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WEED SCIENCE

Evaluation of WideStrike® Cotton (*Gossypium hirsutum* L.) Injury from Early Season Herbicide and Insecticide Tank Mixes

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ABSTRACT

A study was conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN to evaluate the tolerance of Phytogen® 375 WRF (WideStrike®) cotton to glufosinate or a premix of glyphosate and *s*-metolachlor alone or when tank mixed with selected insecticides used for the control of thrips. Significant differences in visual injury were caused by the herbicides and the insecticides in 2010, but not in 2011. Glufosinate delayed crop maturity in 2010, but did not delay maturity in 2011. Total yield was reduced by glufosinate application but not by insecticide treatment in 2010. Herbicide treatment did not affect yield in 2011 (*P* = 0.3496) but insecticide application increased yield. There was no interaction between herbicide and insecticide on total yield in 2010 or 2011. These data show that maturity might be delayed and yield decreased by an early-season glufosinate or glufosinate + insecticide application to WideStrike cotton. This measurable level of yield loss from glufosinate on WideStrike cotton differs from most previous research. The measurable level of yield loss in WideStrike cotton in this case might be due to early-season stress injury just prior to the glufosinate application.

Managing glyphosate-resistant (GR) weeds in cotton has become a serious challenge for producers from the Mississippi Delta region of the U.S. to the Atlantic Coast (Heap, 2011). Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Watson) has in the last 5 yr become problematic in the Mid-South region (Heap, 2012). Glyphosate has been the dominant herbicide in cotton production because applications provide broad-spectrum control of most broadleaf and grass weed species (Askew et al., 2002; Baylis, 2000; Duke and Powles, 2009). Glyphosate systems are also less labor intensive (Culpepper and York, 1998) and less expensive than conventional systems (Baylis, 2000; Duke and Powles, 2009; Gianessi, 2005). Prior to the development of resistant weeds, applying glyphosate alone two to three times post emergence (POST) throughout the growing season was easy, effective, and ultimately profitable (Culpepper and York, 1998; Duke and Powles, 2009). However, this over-reliance on one herbicide has contributed to the spread of GR Palmer amaranth (Duke and Powles, 2009, 2008).

Glyphosate-resistant weeds did not appear until after GR crops were introduced, due to the heavy selection pressure placed on the weeds by one herbicide (Culpepper, 2006; Duke and Powles, 2008; Powles, 2008). Glyphosate-resistant Palmer amaranth was first confirmed in Georgia in 2005 (Culpepper, 2006), but now can be found throughout most of the U.S. cotton belt including Tennessee, Arkansas, Mississippi, Missouri, New Mexico, Alabama, North Carolina, South Carolina, Texas, and Louisiana (Heap, 2012. Palmer amaranth has a rapid growth rate and is competitive with many agronomic crops, including cotton (Culpepper, 2006). The presence of GR Palmer amaranth is a serious threat to the utility of glyphosate systems on GR crops and is a concern for growers (Steckel et al., 2008).

Glufosinate is a nonselective herbicide that is effective in controlling troublesome weed species such as GR Palmer amaranth (Culpepper et al., 2000, 2009; MacRae et al., 2007; Norsworthy et al., 2008). Glufosinate can provide effective control of GR Palmer amaranth when applied at the appropriate time, and is a good alternative to a glyphosate-based system when GR weeds are present (Culpepper et al., 2009; Everman et al., 2007; Steckel et al., 1997).

Varieties of glufosinate-resistant cotton (trade name LibertyLink®) were introduced as an alternative to GR cotton varieties. LibertyLink cotton was developed through insertion of the bar gene derived from *Streptomyces hygroscopicum* (Castle et al., 2006; Green, 2009; Tan et al., 2006), which confers tolerance to glufosinate (Herouet et al., 2005; OECD, 2002).

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Glufosinate can be applied to LibertyLink varieties for broad-spectrum weed control with no crop injury. Despite the need for an alternative technology to help control GR weeds, LibertyLink varieties have not been adopted quickly by growers in the Mid-South, in part because these varieties have not performed as well as other cotton varieties (UT, 2010). Thus, most current varieties are not glufosinate-resistant, limiting herbicide options for non-GR weed species.

