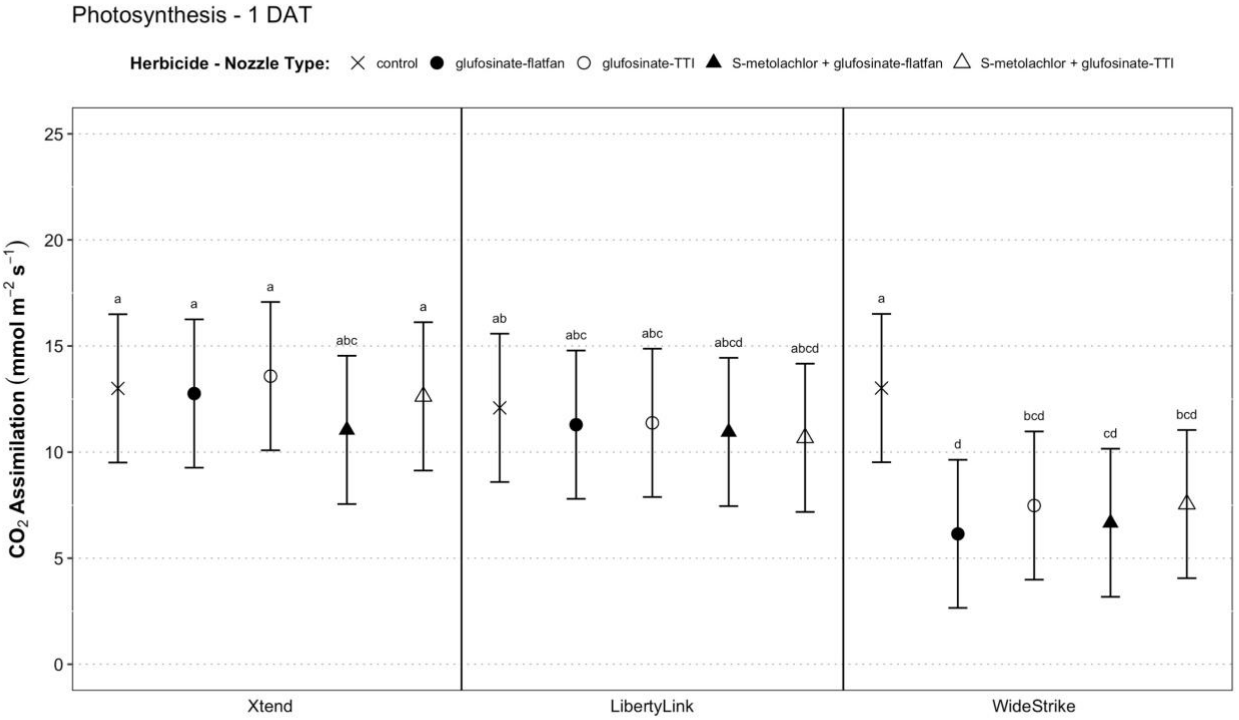


Figure 2.8. Leaf photosynthesis 2 DAT

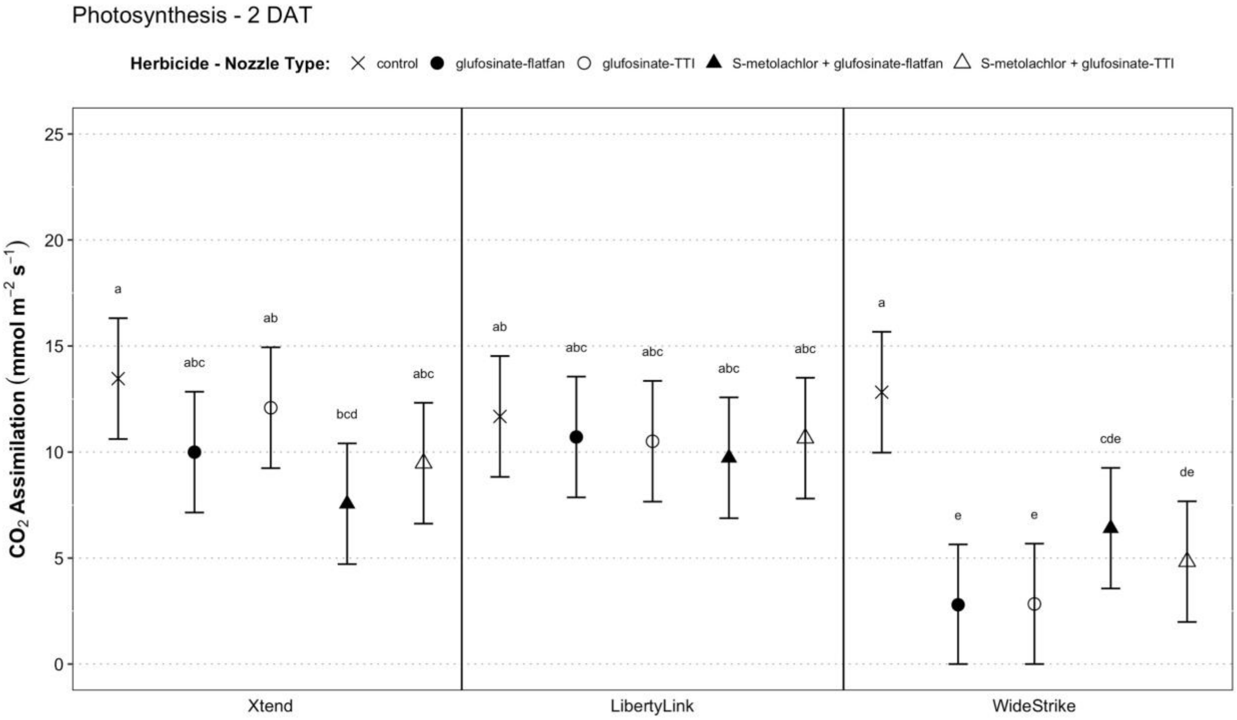


