Glufosinate Safety in WideStrike® Acala Cotton

Steven D. Wright, Anil Shrestha, Robert B. Hutmacher, Gerardo Banuelos, Kelly A. Hutmacher, Sonia I. Rios, Michelle Dennis, Katherine A. Wilson, and Sara J. Avila*

WideStrike® Acala cotton is a two-gene, in-plant trait that provides broad-spectrum and season-long control of lepidopteran insect pests, and the varieties available in California also have resistance to glyphosate. There have been indications that WideStrike cotton has some glufosinate tolerance as well, so the level of tolerance to glufosinate needed to be ascertained. A 2-yr (2008 and 2009) study was conducted in California to evaluate the potential crop injury caused by three different rates (0.59, 0.88, and 1.76 kg ai ha^{-1}) of glufosinate–ammonium at four different growth stages (cotyledon, 2-node, 5- to 6-node, and 18- to 19-node stages) of WideStrike Acala cotton. The effects of these treatments on the cotton plants and yield were closely monitored. Glyphosate at 1.54 kg ae ha^{-1} was applied at all cotton growth stages as a standard application, and a nontreated control was included. The greatest level of injury (58%) was observed with the highest rate of glufosinate applied at both the cotyledon and the two-node stage of cotton. However, injury was less than 10% following glufosinate at 0.59 kg ha^{-1} applied at the 18- to 19-node stage. The level of injury increased with the higher application rate of glufosinate at all crop growth stages. In 2008 and 2009, the glufosinate treatments had no effect on cotton lint yield. Therefore, the study showed that glufosinate can be applied safely topically at 0.59 kg ha^{-1} at the cotyledon- to 2-node stage or as POST-directed spray between the 5- to 19-node stages. Although injury occurred at this rate, the plants recovered within 2 to 3 wk of the treatment. Increasing glufosinate rates beyond 0.59 kg ha^{-1} can increase the possibility of greater crop injury.

Nomenclature: Glufosinate-ammonium; glyphosate; cotton, *Gossypium hirsutum* L.

Key words: Glyphosate, herbicide resistance, insect protection, Acala, application timings, herbicide tolerance, crop injury.

El algodón Acala WideStrike® posee dos genes que brindan control de amplio espectro de plagas insectiles-lepidóptera a lo largo de la temporada de crecimiento, y las variedades disponibles en California también tienen resistencia a glyphosate. Han habido indicaciones de que el algodón WideStrike también tiene algo de tolerancia a glufosinate, así que es necesario definir el nivel de tolerancia a este herbicida. Se realizó un estudio de dos años de duración (2008 y 2009) en California, para evaluar el potencial de daño al cultivo causado por tres dosis diferentes (0.59, 0.88, y 1.76 kg ai ha^{-1}) de glufosinate ammonium en cuatro estadios de crecimiento (cotiledón, 2 nudos, 5 a 6 nudos, y 18 a 19 nudos) de algodón Acala WideStrike. Se le dio seguimiento detallado a los efectos de estos tratamientos en las plantas y el rendimiento del algodón. Se aplicó glyphosate a 1.54 kg ae ha^{-1} en todos los estadios de crecimiento como estándar de aplicación, y se incluyó un testigo sin tratamiento. El mayor nivel de daño (58%) se observó con la dosis mayor de glufosinate aplicada en los estadios de cotiledón y 2 nudos del algodón. Sin embargo, el daño fue menos de 10% después de aplicaciones de glufosinate a 0.59 kg ha^{-1} en el estadio de 18 a 19 nudos. El nivel de daño incrementó con la dosis mayor de glufosinate en todos los estadios de crecimiento del cultivo. En 2008 y 2009, los tratamientos de glufosinate no tuvieron ningún efecto en el rendimiento de fibra del algodón. Así, el estudio mostró que se puede aplicar glufosinate tópicamente en forma segura a 0.59 kg ha^{-1} en los estadios de cotiledón y de 2 nudos, o en forma POST-dirigida en los estadios de 5 a 19 nudos. Aunque hubo daños con esta dosis, las plantas se recuperaron 2 a 3 semanas después del tratamiento. Aumentar las dosis de glufosinate más allá de 0.59 kg ha^{-1} puede incrementar la posibilidad de observar un mayor daño en el cultivo.

