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Tolerance of Pima and Upland cotton to trifloxysulfuron (Envoke) herbicide under field conditions

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Abstract

Trifloxysulfuron (Envoke) is an acetolactate synthase-inhibitor herbicide and can be used to control many broadleaf weeds and nutsedges in cotton production. However, there is a lack of information on genotypic variation in response to the herbicide. In this field study, 60 Pima (*Gossypium barbadense* L.) lines, 122 Upland (*G. hirsutum* L.) lines, and 9 Upland × Pima segregating populations were divided into five tests (18A, 18B, 18G, 18RB, and 18HQ) to evaluate trifloxysulfuron tolerance at the 7-true leaf stage (42 days after planting) under the same field conditions in 2018. Across the five tests, Pima cotton genotypes tested in this study did not show any visual crop injury based on percentage of plants with chlorosis at 6 days after treatment (DAT), indicating consistent and high levels of trifloxysulfuron tolerance. However, the response to trifloxysulfuron within Upland cotton is highly variable. While Upland cotton is overall more sensitive to trifloxysulfuron with crop injury up to 80% than Pima cotton, 19 lines had injury below 5% including one line with no visual injury, and 19 lines had injury between 5% and 10%. In test 18HQ with 15 transgenic Upland cultivars and 17 non-transgenic Upland lines, the analysis of variance detected a significant genotypic difference. The broad-sense heritability estimates for trifloxysulfuron tolerance based on crop injury at 6 DAT was 0.555, suggesting that trifloxysulfuron tolerance in Upland cotton is moderately heritable. This study represents the first report that Pima cotton and many Upland cotton lines are highly tolerant to trifloxysulfuron with no or little crop injury.

Keywords: Upland cotton, Pima cotton, Glandless, Trifloxysulfuron (Envoke), Herbicide tolerance

Introduction

Trifloxysulfuron (Trifloxysulfuron®, manufactured by Syngenta Crop Protection, Greensboro, NC, USA) belongs to the acetolactate synthase (ALS)-inhibitor herbicide class, and it can control many broadleaf weeds and nutsedges in cotton fields when applied post-emergence at or after the 5-true leaf stages (Brecke and Stephenson 2006; Burke and Wilcut 2004; O'Berry et al. 2008; Porterfield et al. 2003; Troxler et al. 2003). It prevents the synthesis of branched-chain amino acids such as leucine, isoleucine, and valine, which in turn leads to the inhibition of protein synthesis and termination of plant growth. Therefore, as with other ALS-inhibiting herbicides, visual crop injury such as chlorosis, necrosis, and stunting within a week or so of trifloxysulfuron application is often observed in Upland cotton, *Gossypium hirsutum* L. (Koger et al. 2005; Richardson et al. 2003b, 2004a, b; Thomas et al. 2006; O'Berry et al. 2008). Applications of trifloxysulfuron before the 5-true leaf stages caused higher crop injury (Branson et al. 2002; Porterfield et al. 2002a; Richardson et al. 2006, 2007a, b), than after the stage (Branson et al. 2005; Koger et al. 2005; Porterfield et al. 2002a; Richardson et al. 2006). There may be genotypic differences in crop injury (Porterfield et al. 2002b). Thyssen et al. (2014) reported that, Upland cotton

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cultivar HS 26 is highly sensitive to trifloxysulfuron with no crop recovery, and the tolerance of trifloxysulfuron in a cross between tolerant cultivar ST 474 (with only transient crop injury) and sensitive cultivar HS 26 is conferred by a single dominant gene on chromosome c20 (D10) based on SSR markers. Thyssen et al. (2018) have subsequently identified that the gene *Gh_D10G1401*, encoding for the cytochrome P450 protein CYP749A16, is responsible for the natural tolerance to trifloxysulfuron and a 1-bp (base pair) frameshift insertion in its third exon leads to the loss of tolerance. Thyssen et al. (2018) further reported that, of 384 obsolete Upland cultivars evaluated, 3 (including HS 26) were homozygous sensitive to trifloxysulfuron and 10 were segregating for the sensibility. However, there is a lacking of information on genotypic differences in trifloxysulfuron tolerance among current commercial Upland and advanced breeding lines, and no report is available on the sensitivity of Pima cotton (*G. barbadense* L.) to the herbicide.

