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WEED SCIENCE

Injury Potential from Herbicide Combinations in Enlist® Cotton

Chase Allen Samples, Greg R. Kruger, Darrin M. Dodds*, J. Trenton Irby,
Daniel B. Reynolds, and Angus L. Catchot

ABSTRACT

Enlist® cotton with tolerance to 2,4-D choline, glyphosate, and glufosinate became publicly available in 2016 to aid growers in controlling glyphosate-resistant weed species. Little data exist regarding the tolerance of Enlist cotton to herbicide tank mixtures containing glyphosate, glufosinate, 2,4-D choline, and *S*-metolachlor. The objective of this study was to evaluate the tolerance of Enlist cotton to herbicide tank mixtures including these herbicides. Field studies were conducted in 2016 and 2017 where cotton was sprayed with herbicide combinations containing glyphosate, glufosinate, *S*-metolachlor, 2,4-D choline, and a premix formulation of glyphosate and *S*-metolachlor. Crop injury consisted of necrosis, chlorosis, visual stunting, injury on new growth, and total injury at 7, 14, and 28 days after application (DAA). Cotton lint yield was recorded at the conclusion of each growing season. The greatest levels of necrosis and total injury at 7 DAA were observed following applications of glufosinate + *S*-metolachlor, alone or in combination with glyphosate or glyphosate + 2,4-D choline. The least amount of necrosis and total injury at 7 DAA was observed following applications of glyphosate, glufosinate, *S*-metolachlor, glyphosate + glufosinate, or glyphosate + *S*-metolachlor, which produced less than 13% injury. Visual injury at 14 DAA ranged from 8 to 16% across herbicides applied. At 28 DAA, no differences in visual injury were reported. Lint yield was unaffected by herbicide application. Although transient visual injury is expected, Enlist cotton withstood herbicide applications with up to four modes of action in tankmixture without suffering yield reduction.

Enlist® cotton (*Gossypium hirsutum* L.) offered by PhytoGen Cottonseed became available to growers across the Cotton Belt in 2016. The Enlist platform in cotton confers tolerance to 2,4-D choline, glyphosate, and glufosinate (ISAAA, 2016). Recent cultivar releases from Phytogen also contain WideStrike® 3 technology, which confers tolerance to lepidopteran pests (Jacobson et al., 2016). The combination of these traits in a single technology package are coded as four events: 3006-210-23 x 281-24-236 x MON88913 x COT102 x 81910 (ISAAA, 2016). The trait 281-24-236 codes for a synthetic form of phosphinothricin N-acetyltransferase (PAT) that confers tolerance to glufosinate by acetylation. This event codes for the Cry1F delta endotoxin, which was derived from *Bacillus thuringiensis* var. *aizawai* and confers tolerance to lepidopteran insects by damaging the insect's midgut lining (ISAAA, 2016). The event 3006-210-23 codes for a synthetic form of the PAT enzyme that serves as a selectable marker, and codes for Cry1Ac delta-endotoxin, which also confers tolerance to lepidopteran insects by damaging the midgut lining (ISAAA, 2016). The event COT102 codes for the VIP3A vegetative insecticidal protein that was derived from *Bacillus thuringiensis* strain AB88, and confers tolerance to feeding damage caused by lepidopteran pests in a similar manner to Cry1Ac and Cry1F. It also codes for the hygromycin-B phosphotransferase (hph) enzyme, which is introduced by the gene aph4 (hpt) and allows selection for tolerance to the antibiotic hygromycin B. Event 81910 codes for the introduction of aad-12, which produces aryloxyalkanoate di-oxygenase 12 (AAD-12) protein that catalyzes the side-chain degradation of 2,4-D. It also codes for the PAT enzyme, which eliminates the herbicidal activity of glufosinate (ISAAA, 2016).

C.A. Samples, D.M. Dodds, J.T. Irby, and D.B. Reynolds, Department of Plant and Soil Sciences, Mississippi State University, 32 Creelman St., Mississippi St., MS 39762; G.R. Kruger, West Central Research and Extension Center, University of Nebraska-Lincoln, 402 W State Farm Road, North Platte, NE 69101-7751; and A.L. Catchot, Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, 100 Twelve Lane, Mississippi State, MS 39762.
*Corresponding author: dmd76@msstate.edu