WideStrike® cotton varieties contain two genes derived from *Bacillus thuringiensis* that confer resistance to lepidopteran pests (Castle et al., 2006; Dow Chemical Company, 2006). These varieties express Cry1Ac and Cry1F Bt insecticidal proteins; the Bt genes are linked with the *pat* gene. The *pat* gene also confers tolerance to the herbicide glufosinate. However, the tolerance of WideStrike varieties to glufosinate is incomplete compared with LibertyLink varieties (OECD, 2002; Tan et al., 2006). WideStrike varieties designated WRF also contain the CP4 EPSPS enzyme, which confers resistance to glyphosate. This allows growers the option of using both glyphosate and glufosinate as a part of their weed control program (Culpepper et al., 2009).

Injury from glufosinate applied to WideStrike varieties can reach 15 to 25% with one to two applications without decreasing yield (Barnett et al., 2011; Culpepper et al., 2009; Dodds et al., 2011; Whitaker et al., 2011). These varieties have performed well in the Mid-South and as a result, are being used by a large percentage of the growers in this region (Anonymous, 2012). In Tennessee, Phytogen 375 WRF and Phytogen 499 WRF were in the top performing group of five varieties across six different locations from 2010 to 2012 (UT, 2012). WideStrike varieties comprised 63% of the Tennessee cotton acres in 2010 and approximately 70% in 2011 (Anonymous 2010, 2012).

Thrips (Thysanoptera: Thripidae) primarily *Frankliniella* spp. and *Thrips tabaci* Lindeman, are common pests of seedling cotton (Stewart, 2011). Several species of thrips can be found in Tennessee including tobacco thrips, *Frankliniella fusca* (Hinds), western flower thrips, *Frankliniella occidentalis* (Pergande), flower thrips, *Frankliniella tritici* (Fitch), onion thrips *Thrips tabaci*, and soybean thrips *Neohydatothrips variabilis* (Beach)(Cook et al., 2003; Reed et al., 2010). *Frankliniella* spp. are usually predominant in Tennessee (Stewart, 2011). Thrips are consistently among the most important pests of cotton in Tennessee, and they inflict economic damage to some fields on an annual basis (Stewart, 2011). Thrips damage cotton seedlings by feeding on the leaves and the plant growing point in the terminals (Watts, 1937). Injury to apical meristem can result in loss of apical dominance resulting in excessive vegetative branching (Cook et al., 2011). Moreover, thrips injury to cotton can delay maturity and yield can be lost (Cook et al., 2011). Environmental conditions that result in poor seedling growth and vigor increase the chance of economic damage. Seedlings can be killed if heavy infestations persist unchecked.

At-planting treatments such as in-furrow aldicarb or insecticide seed treatments (Centric®, thiamethoxam, Gaucho®, imidacloprid) are used ubiquitously in Tennessee to prevent thrips injury and yield loss. Residual activity of at-planting insecticides is variable and can range from 2 to 4 wk after planting (Cook et al., 2011). Rummel et al. (1988) found an interaction (correlation) between the weather and thrips injury in seedling cotton. Therefore, supplemental foliar treatments might be justified even when at-planting insecticides were used, especially if conditions favor poor seedling growth and vigor (i.e., cool, wet weather). Despite the near universal use of insecticide seed treatments for the control of thrips, foliar insecticide applications for the control of thrips were made on 60% and 36% of cotton grown in Tennessee in 2009 and 2010, respectively (Williams 2010, 2011). The University of Tennessee recommends foliar treatment when thrips numbers average one or more per plant and injury is observed (Stewart et al., 2010). Once cotton reaches the fourth true leaf stage, thrips control is generally no longer necessary (Stewart, 2011). Supplemental foliar applications of insecticides that are often used for thrips control include acephate, dicrotophos, and dimethoate (Stewart et al., 2010). The optimum timing of insecticide for thrips control and herbicide for early weed control often coincide (Pankey et al., 2004). Therefore, tank mixtures of insecticides and herbicide are common in Tennessee.

Glufosinate can be applied post emergence (POST) to transgenic cotton beginning as early as cotyledon stage (Anonymous, 2011a). Weed species that can be present alongside the seedling cotton crop are also in the early developmental stages and thus more susceptible to control with glufosinate at this time.