The objective of this study was to evaluate Pima cotton, commercial Upland cotton cultivars, advanced breeding lines and glandless cotton for response to over the top application of trifloxysulfuron in the field. Consistent results were obtained, providing useful information for cotton breeding or production when selection for tolerance to trifloxysulfuron is one of the objectives.

Materials and methods

Materials and experimental designs

This field study consisted of five different tests (18A, 18B, 18G, 18RB, and 18HQ) and was performed in the same field of the New Mexico cotton breeding nursery, Leyendecker Plant Science Center, New Mexico State University, Las Cruces, New Mexico, USA. Tests 18A and 18B were Pima and Upland progeny row tests with 68 and 28 genotypes, respectively. Test 18G was an advanced yield test with 32 Upland breeding lines developed in the New Mexico cotton breeding program. Test 18RB involved 32 advanced breeding lines of Upland cotton newly developed in the US public cotton breeding programs. Test 18HQ was composed of 32 commercial transgenic cultivars from seed companies and advanced breeding lines of Upland cotton from several US public breeding programs. Each of the above three tests, i.e., 18G, 18RB and 18HQ, was arranged in a randomized complete block design with 3 (18G) or 4 replications (18RB and 18HQ). On May 9, 2018, seeds (at the seeding rate of 10 seed·m^{-1}) for the above five tests were mechanically planted to 1-row (18A, 18B, and 18G) or 2-row (18RB and 18HQ) × 10 m long plots using a 4-row plot planter. The row spacing was 1.0 m. A furrow irrigation was applied immediately after planting to achieve a uniform germination and seedling stand, which was followed by another furrow irrigation in June. Other crop management practices followed local recommendations for cotton production, but no insecticide was applied during the production season.

Crop injury in response to trifloxysulfuron and data analysis

The field was sprayed over the top with trifloxysulfuron at a recommended rate of 10.6 g·ha^{-1}, on June 20, 2018 (42 days after planting, DAP), when the seedlings from the five tests were at the 7-true leaf stage. Plant responses to trifloxysulfuron were observed 1, 2 and 3 days after treatment (DAT). Screening for crop injury in cotton response to trifloxysulfuron was conducted on June 26, 2018, i.e., 6 DAT. In each plot of tests 18HQ and 18RB, an average of 70 plants were evaluated for crop injury, while 30 seedlings in each plot of the other three tests were consecutively selected to count for plants with chlorosis. The percentage of seedlings with chlorosis was calculated and used as the measurement for trifloxysulfuron tolerance in cotton. A least significant difference (LSD) at $P<0.05$ was used to compare genotypes, following an analysis of variance (ANOVA). Broad-sense heritability (Hb) for the response to trifloxysulfuron was estimated on a genotypic mean basis for each test with a significant genotypic variation based on ANOVA, as the following, $\mathrm{Hb}=(\mathrm{MS_G}-\mathrm{MS_E})/\mathrm{MS_G}$. Here, $\mathrm{MS_G}$ is the mean square for genotype, and $\mathrm{MS_E}$ is the mean square for experimental error.

Results and analysis

Analysis of variance and estimation of broad-sense heritability of cotton response to trifloxysulfuron

The trifloxysulfuron herbicide was applied over the top at the 7-true leaf stage (i.e., 42 DAP) in the five field tests. The ANOVA (Table 1) showed that highly significant genotypic variation ($P<0.01$) in crop injury (as measured by % of plants with chlorosis) at 6 DAT was detected in the 18HQ test. Since all the lines tested in this test were unknown for their response to trifloxysulfuron, the choosing of the genotypes in each test can be considered

Table 1 An analysis of variance for crop injury (% of plants with chlorosis) caused by herbicide trifloxysulfuron in replicated field test 18HQ conducted in a randomized complete block design with 3 replications, Las Cruces, NM, US, June 2018

Source	*df*	MS	F	Hb
Replication	2	565.316	5.237	
Genotype	31	242.277	2.245**	0.555
Error	62	107.945		

ns not significant. Hb broad-sense heritability, **$P<0.01$

random samples. Therefore, the broad-sense heritability in cotton response to trifloxysulfuron was estimated (Table 1). About 55.5% of the phenotypic variation in trifloxysulfuron tolerance was due to genetic factors in this study, indicating that the trifloxysulfuron tolerance in Upland cotton is moderately heritable.