Braxton et al. (2017) observed that when glufosinate was applied to 6-8–leaf Enlist cotton (DAS-81910-7) at 542 g ha^{-1} (1X label rate), 1,084 g ha^{-1} (2X label rate), and 2,168 g ha^{-1} (4X label rate) in 2010, visual injury was 3, 7, and 13%, respectively, at 3 d after application (DAA). Additionally, crop injury decreased over time and herbicide injury at 7 DAA was minimal with the highest level of injury persisting being 2% following applications at the 4X rate. Similar injury levels were observed on 6-8–leaf cotton as well as 10-12–leaf cotton. Additionally, injury observed 3 DAA was no greater on cotton subjected to sequential applications of glufosinate when compared to cotton receiving a single application of glufosinate at the 10-12–leaf growth stage (Braxton et al., 2017). However, greater cotton injury was observed 7 d after the sequential glufosinate application was made compared to treatments that received a single application at the 10-12–leaf growth stage. Additionally, four formulations of glufosinate were evaluated. Cotton treated with Liberty® or Interline™ resulted in greater injury 3 DAA when compared to cotton treated with Kong™. Injury observed from applications of Cheetah™ did not differ from injury observed from Liberty, Interline, or Kong. Additionally, greater injury persisted 14 DAA following Interline application compared to Kong (Braxton et al., 2017). However, no impact on cotton yield was observed and Enlist cotton appears to have a similar level of tolerance to glufosinate to that observed in LibertyLink® or GlyTol™ + LibertyLink cotton (Dodds et al., 2015; Irby et al., 2013; Sweeney and Jones, 2014; Wallace et al., 2011).

Published data on the response of Enlist cotton to herbicide applications containing multiple modes of action (MOAs) are lacking. However, the response of WideStrike® cotton to applications containing multiple MOAs has been documented. Steckel et al. (2012) found that tank mixtures containing glufosinate resulted in significantly greater injury when applied to WideStrike cotton when compared to tank combinations containing glyphosate. In addition, application of *S*-metolachlor alone, dimethoate alone, or the combination of the two resulted in less injury (3-9%) compared to applications containing glufosinate, glyphosate, *S*-metolachlor, and dimethoate (20-33%) (Steckel et al., 2012). The addition of *S*-metolachlor to applications of glufosinate increased the level of injury observed on WideStrike cotton (Cahoon et al., 2015). Similar results have been observed by Culpepper et al. (2009), Whitaker et al. (2011), and Steckel et al. (2012). Cahoon et al. (2015) found that visual injury on WideStrike cotton increased when glufosinate and glyphosate were co-applied compared to either herbicide applied alone. These findings agree with previous research from Steckel et al. (2012), who found that applications of glufosinate + glyphosate, glufosinate + *S*-metolachlor, as well as three- and four-way combinations of glufosinate, glyphosate, *S*-metolachlor, and dimethoate resulted in reduced cotton height. Treatments of glufosinate + *S*-metolachlor also resulted in delayed cotton maturity and increased yield loss.

Although previous research documented the herbicide injury response observed when multiple MOAs were applied to WideStrike cotton, currently there are little data available regarding crop injury response of Enlist cotton to herbicide applications containing multiple MOAs. Therefore, this research was conducted to quantify the effects of herbicide tank-mix combinations on Enlist cotton injury and yield.

MATERIALS AND METHODS

Experiments were conducted in 2016 and 2017 at the R.R. Foil Plant Science Research Center at Starkville, MS, and the Black Belt Branch Experiment Station in Brooksville, MS. Planting date, application date, and harvest date are given in Table 1. Phytogen 490 W3FE was planted at 13.1 seeds per meter of row on conventionally tilled beds spaced 97 cm apart in all experiments. Cottonseed treatment included azoxystrobin (0.018 mg ai/seed), fludioxonil (0.005 mg ai/seed), mefenoxam (0.016 mg ai/seed), imidacloprid (0.493 mg ai/seed), abamectin (0.15 mg ai/seed), myclobutanil (0.023 mg ai/seed), and sedaxane (0.009 mg ai/seed). Cotton maintenance including applications of plant growth regulators and harvest aids were conducted based on Mississippi State University Extension (MSU Extension, 2020) recommendations. Each plot consisted of four rows 12 m in length. Herbicide applications were made to 3-6–leaf cotton with the center two rows being treated and the outside rows remaining untreated to serve as a buffer between plots. Experiments located at the Starkville location in 2016 and 2017 were furrow irrigated as needed, whereas cotton at the Brooksville location was grown under rainfed conditions.