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* First, fourth, fifth, sixth, eighth, and ninth authors, Farm Advisor, Staff Research Associate III, Staff Research Associate II, Student Research Assistant III, Staff Research Associate II, and Student Research Assistant, University of California Cooperative Extension, 4437B South Laspina, Tulare, CA 93274; second and seventh authors: Associate Professor and Graduate Student, Department of Plant Science, California State University, Fresno, 2451 East San Ramon Avenue M/S AS72, Fresno, CA 93740; third author: Cooperative Extension Cotton Specialist and Center Director, West Side REC, University of California Cooperative Extension, 17353 West Oakland Avenue, Five Points, CA 93624. Corresponding author's E-mail: sdwright@ucdavis.edu

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Cotton is an important crop grown in California's San Joaquin Valley (SJV). Currently, a majority of cotton growers in the SJV choose glyphosate-resistant (GR) varieties because of wide-spectrum control of broadleaf and grass weeds by over-the-top applications with glyphosate during the cotton growing season. However, this weed management technology alone is not enough to achieve optimum control of some of the more troublesome weeds of cotton in the SJV, including pigweeds (*Amaranthus* spp.), common lambsquarters (*Chenopodium album* L.), and nightshades (*Solanum* spp.) (Wright 2009). In addition, relying on a sole method for weed control can lead to selection of herbicide-resistant weeds (Norsworthy et al. 2010). Weed control may gradually become more difficult as continuous use of the same herbicide causes weed species shifts or selects for weeds that are resistant to that particular herbicide (Wright 2009). Glyphosate-resistant weeds were never seen before the introduction of genetically modified glyphosate-resistant crops (Culpepper 2006). The first case of a GR weed was reported in Delaware in 2000 when a population of horseweed (*Conyza canadensis* L. Cronq.) showed 8- to 11-fold resistance to glyphosate compared to a glyphosate-susceptible population (VanGessel 2001). Since then the number of GR weed species has grown to 24 (Heap 2013). These GR weeds in some cases have caused devastating crop losses, a recent example of which is the case of Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the U.S. Cotton Belt (Steckel et al. 2012). Although California has not reported GR weed populations specifically in glyphosate-resistant cotton cropping systems, care must be taken to prevent the onset of GR weeds in these systems.

WideStrike® cotton, which has an insect resistance trait, was released by Dow AgroSciences in 2005 and was introduced into California in 2006. WideStrike is available in different varieties; but it was initially used on elite Phytogen brand cottonseed. The variety of WideStrike that is predominantly used in the SJV is Acala Phytogen 755 WRF (Dow Agro Sciences 2013). In addition to being a glyphosate-resistant variety in California, WideStrike cotton expresses two forms of insecticidal proteins from *Bacillus thuringiensis* var. *aizawai* and *B. thuringiensis* var. *krustaki*, Cry1F and Cry1Ac, respectively (Dow Chemical Company 2006). The expression of these proteins controls lepidopteran pests in cotton such as bollworm (*Helicoverpa zea* Boddie), pink bollworm (*Pectinophora gossypiella* Saunders), beet armyworm (*Spodoptera exigua* Hübner), and cabbage looper (*Trichoplusia ni* Hübner), along with several others (Dow Chemical Company 2006). The genes that express the Cry1F and Cry1Ac proteins also contain the *pat* gene, which confers tolerance to glufosinate (Steckel et al. 2012). However, the *pat* gene activity is lower in WideStrike varieties than in LibertyLink varieties; thus, glufosinate tolerance in WideStrike is incomplete as compared to LibertyLink (Steckel et al. 2012). As a result, injury up to 25% has been reported with glufosinate applications of 0.59 kg ai ha^{-1} in WideStrike cotton (Culpepper et al. 2009; Whitaker et al. 2011). Although such transient injuries to the crop have been reported, none of the studies found injury causing detrimental effect on cotton yield or fiber quality (Culpepper et al. 2009; Dodds et al. 2011; Steckel et al. 2012; Whitaker et al. 2011). Thus, a coapplication of glyphosate and glufosinate to WideStrike cotton may widen the spectrum of weed control, and would also assist in preventing herbicide resistance. POST over-the-top applications of glufosinate are being tested in WideStrike cotton in the United States to control GR populations of giant ragweed (*Ambrosia trifida* L.) (Barnett et al. 2012) and Palmer amaranth (Whitaker et al. 2011). In a study that compared glufosinate-containing tank mixes, the mixture of glufosinate and *S*-metolachlor significantly increased injury in addition to decreasing cotton yield (Steckel et al. 2012). Also, tank-mixing glufosinate with the insecticide dimethoate injured cotton greater than glufosinate alone but did not decrease yield. However, all previous studies (Culpepper et al. 2006, 2011; Steckel et al. 2012) with glufosinate applied topically in WideStrike were conducted on non-Acala upland cotton varieties at different timings. It was not known if the upland Acala WideStrike cotton would have similar responses when applied at various growth stages under SJV environmental conditions. Therefore, the objective of this study was to assess the tolerance of Acala WideStrike upland cotton to increasing rates of glufosinate, and also to compare topical applications at early growth stages with directed sprays at later growth stages.