Trifloxysulfuron tolerance in Pima cotton

A total of 69 lines were tested in 18A, including 3 Upland, 60 Pima, and 6 Upland × Pima interspecific segregating populations, and the results are shown in Table 2. The three Upland cotton lines had crop injury ranging from 16.67 to 46.67% with an average of 26.67%. Among the 60 Pima lines, 56 had no apparent crop injury, while 4 lines each had injury at 3.33% (i.e., 1 out of 30 plants evaluated) due most likely to seed impurity or outcrossing with Upland cotton. Of the six Upland × Pima segregating populations, the crop injury ranged from 3.33 to 20.00% with an average of 9.95%. The results clearly demonstrate that Pima cotton is highly tolerant to trifloxysulfuron when applied at the 7-true leaf stage.

As a comparison, in test 18B with 28 lines (Table 3), similar results were observed in that, the only Pima line was also highly tolerant with no visual crop injury, while the two Upland × Pima segregating populations also had lower injury (13.33%). However, among the remaining 25 Upland lines with injury between 3.33% and 80.00% (with a mean of 45.33%), only three lines (17Y2004-1, 17Y2008-1,2,3, and 17Y2009-1) were highly tolerant (with a minimal crop injury of 3.33%–6.67%) to trifloxysulfuron. However, 10 lines had crop injury ranging from 20.00% to 50.00%, and 12 lines had injury between 53.33% and 83.33%, indicating their moderate to high sensitivity to trifloxysulfuron.

Trifloxysulfuron tolerance in advanced breeding lines developed in the US public breeding programs

In test 18RB (Table 4), 28 non-transgenic lines from eight US public cotton breeding programs including New Mexico State University were tested, together with four non-transgenic commercial checks (DP 393, DP 493, FM 958, and UA 222, Bourland and Jones 2012). The crop injury ranged from 1.79% for DP 393 to 32.26% for TAM LBB150107 with a mean of 12.41% (Table 4). A total of 15 lines incurred < 10% crop injury, including three of the four commercial checks, all the five lines from the University of Arkansas, and 12 lines from the University of Georgia, Louisiana State University, Mississippi State University and Texas A & M University at the Lubbock location. Although all the above lines with lower injury were glanded, 8 other glanded lines had higher injury (13.95%~32.26% with a mean of 20.07%). As a comparison, the 9 lines including NuMex COT 15 GLS (Zhang

Table 2 Mean crop injury (% of plants with chlorosis) caused by herbicide trifloxysulfuron in test 16A with 3 Upland (AD1), 59 Pima (AD2) genotypes, and 7 AD1 × AD2 interspecific segregants, Las Cruces, NM, US, June 2018

18 Field ID	Line	Type	% chlorosis
18A1940	17C1299-7	AD1	16.67
18A1945	17C1306-W	AD1	46.67
18A1987	17NV3028-3	AD1 okra segregants	16.67
18A1937	17C1298-4	AD1 × AD2	19.70
18A1955	17C1126-W	AD1 × AD2	3.33
18A1992	17NV4021-B2	AD1 × AD2	20.00
18A1995	17NV4029-B2	AD1 × AD2	10.00
18A1939	17C1299-4	AD1 × AD2	3.33
18A1942	17C1302-1	AD1 × AD2	3.33
18A1950	17C1337-10	AD1 × AD2	0
18A1927	17C1283-W	AD2	0
18A1928	17C1285-W	AD2	0
18A1929	17C1288-W	AD2	0
18A1930	17C1289-W	AD2	0
18A1931	17C1290-W	AD2	0
18A1932	17C1296-1	AD2	0
18A1933	17C1296-W	AD2	0
18A1934	17C1297-W	AD2	0
18A1935	17C1297-1	AD2	0
18A1936	17C1298-1,2	AD2	0
18A1938	17C1299-1,2,3	AD2	0
18A1941	17C1299-W	AD2	0
18A1943	17C1303-W	AD2	0
18A1944	17C1304-W	AD2	0
18A1946	17C1307-W	AD2	0
18A1947	17C1309-W	AD2	0
18A1948	17C1310-1,2	AD2	0
18A1949	17C1336-9	AD2	0
18A1951	17C1344-W4	AD2	0
18A1952	17C11362-W2	AD2	0
18A1953	17C1362-W	AD2	0
18A1954	17C1362-1	AD2	0
18A1956	17C1363-W2	AD2	0
18A1957	17NV1016-1	AD2	0
18A1958	17NV1016-B	AD2	0
18A1959	17NV1016-B2	AD2	0
18A1960	17NV1021-1	AD2	0
18A1961	17NV1021-2,3,4	AD2	0
18A1962	17NV1021-5	AD2	0
18A1963	17NV1021-B	AD2	0
18A1964	17NV1025-B	AD2	0
18A1965	17NV1026-1	AD2	0
18A1966	17NV1026-3	AD2	0
18A1967	17NV1026-B	AD2	0
18A1968	17NV2016-1	AD2	0
18A1969	17NV2016-2,3,4,5	AD2	0
18A1970	17NV2024-1,2,3,4	AD2	0