Table 1. Dates of planting, visual rating, and harvest evaluation of multiple herbicide mode of actions applied to W3FE cotton

	Brooksville		Starkville	
	2016	2017	2016	2017
	Date			
Planting Date	12 May	09 May	11 May	02 May
Stand Counts	25 May	25 May	25 May	24 May
Application Date	07 June	13 June	08 June	13 June
7 days after application	15 June	20 June	15 June	20 June
14 days after application	21 June	27 June	21 June	27 June
28 days after application	05 July	07 July	05 July	09 July
Harvest	27 October	10 November	10 October	25 October

In 2016 at Starkville, nitrogen (N) fertilizer (32% UAN) was injected, whereas in 2017, AMS (30-0-0-2.5 S) was utilized. In both years at the Starkville location, 56 kg N ha^{-1} was applied at planting and 78 kg N ha^{-1} was applied at the third week of squaring. In both years at the Brooksville location, N fertilizer was applied in a single application of 134 kg N ha^{-1} at the third week of squaring. All applications were made using a ground-driven knife applicator. All other fertilizer was applied based on soil test recommendations. Cotton fields were scouted weekly at both locations in both years using the appropriate methodology for weed and insect pests with pesticide applications and harvest aid applications applied based on Mississippi State University Extension (MSU Extension, 2020) recommendations.

Experiments were conducted using a randomized, complete block design with four replications. Treatment combinations consisted of an untreated check and herbicide(s) applications containing glyphosate (Roundup Powermax®, Monsanto Company, St. Louis, MO), glufosinate (Liberty® 280 SL, Bayer CropScience, Durham, NC), *S*-metolachlor (Dual Magnum®, Syngenta Crop Protection, Greensboro, NC), and 2,4-D choline (Enlist® One with Colex D Technology, Dow AgroSciences LLC, Indianapolis, IN), and a premix formulation of glyphosate + *S*-metolachlor (Sequence®, Syngenta Crop Protection, Greensboro, NC) Specific treatment combinations and herbicide rates are included in Table 2. Applications were made using a CO_2 pressurized backpack sprayer calibrated to deliver 140 L/ha with TeeJet AIXR 110015 nozzles at 317 kPa.

Data collection included visual injury ratings at 7, 14, and 28 DAA (Table 1). Total visual injury ratings were the summation of % necrosis, % chlorosis, % stunted growth, and % visual injury in comparison to the untreated check following applications. Seed cotton yield was collected using a 2-row spindle picker modified for small plot research. Prior to harvest, 25 boll samples were hand harvested from each plot. Each sample was ginned on a 10-saw Continental Eagle (Lubbock, TX) laboratory gin. Gin turnout was derived by dividing the lint mass after ginning by the seed cotton weight prior to the ginning process and multiplying by 100. Data were pooled over years and locations and were subjected to analysis of variance using the PROC GLIMMIX procedure in SAS (SAS v9.4, SAS Institute Inc., Cary, NC). Means were separated using Fisher's protected LSD ($\alpha \leq 0.05$).

Table 2. List of herbicide combinations used as well as rates applied to W3FE cotton

Herbicides	Rates (kg ae/ai ha^{-1})
Glyphosate	1.1
Glufosinate	0.6
S-metolachlor	1.1
Glyphosate + glufosinate	1.1 + 0.6
Glyphosate + *S*-metolachlor[z]	0.8 + 1.1
Glufosinate + *S*-metolachlor	0.6 + 1.1
Glyphosate + glufosinate + *S*-metolachlor	1.1 + 0.6 + 1.1
Glyphosate + glufosinate + 2,4-D	1.1 + 0.6 + 1.1
Glyphosate + glufosinate + 2,4-D + *S*- metolachlor	1.1 + 0.6 + 1.1 + 1.1

[z] Premix formulation

RESULTS AND DISCUSSION

Herbicide treatments evaluated did not differ on chlorosis injury visually observed on foliage at 7, 14, and 28 DAA (Table 3). Mean level of chlorosis injury observed ranged from 0 to 3% at 7 and 14 DAA and 0 to 6% at 28 DAA (data not shown). Similarly, herbicide combinations applied did not result in stunted growth at 7, 14, and 28 DAA (Table 3). Ratings of stunted growth did not exceed 3% at any evaluation period (data not shown). Although statistical differences were detected between the untreated and some of the herbicide treatments, the actual amount of chlorosis or stunting present was not realistically or biologically significant (1-3%).