Figure 2.9. Leaf photosynthesis 3 DAT

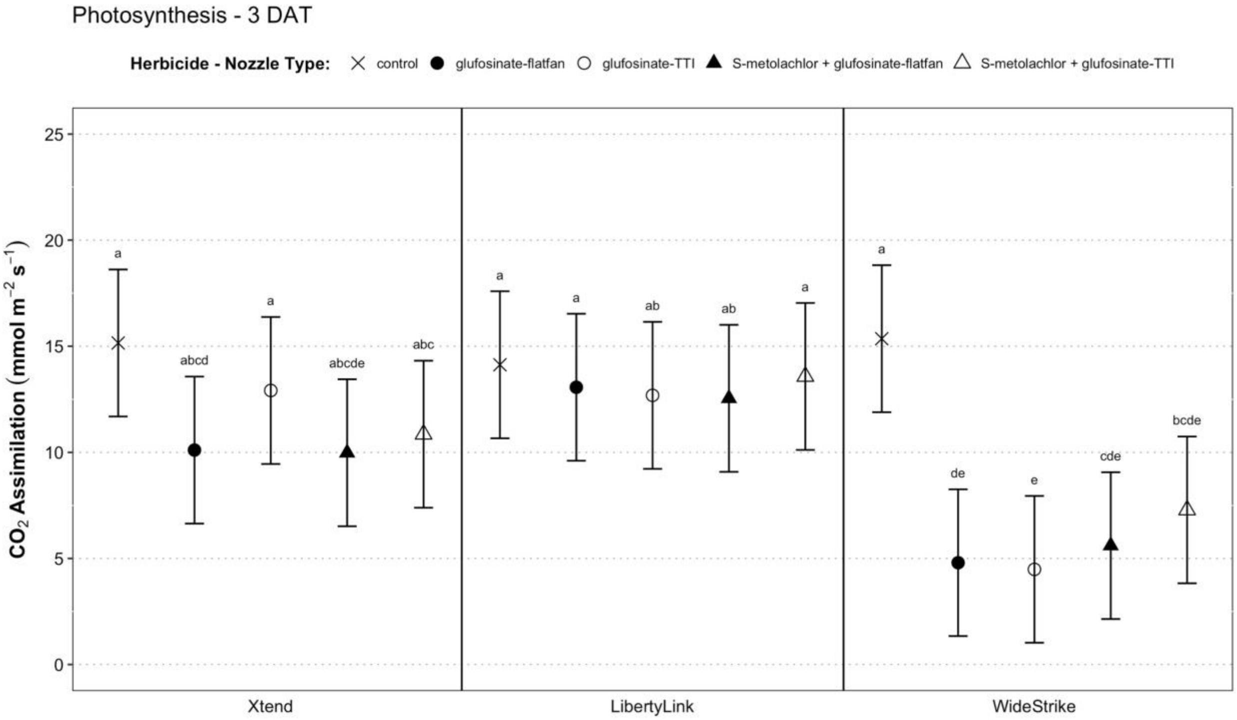


Figure 2.10. Leaf photosynthesis 7 DAT