Table 2 (continued)

18 Field ID	Line	Type	% chlorosis
18A1971	17NV2024-B	AD2	0
18A1972	17NV2024-B2	AD2	0
18A1973	17NV2026-1	AD2	0
18A1974	17NV2026-B	AD2	0
18A1975	17NV3015-B	AD2	0
18A1976	17NV3017-1,2,3,4	AD2	0
18A1977	17NV3017-B	AD2	0
18A1978	17NV3025-1	AD2	0
18A1979	17NV3025-7	AD2	0
18A1980	17NV3025-B	AD2	0
18A1981	17NV3026-1	AD2	0
18A1982	17NV3026-4	AD2	3.33
18A1983	17NV3026-6	AD2	0
18A1984	17NV3026-B	AD2	0
18A1985	17NV3028-1	AD2	0
18A1986	17NV3028-2	AD2	3.33
18A1988	17NV3028-5,6	AD2	0
18A1989	17NV3028-B	AD2	0
18A1990	17NV4015-1,2	AD2	3.33
18A1991	17NV4021-B	AD2	3.33
18A1993	17NV4029-1	AD2	0
18A1994	17NV4029-5	AD2	0

et al. 2016) from New Mexico State University were all glandless and had similar trifloxysulfuron injury, ranging from 10.00% to 27.85% with a mean of 18.71%.

In test 18G (Table 5), of all the 32 lines developed from the New Mexico cotton breeding program, 15 lines had injury at 10% (3 out of 30 plants) or below, including one line (17V2008) without apparent injury and five lines with only 1 injured plant (out of 30 plants screened). Acala 1517-08 (Zhang et al. 2011) also had very lower injury (5%). However, 8 lines incurred a much higher injury ranging from 23.33% to 40.00%. The results from both 18RB and 18G indicate that, high levels of trifloxysulfuron tolerance often exist in the current advanced breeding lines developed from the public cotton breeding programs in the US. It suggests that many parental lines used in cross breeding to create these lines were tolerant to trifloxysulfuron herbicide, although their tolerance is currently unknown.

Table 3 Mean crop injury rating caused by herbicide trifloxysulfuron in test 16B with 25 Upland and 1 Pima cotton genotypes, and 2 AD1 × AD2 segregants, Las Cruces, NM, US, June 2018

18 Field ID	Line	Type	% chlorosis
18B1617	17Y2003-1	AD1	40.00
18B1618	17Y2004-1	AD1	6.67
18B1619	17Y2006-1	AD1	56.67
18B1620	17Y2006-3,4	AD1	60.00
18B1621	Acala 1517-08 GLS	AD1	60.00
18B1622	17Y2006-B	AD1	50.00
18B1623	17Y2008-1,2,3	AD1	6.67
18B1625	17Y2009-1	AD1, pilose	3.33
18B1626	17Y2009-B	AD1	30.00
18B1627	17Y2010-1,2	AD1	83.33
18B1628	17Y2012-1,2,3	AD1	20.00
18B1629	17Y2015-1	AD1	60.00
18B1630	17Y2020-1	AD1	40.00
18B1631	Acala 1517-18 GLS	AD1, glandless	66.67
18B1632	17Y2021-1	AD1	56.67
18B1633	17Y2026-1	AD1	40.00
18B1634	17Y1031-B	AD1	46.67
18B1635	17V1015-B	AD1	53.33
18B1636	17V1015-B3	AD1	23.33
18B1637	17A1052-3-2	AD1	56.67
18B1638	17NF-R2-6	AD1	43.33
18B1641	17NF-R2-1	AD1	53.33
18B1642	17NF-R2-2	AD1	63.33
18B1643	17NF-R2-7	AD1	80.00
18B1644	17NF-R2-8	AD1	33.33
18B1640	NM O TYPE C	AD2	0.00
18B1639	NM O TYPE C	AD1 × AD2	13.33
18B1624	17Y2008-1,2,3,4	AD1 × AD2, smooth	13.33