As GR Palmer amaranth continues to spread across the U.S. Cotton Belt, applying combinations of herbicides to control these weeds is becoming more common. Glufosinate can be an effective tool; however, more timely applications as well as residual herbicides, are needed to control GR Palmer amaranth (Culpepper et al., 2007, 2009; Everman et al., 2009; Whitaker et al., 2008). Miller et al. (2008) found that some insecticides in a tank mixture with glyphosate and mepiquat chloride could increase cotton injury, but these treatments had no effect on yield. There were no interactions on stand, plant weight, or cotton yield in a study examining aldicarb and trifluralin plus diuron (Micinski, 1985). Additionally, pyrithiobac (Staple®), combined with various insecticide treatments (including dimethoate) did not reduce cotton leaf area, height, main stem node count, main stem nodes to first square, days to first square or flower, main stem nodes above white flower, or seed cotton yield (Costello et al., 2005). However, the stress of a potentially injurious herbicide applied alone or in combination with insecticide applications to seedling cotton might increase crop injury and ultimately affect crop yield. The effect of insecticides in a tank mixture with glufosinate is unknown, but insecticides potentially could worsen the injurious effects of glufosinate (herbicides). The objectives of this experiment were to 1) determine the tolerance of WideStrike cotton to glyphosate + s-metolachlor or glufosinate applied alone or in a tank mixture with acephate, dimethoate, or dicrotophos in the presence of thrips and 2) evaluate the effects on yield and maturity.

MATERIALS AND METHODS

Field experiments were conducted in 2010 and 2011 at the West Tennessee Research and Education Center in Jackson, TN. Soil at the Jackson location is a Lexington silt loam (fine-silty, mixed, thermic, Typic Paleudalfs) with organic matter of 1.5% and a pH of 6.6. Cotton variety Phytogen 375 WRF with only a base fungicide treatment was planted at a rate of 14 seeds per meter of row on 14 May 2010 and 10 May 2011. Plot size was four 97-cm rows by 9 m long. Cotton was planted by conventional till onto bedded ground that was planted to cotton the previous year. Agronomic practices such as fertilization, seeding rates, insect control, and harvest aides followed University recommendations. Treatments were applied with a high-clearance sprayer calibrated to apply (deliver) 74.8 L/ha with 8001 flat fan nozzles with a boom width of 3.86 m.

Plots were arranged as a four by two factorial in a randomized complete block design with four replications. Main effects were four levels of insecticide treatments by two herbicide treatments. Herbicide treatments included Sequence (Syngenta Crop Protection, 3411 Silverside Rd., Wilmington, DE 19810) (a premix of glyphosate + s-metolachlor) at 1.46 ai kg/ha or glufosinate (Bayer Crop Science, 7616 Moore Rd. Memphis, TN 38138) at 0.59 ai kg/ha alone or in a tank mixture with an insecticide. Insecticide treatments included dimethoate at 0.21 ai kg/ha, dicrotophos at 0.21 ai kg/ha, or acephate at 0.25 ai kg/ha. All insecticides are labeled and commonly applied for foliar control of thrips in Tennessee cotton. Application was made 1 June 2010 and 30 May 2011 to cotton at the two-leaf stage, which is within the typical application window for the control of thrips (Stewart et al., 2010). There were considerable visual symptoms of thrips injury at the time of application. Thrips injury was rated 2 d after application (DAA) using a 1 to 5 scale with 1 = no injury and 5 = severe injury. Thrips densities were determined 2 DAA by randomly selecting five plants per plot. Plants were cut at the soil level and immediately inverted into jars of 70% ethanol solution, vigorously agitated, and sealed. The jars were taken to the laboratory and plants were given an ethanol wash as they were removed from the jars. Samples were sieved through a fine-mesh sieve (125-µm openings) and transferred to ruled petri dishes where adult and immature thrips were counted under a dissecting microscope. Visual ratings were conducted 2 DAA to assess herbicide injury, primarily leaf burn (leaf chlorosis and necrosis), on a 0 to 100 scale (0 = no crop injury and 100 = complete plant death). Cotton plants were also sampled to determine differences in maturity from application treatments. Total squares were counted in 1 m of row on 24 June 2010 and 24 June 2011. Cotton was defoliated at optimum maturity according to University of Tennessee recommendations. The two center rows of each plot were mechanically harvested with a spindle picker on 16 September 2010 and 23 September 2011 and seed cotton yield data were recorded. A second picking was conducted 15 d after first harvest.

The study was a four by two factorial arranged design. Results were analyzed by analysis of variance (ANOVA) procedures conducted with PROC GLM in SAS (ver. 9.2; SAS Institute, Cary, NC). Means for significant main effects and interactions were separated using Fisher's protected least significant difference (LSD) at $P = 0.05$.