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Materials and Methods

A 2-yr (2008 and 2009) field study was conducted at the University of California's Westside Research and Extension Center (36.34°N, 120.11°W) in Five Points, CA. The soil was a Panoche clay loam (fine–loamy, super active, thermal typic haplocambids) with a pH of 7.6 to 7.8 (Beaudette and O'Geen 2009). The experimental design was a two-factor factorial arranged as a randomized complete block with four replications. Factors consisted of four herbicide treatments and application timings that corresponded to specific cotton growth stages. Glyphosate (Roundup WeatherMax®, 540 g ae L^{-1}; Monsanto, St. Louis, MO) at 1.26 kg ae ha^{-1} and glufosinate (Rely 280® 280 g ai L^{-1}; Bayer CropScience, Research Triangle Park, NC) at 0.59, 0.88, and 1.79 kg ai ha^{-1} were applied to cotyledon, 2-, 5- to 6-, and 18- to 19-node cotton. A nontreated control was also included for comparison. All herbicides were applied POST over-the-top to cotyledon and two-node cotton and POST-directed to 5- to 6- and 18- to 19-node cotton. The last two timings where applied as a directed spray in order to minimize the potential risk of crop injury. This application method is standard in California layby applications.

Prior to defoliation, plant mapping was conducted to determine cotton node number and plant height. All data were collected from the center two rows of each plot. Plant mapping data were evaluated with the use of the software program CottonPro (Plant 1997). The center two rows of each plot were harvested on October 28 and October 15 in 2008 and 2009, respectively, with the use of a two-row commercial-type spindle picker. Seed cotton subsamples weighing 2 to 3 kg were collected from each plot and sent to a commercial saw type gin at the University of California, Shafter, CA research station to determine gin turnout and lint yield.

Data for all parameters were tested to verify if the assumptions of analysis of variance (ANOVA) were met with the use of the Shapiro-Wilks test. Data that failed to meet the assumptions of ANOVA were log transformed and analyzed with the use of the generalized linear model (GLM) procedure in SAS (version 9.2; SAS Institute Inc., Cary, NC). The significance level was set at $\alpha = 0.05$ and means were separated with the use of Fisher's LSD test. For the transformed data, when the ANOVA showed significant differences, the mean separation test was conducted on the transformed data but nontransformed means were reported for ease in presentation. Interactions between year and treatments were tested, and whenever these interactions were significant ($P < 0.05$) analysis was conducted separately for each year. Interactions between herbicide rate and application timing were also tested.

Results and Discussion

Crop Injury. A year-by-treatment interaction occurred for crop injury, so data were analyzed separately for each year. Glufosinate applications at all stages of cotton caused injury to the crop in both years of the study; however, the injury was more prominent when applied at 0.59 and 0.88 kg ha^{-1} in 2009 than in 2008 when applied to two-node cotton (Tables 1 and 2). Spring temperatures were generally warmer in 2009 than in 2008 (data not shown); however, it is uncertain if this difference attributed to slightly higher crop injury in 2009.