Trifloxysulfuron tolerance in commercial US cotton cultivars

18HQ contained 15 commercial transgenic (carrying insect resistant B2, B3, or W3 genes or herbicide tolerant RF, XF, FE, or GLT genes) Upland cotton cultivars from five seed companies, in addition to 17 non-transgenic breeding lines from New Mexico State University and four other public breeding programs (Table 6). The crop injury ranged from 6.00% for PHY 440 W3FE to 56.47% for NG 4545 B2XL with an average of 19.24%. There were 5 genotypes with injury below 10%: LA 14063001, LA 14603038, PHY 480 W3FE, PHY 440 W3FE, and ST 5020 GLT; and 8 genotypes with injury between 10 and 15%: ARK 1002-40, ARK 1019-36, ARK 1019-14, DG 2355 B2RF, DP 1549 B2XF, NM 17T1364, NM 17T106, and TAM KJ-Q14. Two cultivars, i.e., FM 2574 GLT and NG 4545 B2XL, incurred the highest injury (50%–56%). Eleven other genotypes including DC 375, DP 1845 B3XF, and DP 1820 B3XF also had above-average crop injury. Therefore, the results in test 18HQ showed that, similar to test 18G and 18RB, trifloxysulfuron tolerance also exists in commercial transgenic cultivars. Five glandless cotton lines including Acala 1517-18 GLS (Zhang et al.

Table 4 Mean crop injury rating caused by herbicide trifloxysulfuron in test 18RB with 32 Upland cotton genotypes, Las Cruces, NM, US, June 2018

Genotype	Source	Trait	% plants with chlorosis
ARK 1004-38	University of Arkansas	Glanded	3.33
ARK 1005-35	University of Arkansas	Glanded	3.66
ARK 1005-41	University of Arkansas	Glanded	4.35
ARK 1007-15	University of Arkansas	Glanded	4.62
ARK 1015-42	University of Arkansas	Glanded	4.05
GA 2012141	University of Georgia	Glanded	3.77
GA 2015024	University of Georgia	Glanded	16.98
LA1 1309040	Louisiana State University	Glanded	13.95
LA 14063075	Louisiana State University	Glanded	5.19
LA 14063083	Louisiana State University	Glanded	3.53
MS 2010-87-5	Mississippi State University	Glanded	3.39
PD 2011021	USDA-ARS, Florence, SC	Glanded	21.13
PD 2011026	USDA-ARS, Florence, SC	Glanded	26.56
PD 2011081	USDA-ARS, Florence, SC	Glanded	19.23
TAM 12J-39	Texas A&M University, College Station	Glanded	14.29
TAM 13S-03	Texas A&M University, College Station	Glanded	16.13
TAM LBB150107	Texas A & M University, Lubbock	Glanded	32.26
TAM LBB150824	Texas A & M University, Lubbock	Glanded	2.08
TAM LBB150921	Texas A & M University, Lubbock	Glanded	8.82
DP 393 (Check)	Monsanto	Glanded	1.79
DP 493 (Check)	Monsanto	Glanded	6.94
FM 958 (Check)	Bayer Crop Science	Glanded	3.51
UA 222 (Check)	University of Arkansas	Glanded	9.09
NM 17T1002	New Mexico State University	Glandless	19.32
NM 17T1003	New Mexico State University	Glandless	19.32
NM 17T1009	New Mexico State University	Glandless	26.47
NM 17T1014	New Mexico State University	Glandless	10.00
NM 17T1069	New Mexico State University	Glandless	12.68
NM 17T1125	New Mexico State University	Glandless	20.78
NM 17T1217	New Mexico State University	Glandless	12.90
NM 17T1249	New Mexico State University	Glandless	19.10
NuMex COT 15 GLS	New Mexico State University	Glandless	27.85
LSD (0.05)			10.02

2019b) had similar crop injury to most of the glanded cotton in response to trifloxysulfuron (Table 6).