RESULTS

Significant treatment-by-year interactions prevented the data from being analyzed across tests. Therefore, data are presented separately for each year. The two-way interactions between herbicide and insecticide were not significant for any variable in either year, including yield in either year Therefore, data for the main effects of herbicide were combined and analyzed across insecticide treatments. Likewise, data for insecticides were combined and analyzed across herbicide treatments.

Insecticides. Thrips populations exceeded the recommended threshold of one or more thrips plant^{-1} and injury present each year in the non-insecticide treated plots (Stewart et al., 2010). All insecticide treatments similarly reduced thrips numbers and thrips injury in both years (Table 1). As expected, herbicide did not affect thrips numbers in 2010 and 2011 (Table 1) nor was there an interaction between herbicide or insecticide treatments ($P = 0.8178$). It is notable that thrips densities were much heavier in 2010 than 2011. Insecticide treatment had no effect on total seed cotton yield in 2010 ($P = 0.1969$), but dicrotophos and acephate application increased yield the following year by more than 395 lbs/acre ($P = 0.0165$)(Table 1).

Cotton Injury. There were significant differences in phytotoxicity injury between herbicides and also between insecticides in 2010, but not in 2011. Glufosinate caused 23% more injury compared with glyphosate + *s*-metolachlor in 2010 ($P = 0.0001$), but there were no differences in injury between herbicide application in 2011 ($P = 0.2796$), nor a delay in maturity ($P = 0.8434$). We speculate that the higher thrips numbers in 2010 stressed the cotton more and magnified the glufosinate injury. Dimethoate increased cotton injury 3 to 4% compared with the other insecticides in 2010 ($P = 0.0356$). However, insecticide had no effect on degree of herbicide injury in 2011 ($P = 0.8098$).

Herbicides. Glufosinate delayed maturity compared with glyphosate + *s*-metolachlor 2010 as evidenced by square counts (data not shown) and first and second harvest data (Table 2). Glufosinate had 34% fewer squares in 1 row m^{-1} in 2010 than did glyphosate + *s*-metolachlor (32 vs 50, respectively). However, there was no difference in square counts between herbicides in 2011. Glyphosate + *s*-metolachlor yielded 21% more seed cotton at the first harvest compared with glufosinate ($P = 0.0001$, Table 1). Second harvest data showed glufosinate yielded 23% more than did glyphosate + *s*-metolachlor ($P = 0.0001$) (Table 1). As evidenced by similar first harvest yield amounts, there was no observable difference in maturity caused by herbicide application in 2011 (Table 2). Although total yield was reduced by application of glufosinate in 2010 ($P = 0.0007$), herbicide treatment did not affect yield in 2011 ($P = 0.3496$).

Table 1. Mean thrips numbers, leaf injury (chlorsis, necrosis), and seed cotton weights of herbicide and insecticide treatments, 2010 and 2011.

Main Effect	Treatment	2010			2011		
		Thrips^{z}	Injury %^{Y}	kg ha^{-1}	Thrips^{z}	Injury %	kg ha^{-1}
Herbicide	glyphosate + *s*-metolachlor	60	6	2120^{X}	38	10	1380
	Glufosinate	66	29	1870	40	11	1420
	Factorial Analysis	*P = 0.6261*	*P= 0.0001*	*P = 0.0007*	*P = 0.6885*	*P = 0.2796*	*P = 0.3496*
Insecticide	Untreated	200 a	17 a	2080 a	70 a	10	1290 a
	Dimethoate	19 b	20 b	1900 a	38 b	12	1380 ab
	Dicrotophos	18 b	17 a	1960 a	19 b	11	1470 b
	Acephate	14 b	17 a	2040 a	28 b	11	1470 b
	Factorial Analysis	*P = 0.0001*	*P = 0.0479*	*P = 0.1969*	*P = 0.0001*	*P = 0.8098*	*P = 0.0165*

^{Z}Thrips numbers per five plants at 2 days after application.

^{Y}Injury with 0= no visible injury and 100%= complete plant death.

^{X}Seed cotton yield.

Table 2. First harvest and second harvest seed cotton in 2010, averaged across all insecticide treatments. There was no difference between first and second harvest for 2011 (data not shown).