In 2008, crop injury increased with increasing rates of glufosinate regardless of growth stage. Injury ranged from 25 to 41% when glufosinate at 1.76 kg ha^{-1} was applied to cotyledon, 2-, and 5- to 6-node cotton; but only 16% injury was observed when it was applied to 18- to 19-node cotton 1 and 2 wk after treatment (WAT) (Table 1). Injury was 8% or less following glufosinate at 0.59 and 0.88 kg ha^{-1} 4 WAT regardless of cotton growth stage at application. However, 19% injury was still observed 4 WAT following glufosinate at 1.76 kg ha^{-1} applied to two-node cotton. Little to no injury was observed following the glyphosate application regardless of cotton growth stage (Table 1). Barnett et al. (2012) reported crop injury of up to 7% with glufosinate at 0.59 kg ha^{-1} when applied to one-leaf cotton. We observed injury levels similar to those reported by Barnett et al. (2012) when glufosinate was applied at 0.59 kg ha^{-1} to cotyledon cotton. Our study demonstrated that the injury level can initially be greater than 23% with the 0.88 and 1.76 kg ha^{-1} rates of glufosinate and the plants can take up to 4 wk to recover.

Similar to 2008, cotton injury increased with increasing glufosinate rates in 2009. Glufosinate at 0.59 kg ha^{-1} applied to two-node cotton caused 21% injury 1 WAT in 2009 (Table 2). This result is similar to that of Steckel et al. (2012), who reported

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Table 1. Response of WideStrike® cotton to herbicides applied at the cotyledon, 2-node, 5- to 6-node, and 18- to 19-node growth stages in 2008.[a]

Treatment	Rate[b]	Cotton growth stage	1 WAT	2 WAT	4 WAT	8 WAT
	kg ai or ae ha^{-1}		Cotton injury (%)			
Glyphosate	1.54	Cotyledon	0	0	0	0
Glufosinate	0.59	Cotyledon	13	10	0	0
Glufosinate	0.88	Cotyledon	25	17	5	0
Glufosinate	1.76	Cotyledon	33	25	5	0
Glyphosate	1.54	2 node	0	0	0	0
Glufosinate	0.59	2 node	11	6	4	0
Glufosinate	0.88	2 node	28	13	8	0
Glufosinate	1.76	2 node	41	28	19	0
Glyphosate	1.54	5–6 nodes	0	0	0	0
Glufosinate	0.59	5–6 nodes	8	4	1	0
Glufosinate	0.88	5–6 nodes	23	18	5	0
Glufosinate	1.76	5–6 nodes	36	28	6	0
Glyphosate	1.54	18–19 nodes	2	–	0	0
Glufosinate	0.59	18–19 nodes	4	–	0	0
Glufosinate	0.88	18–19 nodes	8	–	0	0
Glufosinate	1.76	18–19 nodes	16	–	0	0
LSD[c]			11	7	5	NS
P value			0.0001	0.0001	0.0582	1.000

[a] Abbreviations: NS, nonsignificant; WAT, wk after treatment.
[b] Glyphosate rates are in kg ae ha^{-1}, glufosinate rates are in kg ai ha^{-1}.
[c] LSD (0.05) value compares means within column.

Table 2. Response of WideStrike® cotton to herbicides applied at the cotyledon, 2-node, 5- to 6-node and 18- to 19-node growth stages in 2009.[a]

Treatment	Rate[b]	Cotton growth stage	1 WAT	2 WAT	4 WAT	8 WAT
	kg ai or ae ha^{-1}		Cotton injury (%)			
Glyphosate	1.54	Cotyledon	0	0	0	0
Glufosinate	0.59	Cotyledon	7	6	0	0
Glufosinate	0.88	Cotyledon	24	16	5	0
Glufosinate	1.76	Cotyledon	58	54	10	0
Glyphosate	1.54	2 node	0	0	0	0
Glufosinate	0.59	2 node	21	15	5	0
Glufosinate	0.88	2 node	45	35	21	0
Glufosinate	1.76	2 node	58	48	36	0
Glyphosate	1.54	5–6 nodes	0	0	0	0
Glufosinate	0.59	5–6 nodes	18	10	1	0
Glufosinate	0.88	5–6 nodes	23	16	4	0
Glufosinate	1.76	5–6 nodes	38	24	9	0
Glyphosate	1.54	18–19 nodes	–	0	0	0
Glufosinate	0.59	18–19 nodes	–	1	1	0
Glufosinate	0.88	18–19 nodes	–	5	5	0
Glufosinate	1.76	18–19 nodes	–	13	11	0
LSD[c]			15	10	7	NS
P value			0.0001	0.0001	0.0001	1.000