Recovery from crop injury

For all the tested lines with varied percentage of seedlings showing crop injuries after the application of trifloxysulfuron, no permanent damage was observed. The crop injury symptoms were all transient and disappeared within 34 weeks after the herbicide application. It appeared that all the cultivars or lines tested achieved a complete recovery; however, whether there were long-term deleterious effects on cotton growth, maturity and yield is currently unknown.

Discussion

In this field study, a total of 60 Pima lines, 122 Upland lines, and 9 Upland × Pima segregating populations were evaluated for trifloxysulfuron tolerance at the 7-true leaf stage (42 DAP) in the same field with the same crop management conditions in 2018. No apparent crop injury was observed in Pima cotton, indicating a high level

Table 5 Mean crop injury rating caused by herbicide trifloxysulfuron in test 18G with 32 Upland cotton genotypes, Las Cruces, NM, US, June 2018

Genotype	Source	ID	% plants with chlorosis
17P1007	New Mexico State University	18PYT01	3.33
17P1017	New Mexico State University	18PYT02	40.00
17P2008	New Mexico State University	18PYT03	16.67
17P2018	New Mexico State University	18PYT04	6.67
17P3007	New Mexico State University	18PYT05	16.67
17P3018	New Mexico State University	18PYT06	36.67
17S1003	New Mexico State University	18PYT07	10.00
17S1008	New Mexico State University	18PYT08	16.67
17S1012	New Mexico State University	18PYT09	30.00
17S1026	New Mexico State University	18PYT10	13.33
17S1032	New Mexico State University	18PYT11	23.33
17V1004	New Mexico State University	18PYT12	13.33
17V2008	New Mexico State University	18PYT13	0.00
17V3003	New Mexico State University	18PYT14	30.00
17V3006	New Mexico State University	18PYT15	6.67
17W1011-W7	New Mexico State University	18PYT16	30.00
17W2002	New Mexico State University	18PYT17	12.50
17W2026	New Mexico State University	18PYT18	6.67
17W3002	New Mexico State University	18PYT19	16.67
17Q1004	New Mexico State University	18PYT20	2.08
17Q1007	New Mexico State University	18PYT21	10.00
17Q1008	New Mexico State University	18PYT22	26.67
17Q2016	New Mexico State University	18PYT23	1.05
17Q2018	New Mexico State University	18PYT24	6.67
17Q2026	New Mexico State University	18PYT25	10.00
17Q3006	New Mexico State University	18PYT26	23.33
17Q3010	New Mexico State University	18PYT27	3.33
17T1011-1,2	New Mexico State University	18PYT28	3.33
17T1081	New Mexico State University	18PYT29	13.33
17T1221	New Mexico State University	18PYT30	10.00
17T1283-1	New Mexico State University	18PYT31	18.52
Acala 1517-08	New Mexico State University	Check	5.00
LSD (0.05)			10.02

of tolerance to the herbicide. On the another hand, the plant response to trifloxysulfuron within Upland cotton was highly variable ranging from no injury in one line to as high as 83% plant injury in another. However, 19 Upland lines showed very low injury (< 5%). The results were repeatable among replications in test HQ, giving a moderate level of heritability in trifloxysulfuron tolerance. This is the first report in evaluating both Upland and Pima cotton for genotypic differences in response to trifloxysulfuron.

One of the important findings from this field study is that almost all the Pima lines tested did not show any crop injury including minor foliar chlorosis to trifloxysulfuron application. This is different from another ALS-inhibiting herbicide Sandea (Zhang et al. 2019a). Recently, we (Zhang et al. 2019a) have reported that eight Pima cotton cultivars tested all incurred moderate to severe crop injury to Sandea when sprayed topically at the 5-true leaf stage. Although both herbicides did not induce transient leaf chlorosis in Pima cotton, Sandea caused moderate to severe necrosis or leaf burning, while trifloxysulfuron did not. The reason for the different responses to the two herbicides in cotton is currently not understood. Seedling growth stages, weather conditions, and the type and application rates of the herbicides maybe in part contribute to the different responses in Pima cotton. Further studies on the two herbicides using the same set of genotypes grown under the same conditions should be compared to discern the differences.