Herbicide	First harvest	Second harvest
	(kg ha^{-1})	
glyphosate + *s*-metolachlor	4150^{Z}	1160
glufosinate	3160	1510
	($P < 0.0001$, LSD 380)	($P < 0.0001$, LSD 150)

^{Z}Seed cotton yield.

DISCUSSION

Yield response of insecticide application to seedling thrips was variable. In 2010, there was no yield response when thrips on seedling cotton were controlled. Similar results have been found in other studies (Beckham, 1970; Cowan et al., 1966; Harp and Turner, 1976; Lentz and Austin, 1994; Newsom et al., 1953; Ratchford et al., 1987, 1989; Roberts, 1994; Terry and Barstow, 1985; Watson, 1965). However, yield was increased in 2011 with foliar application of acephate or dicrotophos. This agrees with other studies that found an increase in yield when cotton seedling infested thrips were controlled (Almand, 1995; Burris et al., 1989; Carter et al., 1989; Davis and Cowan, 1972; Davis et al., 1966; Herbert, 1998; Lentz and Van Tol, 2000; Leser, 1985; Race, 1961; Van Tol and Lentz, 1999; Watts, 1937). Cotton injury data indicated that in 2010, glufosinate-based treatments showed more visual leaf injury than glyphosate and s-metolachlor (Table 1). In 2011 however, there was no difference between any of these treatments. The result from our study in 2010 agreed with previous research, whereas the results in 2011 were different. In previous research (Barnett et al., 2011; Culpepper et al., 2009; Whitaker et al., 2011), glufosinate applications increased phytotoxicity to WideStrike cotton varieties compared to glyphosate.

Application of glufosinate to seedling cotton delayed maturity in 2010 as evidenced by first and second harvest data (Table 2). This is inconsistent with Barnett et al. (2011), Culpepper et al. (2009), Dodds et al. (2011), and Whitaker et al. (2011) who did not find delay in maturity from glufosinate applications to WideStrike cotton despite more visual observed cotton injury from glufosinate. Glufosinate application in 2011 did not delay maturity or adversely affect yield, which is consistent with most previous research (Barnett et al., 2011; Culpepper et al., 2009; Dodds et al., 2011; Whitaker et al., 2011). This difference in response likely was due to the cotton suffering from thrips stress prior to the glufosinate application in 2010 but not 2011. Thrips numbers averaged 40 per plant at time of evaluation 3 DAA, well over the UT recommended threshold of 1 per plant. Similarly, the glufosinate application in 2010 reduced yield compared with the premix of glyphosate + s-metolachlor. This differed from previous research that found no yield penalty for glufosinate application (Barnett et al., 2011; Culpepper et al., 2009; Dodds et al., 2011; Whitaker et al., 2011). Much like the delay in maturity, this response might have been different due to the cotton suffering from thrips stress prior to the glufosinate application. Glufosinate did not cause a delay in maturity or reduce yield in 2011. The results of this study in 2010 disagree and 2011 agrees with previous research: Barnett et al. (2011), Culpepper et al. (2009), Dodds et al. (2011), and Whitaker et al. (2011) found injury to WideStrike cotton with one to two applications of glufosinate without decreasing yield.

There was no herbicide x insecticide interaction in either year of the study. Therefore, this data suggest that acephate, dimethoate, or dicrotophos can be tank mixed with glufosinate. This agrees with other studies that found that herbicide x insecticide co-application had no effect on yield (Costello et al., 2005, Micinski, 1985; Miller et al., 2008)

These data show that maturity can be delayed and yield decreased by an early season glufosinate or glufosinate + insecticide application to WideStrike cotton that is already stressed by thrips. However, the impact of this treatment during the seedling stage of cotton is likely to remain variable because environmental conditions vary between one year and the next. Although this response is inconsistent due to environment, delaying maturity in cotton can increase expenses by extending the amount of time required to protect fruit from other insect pests late in the season. Also, more aggressive measures to defoliate the crop might be needed because some harvest-aid compounds are sensitive to lower temperatures. In addition, this makes the crop susceptible to adverse weather conditions such as rainfall, which can reduce lint quality and yield (Barker et al., 1976; Williford et al., 1995). Excessive rainfall might delay harvest even more, lead to damaging fields with harvest equipment and make fall tillage impossible. On the other hand, early season infestation of thrips and/or GR weeds must be controlled or yield might be lost. Cotton producers must weigh this risk of injury from glufosinate application to WideStrike cotton in the seedling development stage against potential yield loss from GR weeds.

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