[a] Abbreviations: NS, nonsignificant; WAT, wk after treatment.
[b] Glyphosate rates are in kg ae ha^{-1}, glufosinate rates are in kg ai ha^{-1}.
[c] LSD (0.05) value compares means within column.

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Table 3. Final plant mapping data, gin turnout, and lint yield for glufosinate applied at different cotton growth stages.[a]

Treatment	Rate[b]	Stage	2008 Height	2008 Nodes	2008 Gin	2008 Lint yield	2009 Height	2009 Nodes	2009 Gin	2009 Lint yield
	kg ai or ae ha^{-1}		cm	No.	TO%	kg ha^{-1}	cm	No.	TO%	kg ha^{-1}
Glyphosate	1.54	Cotyledon	133	25	31.2	1,190	122	23	32.7	2,000
Glufosinate	0.59	Cotyledon	128	26	31.7	1,200	123	22	32.6	1,990
Glufosinate	0.88	Cotyledon	131	24	31.7	1,210	121	22	33.1	2,020
Glufosinate	1.76	Cotyledon	133	25	32.3	1,170	121	22	32.3	1,860
Glyphosate	1.54	2 node	129	24	32.5	1,430	120	22	32.8	2,090
Glufosinate	0.59	2 node	129	24	31.6	1,310	121	23	33.4	1,980
Glufosinate	0.88	2 node	124	24	32.1	1,230	123	22	32.8	1,980
Glufosinate	1.76	2 node	126	24	31.5	1,160	122	23	33.1	1,990
Glyphosate	1.54	5–6 nodes	128	23	33.9	1,440	122	22	32.8	2,080
Glufosinate	0.59	5–6 nodes	131	24	31.9	1,250	125	22	32.4	2,070
Glufosinate	0.88	5–6 nodes	126	23	31.8	1,230	118	22	32.9	2,000
Glufosinate	1.76	5–6 nodes	129	24	31.8	1,160	123	22	32.8	2,050
Glyphosate	1.54	18–19 nodes	127	24	32.1	1,270	123	23	32.8	1,940
Glufosinate	0.59	18–19 nodes	121	23	32.8	1,450	125	23	33.0	1,940
Glufosinate	0.88	18–19 nodes	123	23	31.4	1,340	127	23	33.0	1,990
Glufosinate	1.76	18–19 nodes	124	24	32.1	1,400	118	22	32.5	1,980
Nontreated	–	–	127	24	31.7	1,170	123	23	33.6	1,960
LSD[c]			7	2	NS	NS	NS	NS	NS	NS
P value			0.1670	0.3180	0.2098	.02098	0.4801	0.1802	0.0840	0.0840

[a] Abbreviations: NS, nonsignificant; WAT, wk after treatment, TO, turnout.

[b] Glyphosate rates are in kg ae ha^{-1}, glufosinate rates are in kg ai ha^{-1}.

[c] LSD (0.05) value compares means within column.

up to 18% injury when glufosinate was applied at 0.59 kg ha^{-1} to two-leaf WideStrike cotton. Less than 10% injury was observed 4 WAT with the 0.59 kg ha^{-1} rate of glufosinate for all growth stages (Table 2). Glufosinate applications to five- to six-node cotton showed injury up to 23% following glufosinate at 0.88 kg ha^{-1}, and almost 40% injury was observed 1 WAT with glufosinate at 1.76 kg ha^{-1} (Table 2). Similar to other cotton growth stages, less than 10% injury was observed 4 WAT following the application of glufosinate at 0.88 and 1.76 kg ha^{-1} to five- to six-node cotton. The glufosinate applications to the 18- to 19-node cotton showed results similar to those of the cotyledon and 5- to 6-node cotton by 4 WAT, with percent injury ratings 11% or less even following glufosinate at 1.76 kg ha^{-1}. By 4 WAT, only the applications of glufosinate at 0.88 and 1.76 kg ha^{-1} to two-node cotton showed injury greater than 11%, and as high as 36%.