Unlike Pima cotton, most Upland lines are sensitive to trifloxysulfuron with varied percentage of plants displaying apparent but transient crop injury. However, one line was identified to be insensitive to trifloxysulfuron with no crop injury, while many lines also had a minimal crop injury with 1~2 injured seedlings out of 30~70 plants evaluated. Zhang et al. (2019a) reported that genotypic variation in response to Sandea also existed in Upland cotton. This present study also detected significant genotypic variation in trifloxysulfuron tolerance within Upland cotton. Since trifloxysulfuron tolerance was not one of the target traits when commercial cultivars and breeding lines were developed, the Upland lines tested may not be highly homozygous for trifloxysulfuron tolerance or sensibility. A thorough pedigree analysis of the most tolerant cotton lines may shed light on possible common ancestors that contributed to the tolerance. However, it appeared that tolerance to trifloxysulfuron is widely spread within Upland cotton, because none of the lines tested had 100% crop injury. Therefore, repeated pedigree selection within these lines for trifloxysulfuron tolerance should increase the frequency of the tolerance alleles and genotypes from the population genetics perspective. As many Pima cotton did not display any transient crop injury to trifloxysulfuron application and many Upland cotton incurred a minimal injury, it is reasonable to believe that the trifloxysulfuron tolerance is a qualitative trait. Based on the performance of 9 Upland × Pima segregating populations in this study, trifloxysulfuron tolerance is likely a dominant trait. Another piece of indirect evidence is provided by Thyssen et al. (2014), who reported that the high and permanent sensitivity of HS 26 to trifloxysulfuron

Table 6 Mean crop injury rating caused by herbicide trifloxysulfuron in test 18HQ with 32 Upland cotton genotypes, Las Cruces, NM, US, June 2018

Genotype	Source	Trait	% plants with chlorosis
ARK 1002-40	University of Arkansas	Glanded, non-GE	12.37
ARK 1019-14	University of Arkansas	Glanded, non-GE	13.62
ARK 1019-36	University of Arkansas	Glanded, non-GE	13.13
DC 180	USDA-ARS, MS	Glanded, non-GE	20.72
DC 375	USDA-ARS, MS	Glanded, Non-GE	26.91
DG 2355 B2RF	Dyna-Gro/All-Tex Seed	Glanded, GE	11.07
DP 1549 B2XF	Monsanto	Glanded, GE	13.17
DP 1646 B2XF	Monsanto	Glanded, GE	17.74
DP 1820 B3XF	Monsanto	Glanded, GE	34.09
DP 1845 B3XF	Monsanto	Glanded, GE	27.15
Acala Daytona RF	Bayer Crop Science	Glanded, GE	22.36
FM 1830GLT	Bayer Crop Science	Glanded, GE	20.37
FM 2574GLT	Bayer Crop Science	Glanded, GE	50.02
ST 5020GLT	Bayer Crop Science	Glanded, GE	9.88
LA 14063001	Louisiana State University	Glanded, non-GE	7.87
LA 14603038	Louisiana State University	Glanded, non-GE	9.33
NG 4545 B2XL	Americot	Glanded, GE	56.47
PHY 440 W3FE	Dow AgroSciences/Phytogen	Glanded, GE	6.00
PHY 444 WRF	Dow AgroSciences/Phytogen	Glanded, GE	20.68
PHY 480 W3FE	Dow AgroSciences/Phytogen	Glanded, GE	7.91
PHY 499 WRF	Dow AgroSciences/Phytogen	Glanded, GE	22.20
PHY 764 WRF	Dow AgroSciences/Phytogen	Glanded, GE	22.28
TAM 13Q-18	Texas A&M University	Glanded, non-GE	17.38
TAM KJ-Q14	Texas A&M University	Glanded, non-GE	13.39
Acala 1517-08	New Mexico State University	Glanded, non-GE	21.04
NM 16W1079	New Mexico State University	Glanded, non-GE	18.90
NM 16W1094	New Mexico State University	Glanded, non-GE	24.27
NM 17T1069	New Mexico State University	Glandless, non-GE	10.45
NM 17T1363	New Mexico State University	Glandless, non-GE	17.17
NM 17T1364	New Mexico State University	Glandless, non-GE	11.85
NM 13P1125	New Mexico State University	Glandless, non-GE	18.42
Acala 1517-18 GLS	New Mexico State University	Glandless, non-GE	17.64
LSD (0.05)			10.02