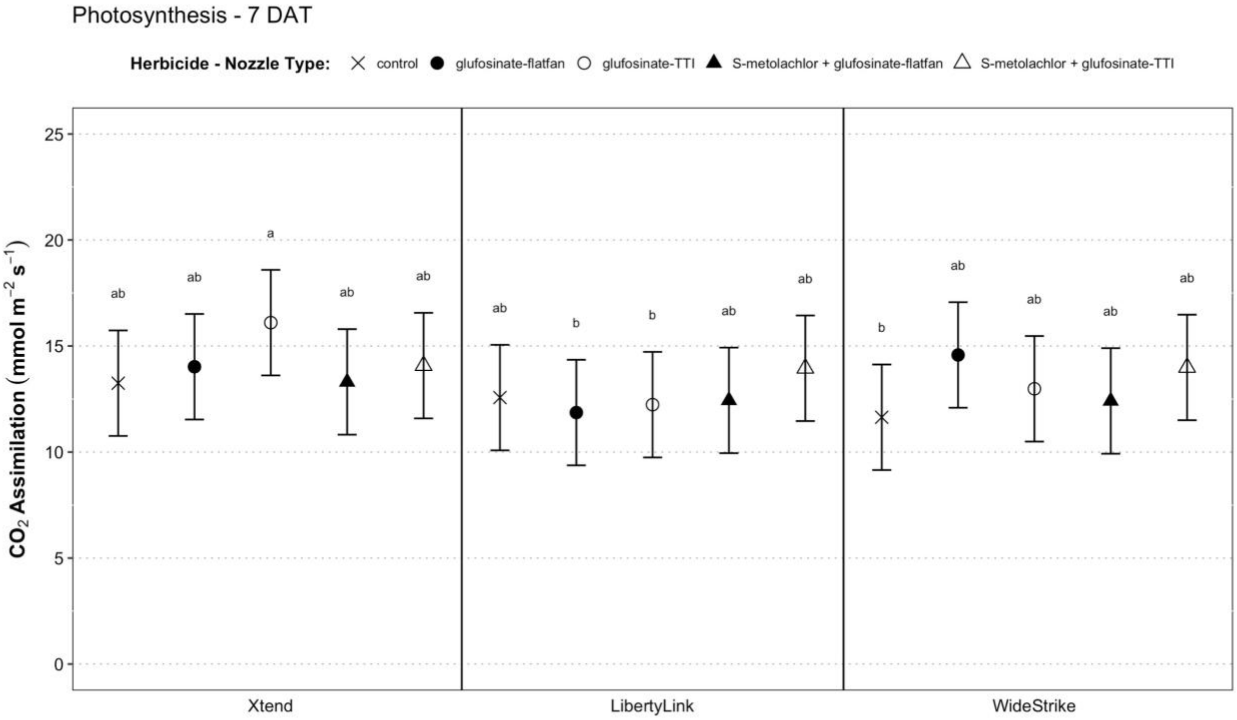


Figure 2.11. Stomatal conductance over time

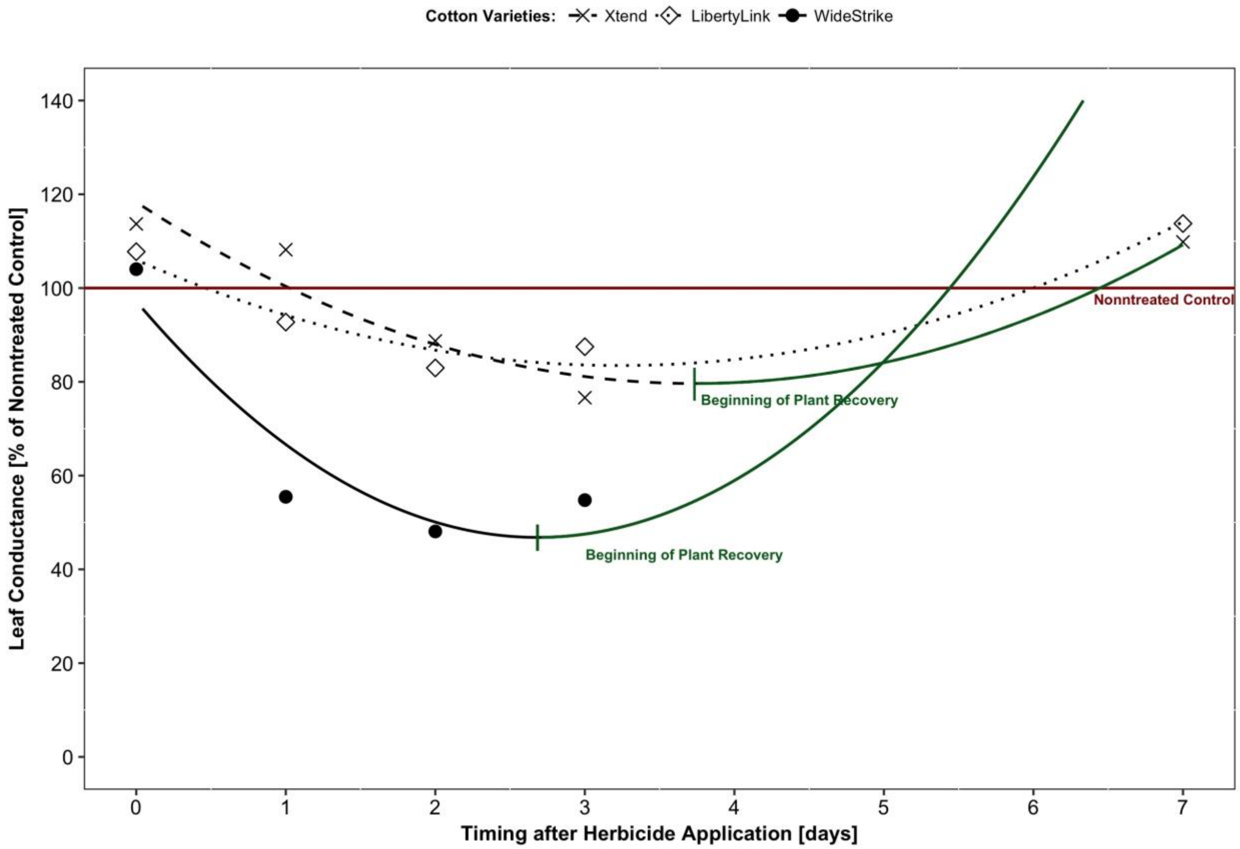