The injury levels at various growth stages with 0.59 kg ha^{-1} glufosinate in our study were lower than in the studies of Culpepper et al. (2009), who reported up to 36% injury with glufosinate at 0.59 kg ha^{-1} at various growth stages. The greatest level of injury with glufosinate at 0.59 kg ha^{-1} in our study was 21% in 1 yr when it was applied to two-node cotton.

Plant Height and Number of Nodes at Harvest. Similar to crop injury, a year-by-treatment interaction also occurred for plant height and number of nodes at harvest; therefore these data were analyzed separately for each year. There was a significant difference in plant height among treatments in 2008 but not 2009 (Table 3). Plants with glufosinate applied at any rate to 18- to 19-node cotton resulted in shorter plants compared to other application timings, but the plants were similar in height to the nontreated control. Differences were not observed when the plants were treated at other growth stages. Therefore, the observed difference in plant height when glufosinate was applied at the 18- to 19-node stage cannot be attributed to glufosinate. The variability could have been due to difference in lygus (*Lygus hesperus*) populations between plots or some other factor that was not assessed in this experiment. It can be concluded that glufosinate did not reduce plant heights in this study. Although

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injury was observed with the highest rate of glufosinate at other growth stages (Table 1), the plants recovered and showed no consequences of this injury in terms of plant height and final number of nodes close to harvest. In 2009, treatments did not have an effect on final plant heights or final number of nodes, and the heights ranged from 118 to 133 cm with 22 to 26 nodes plant^{-1}. Even though injury symptoms were generally more severe in 2009 than in 2008, final plant heights and number of nodes were similar between the treatments. Steckel et al. (2012) also reported that final height and number of nodes above the cracked boll of the cotton plants treated with glufosinate at 0.59 kg ha^{-1} was similar to that of the nontreated control or that treated with glyphosate at 0.84 kg ha^{-1}.

Cotton Yield. Analysis revealed a year-by-treatment interaction for gin turnout and cotton yield; therefore data were separately analyzed for each year. Cotton yield parameters such as gin turnout and lint yield were not affected by the treatments in both years of the study (Table 3). The lint yield was higher in 2009 than in 2008 as it ranged from 1,160 to 1,450 kg ha^{-1} in 2008 and 1,860 to 2,090 kg ha^{-1} in 2009. These differences in yield were most likely due to heavy infestations of lygus in 2008. Steckel et al. (2012) also reported similar cotton lint yield with glufosinate at 0.59 kg ha^{-1} and glyphosate at 0.84 kg ha^{-1} when these treatments were applied at the two-leaf stage of cotton. Culpepper et al. (2009) reported 3.3 times greater seed-cotton yield with glufosinate-based than glyphosate-based systems in WideStrike cotton, but they attributed this difference to better control of GR Palmer amaranth with glufosinate. No cases of GR weeds were present in our research plots, so ineffective weed control was not a possible factor in reducing lint yield. Weeds were adequately controlled by both glyphosate and glufosinate, thus the lack of yield differences among treatments indicates that glufosinate applications to WideStrike Acala cotton do not reduce yield.

Research indicated that glufosinate at 0.59 kg ha^{-1}, regardless of cotton growth stage at application, injured WideStrike Acala cotton 6 to 21% at 1 to 2 WAT, but injury at this rate was 5% or less by 4 WAT. Overall, injury level at all growth stages of cotton increased with the application rate of glufosinate. However, injury did not affect gin turnout or reduce cotton lint yield. The study also showed that glufosinate could be applied topically to cotyledon- to two-node cotton or POST-directed to 5- to 19-node cotton. However, depending on the growth stage at the time of application, 4 to 8 wk were required before no injury was observed. Although better weed control may be obtained with higher rates of glufosinate, increasing glufosinate rates beyond 0.59 kg ha^{-1} can increase the possibility of greater crop injury and add material costs to the grower.

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