Herbicide treatments impacted necrosis injury as well as total injury present at 7 and 14 DAA (Table 3). Foliar necrosis varied by treatment and ranged from 0 to 21% (Table 4). For all three rating periods, total injury primarily consisted of necrosis on foliar tissue. This injury manifested as necrosis speckling on foliage present at the time of application. New growth was not affected by herbicide treatments after application. The greatest level of necrosis and total injury present 7 DAA was observed following application of glufosinate + *S*-metolachlor alone or in combination with glyphosate or glyphosate + 2,4-D choline, as well as glyphosate + glufosinate + 2,4-D choline (Table 4). The least amount of necrosis and total injury 7 DAA was observed following applications of glyphosate, glufosinate, *S*-metolachlor, glyphosate + glufosinate, and glyphosate + *S*-metolachlor, which resulted in less than 10% necrosis and 13% injury (Table 4). These data corroborate with Steckel et al. (2012), who observed that visual injury was higher when glufosinate was applied in combination with *S*-metolachlor compared to individual applications of each herbicide. Authors also observed that injury was greater when glufosinate was applied in combination with *S*-metolachlor compared to the application of glyphosate + *S*-metolachlor. Cahoon et al. (2015) and Steckel et al. (2012) reported an increase in injury when glyphosate and glufosinate were applied in combination with *S*-metolachlor to WideStrike cotton compared to when they were applied alone.

Table 3. Analysis of variance for the effect of herbicide combinations on visual injury estimates at 7, 14 and 28 days as after application (DAA) as well as lint yield

Effect	D.F.[z]	Chlorosis			Necrosis			Stunting			Total Injury			Lint Yield
		7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	
		p-value												
Herbicide combinations	9	0.6103	0.2827	0.5970	0.0003	0.0286	0.7802	0.5571	0.1525	0.4359	<0.0001	0.0054	0.3092	0.9257

[z] Degrees of freedom

Table 4. Effect of herbicide combinations applied to W3FE cotton on percentage foliar necrosis and total foliar visual injury

Herbicide combinations	7 DAA[z]	14 DAA	7 DAA	14 DAA
	Necrosis		Total Injury	
	%			
Untreated	0 d[y]	0 b	0 d	0 c
Glyphosate	6 cd	8 ab	8 cd	8 bc
Glufosinate	9 cd	9 ab	12 bc	10 b
S-metolachlor	6 cd	13 a	9 cd	14 ab
Glyphosate + glufosinate	10 bcd	8 ab	13 bc	10 bc
Glyphosate + *S*-metolachlor	9 cd	13 a	13 bc	14 ab
Glufosinate + *S*-metolachlor	20 ab	17 a	22 ab	18 ab
Glyphosate + glufosinate + *S*-metolachlor	21 a	16 a	24 a	18 ab
Glyphosate + glufosinate + 2,4-D	14 abc	15 a	16 abc	17 ab
Glyphosate + glufosinate + 2,4-D + *S*-metolachlor	21 a	16 a	25 a	20 a

[z] Days after application

[y] Means within a column followed by the same letter are not significantly different ($\alpha \leq 0.05$)

In general, crop injury expressed as necrosis speckling decreased in most cases at 14 DAA. Injury ranged from 8 to 16% depending on herbicide(s) applied (Table 4). Application of all herbicides resulted in similar levels of necrosis injury. Application of glyphosate or glufosinate alone, or in combination, resulted in indistinguishable necrosis compared to the untreated check. Total level of injury from applications of glyphosate + glufosinate + 2,4-D choline + *S*-metolachlor was greater 14 DAA compared to total injury present on cotton treated with glyphosate and glufosinate applied alone and in combination at 14 DAA (Table 4). Similar levels of injury were present 14 DAA on cotton treated with glyphosate, glufosinate, *S*-metolachlor, glyphosate + *S*-metolachlor, glufosinate + *S*-metolachlor, glyphosate + glufosinate, glyphosate + glufosinate + *S*-metolachlor, and glyphosate + glufosinate + 2,4-D choline (Table 4). At 28 DAA no differences in necrosis or total injury were reported (less than 7%, data not shown).

There was no significant impact of herbicide application on cotton lint yield (Table 3). Steckel et al. (2012) observed a decrease in lint yield following applications of glyphosate + glufosinate + *S*-metolachlor applied to WideStrike cotton. Braxton et al. (2017) also reported a lack of yield response of W3FE cotton to herbicide tank-mix combinations. Cotton lint yield ranged from 874 to 987 kg lint ha^{-1} (data not shown). The lack of response on new growth and lint yield reductions indicated that visual injury was transient. We hypothesize that this is due to the additional copy of the PAT enzyme present in the construct coded 81910. Minimal response of WideStrike cotton to a single labeled application of glufosinate has been shown by Culpepper et al. (2009), Whitaker et al. (2011), Steckel et al. (2012), Cahoon et al. (2015), and Dodds et al. (2015).