GE, genetically engineered with insect resistance (B2, B3, or W3) or herbicide tolerance (RF, XF, FE, or GLT)

is a recessive trait relative to the tolerant trait with a transient chlorosis in STV 474 and most Upland lines (Thyssen et al. 2018), and the difference is controlled by one major gene on chromosome c20 (D10) based on SSR markers. Therefore, there are at least three types of cotton genotypes in response to trifloxysulfuron: highly tolerant with no chlorosis (such as Pima), tolerant with a transient chlorosis (such as many Upland lines including STV 474), and highly sensitive with permanent crop injury and no recovery (such as HS 26). In the present study, HS 26 and STV 474 were not used, because the study on tolerance to trifloxysulfuron was not planned before planting, but infestations from sedges promoted the application of trifloxysulfuron which led to the current study. However, results from our study and Thyssen et al. (2018) were consistent. It will be interesting to study the genetic basis of these three types of cotton in response to trifloxysulfuron. Thyssen et al. (2018) showed that chlorosis caused by trifloxysulfuron in tolerant Upland cotton is due to the delayed expression of a cytochrome P450 gene encoding for CYP749A16 (Gh_D10G1401). The expression of this gene will accumulate enough protein over time to metablize the herbicide to achieve recovery, while susceptible Upland cotton with no recovery such as HS 26 does not produce functional protein because of a 1-bp frameshift mutation. However, the tolerant mechanism in Pima cotton with no crop injury has not been investigated. Based on a field study, Portfield et al. (2002b) reported that there were different genotypic responses among seven commercial transgenic Upland cotton cultivars to two different rates of trifloxysulfuron. Since sensitivity to trifloxysulfuron is associated with the absorption, translocation, and metabolism level in plants (Askew and Wilcut 2002; Richardson et al. 2003a), the genetic and physiological basis for the qualitative difference between Pima and Upland cotton and the quantitative variation within Upland cotton is currently not understood and should be studied.

Comparing the results from the current study with our previous report (Zhang et al. 2019a), cotton genotypes responded to the two herbicides differently, as evidenced from different crop injury symptoms and different responses from Pima cotton. A comparison between glandless cotton and glanded cotton also support the above observations. In the previous study (Zhang et al. 2019a), glandless cotton was found to be consistently and highly sensitive to Sandea than glanded cotton. However, in this study, glandless cotton responded to trifloxysulfuron similarly to glanded cotton. In 18RB, 9 glandless cotton lines had crop injury ranging between 10.0% and 27.9% with a mean of 18.7%, while 9 of the 23 glanded lines also had similar responses with injury from 9.1% to 32.3% with a mean of 18.9%. Similar results were also noted in 18HQ, where five glandless lines had injury ranging from 10.6% to 18.4% with a mean of 15.1%, whereas the injury for the 27 glanded lines ranged between 6.0% to 56.5% with a mean of 20.0%. Therefore, the sensitivity of cotton to the two herbicides with relation to the existence of glands and gossypol production in cotton differs and should be further investigated. Whether gossypol in cotton may bind to trifloxysulfuron or Sandea to reduce crop injury is also currently unknown and should be investigated.

As with other herbicides, severity of crop injury due to trifloxysulfuron depends on crop growth stage, rate of herbicide, application method, and environmental conditions. Although chlorosis, necrosis and stunting in cotton from the application of trifloxysulfuron are temporary in Upland cotton, the delay in growth due to crop injury may cause delay in maturity and reduction in lint yield. Since this study did not compare application rates and methods of trifloxysulfuron among different cotton genotypes, whether there was a yield loss in Upland cotton was unknown. However, identification of trifloxysulfuron-tolerant Pima and Upland cotton germplasm and their use in breeding for developing commercial cultivars with no crop injury will minimize any possible yield loss related to the use of this herbicide, and it will also broaden the window in using this herbicide at the seedling stage of cotton.

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Zhang JF collected the data and drafted the manuscript. Abdelraheem A participated in the study. Wedegaertner T edited manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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