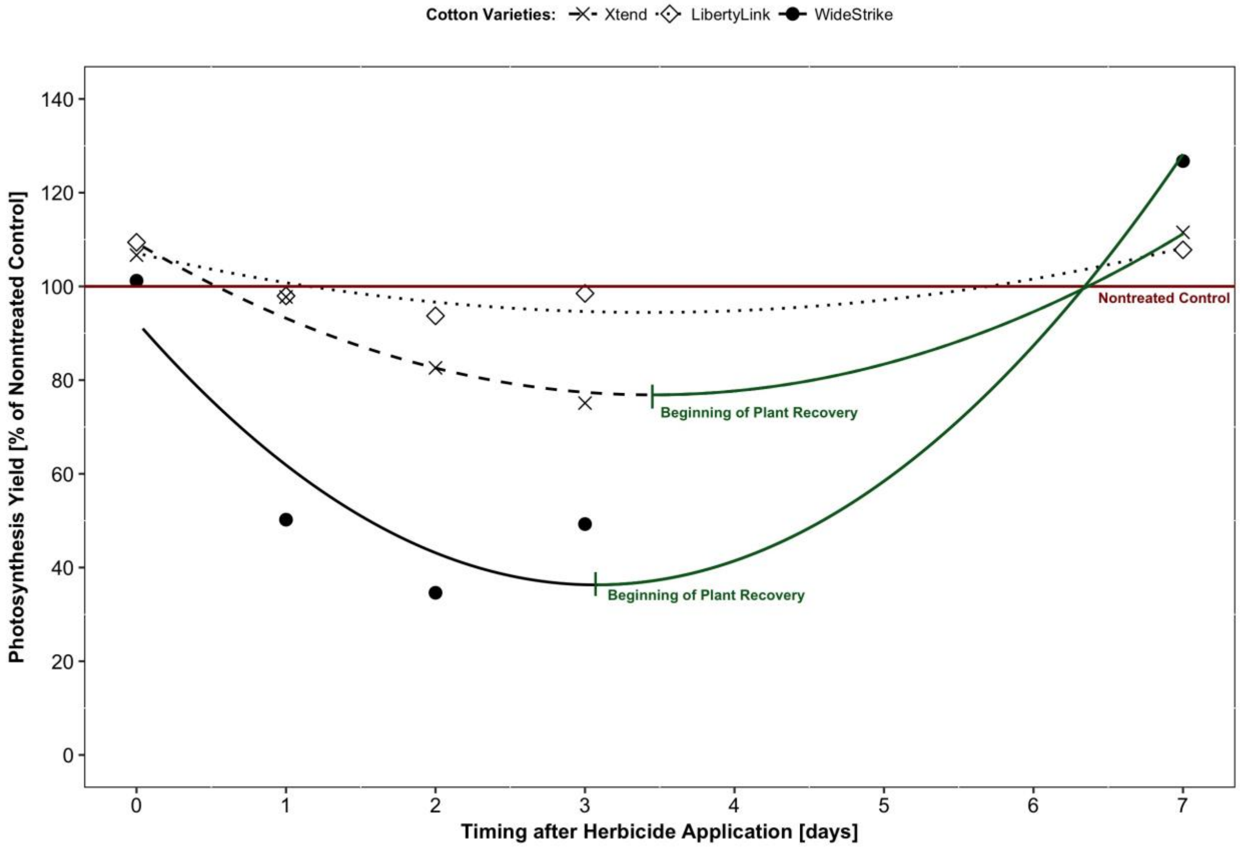


Figure 2.12. Leaf photosynthesis over time



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