Chlorosis observed at 14 DAA on Enlist cotton was influenced only by the number of MOAs applied (Table 5). Chlorosis was 1% or less regardless of the number of MOAs applied (Table 6). Necrosis and total injury present 7, 14, and 28 DAA were influenced by the number of MOAs present in a single application (Table 5). Most of the injury observed at 7, 14, and 28 DAA consisted of necrosis speckling on foliar tissue. Total injury as well as % necrosis present at 7 DAA was greater when three or more MOAs were applied to Enlist cotton. Total injury present at 7 DAA on Enlist cotton treated with applications containing one, two, three, and four MOAs was 10, 16, 20, and 25%, respectively (Table 6).

Table 5. Analysis of variance for the effect of number of mode of actions used in a single application had on visual injury estimates at 7, 14 and 28 days as after application (DAA) as well as lint yield

Effect	D.F.[z]	Chlorosis			Necrosis			Stunting			Total Injury			Lint Yield
		7 DAA[y]	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA	
		p-value												
Number of MOAs[y]	4	0.1541	0.0295	0.1516	<0.0001	0.0059	0.3269	0.1594	0.0250	0.8920	<0.0001	0.0007	0.0468	0.8520

[z] Degrees of freedom

[y] Modes of action

Table 6. Effect of number of mode of actions applied in a single application to 3-6–leaf Enlist® cotton on % foliar necrosis visual injury at 7, 14, and 28 days after application (DAA)

Number of MOAs[z]	Necrosis	Total Injury	Chlorosis	Necrosis	Stunting	Total Injury	Total Injury
	7 DAA		14 DAA				28 DAA
	%						
1	7 c[y]	10 c	0.5 a	10 a	1 b	11 b	5 a
2	13 b	16 b	0.5 a	13 a	1 b	14 ab	5 a
3	17 ab	20 ab	1.0 b	15 a	1 ab	17 a	6 a
4	21 a	25 a	1.0 b	16 a	3 a	20 a	7 a

[z] Modes of action

[y] Means within a column followed by the same letter are not significantly different ($\alpha \leq 0.05$)

Stunted growth at 14 DAA was minimal (1-3%) and had dissipated by 28 DAA (Tables 4 and 6). Total injury present at 14 DAA dissipated when compared to visual injury ratings at 7 DAA (Table 6). Total injury 14 DAA ranged from 11 to 20% depending on the number of MOAs applied. Applications containing three or more MOAs resulted in greater injury on Enlist cotton compared to applications containing one MOA (Table 6). Although the number of MOAs present in the application influenced the level of total injury present 28 DAA, level of injury was less than 7% (Tables 4 and 6). The increased cotton injury observed for applications with three or more herbicides in tank mixture could be a result of increased concentration of formulation adjuvants within the tank-mixture solution. This hypothesis needs to be further investigated as higher concentrations of adjuvants and surfactants in the tank solution generally increase crop injury potential (Falk et al., 1994; Temple et al., 1963).

Yield was not influenced by the number of MOAs present in the herbicide application (Table 5). Steckel et al. (2012) observed that three- and four-way herbicide combinations reduced yield in WideStrike cotton. These data along with Braxton et al. (2017) suggest the additional copy of the PAT enzyme increased the level of tolerance of W3FE cotton to multiple herbicide tank-mix combinations compared to Widestike cotton. However, growers should be aware of the increased injury potential when making these applications even though the injury was transient.

CONCLUSION

Regardless of the herbicide combination used or the number of MOAs contained within the application, Enlist cotton yield was unaffected by herbicide application. Injury was greater at 7 DAA and when two or more herbicides, one of which was glufosinate, were applied in a single application. However, injury was transient and was never observed on new growth or had a negative impact on yield. The additional copy of the PAT enzyme seems to increase the level of tolerance to glufosinate alone as well as when co-applied with glyphosate and *S*-metolachlor. It has been postulated by Stephenson et al. (2013) as well as Cahoon et al. (2015) that the use of multiple MOAs could preserve existing herbicide technologies and slow down the evolution of herbicide resistance in problematic weed species. Based on these findings, cotton cultivars expressing the Enlist trait can withstand herbicide applications containing up to four MOAs without suffering yield reductions. However, growers should be cautioned prior to the use of multiple MOAs that higher levels of visual injury can be expected.

DISCLAIMER

Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U. S. Department of Agriculture and does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

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