**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...
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 \textit{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 (\textit{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, Hb = (MS\(_G\) − MS\(_E\))/MS\(_G\). Here, MS\(_G\) is the mean square for genotype, and 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

| Field ID | Line Type | Type | % chlorosis |
|----------|------------|------|-------------|
| 18A-1940 | 17C1299-7  | AD1  | 16.67       |
| 18A-1945 | 17C1306-W  | AD1  | 46.67       |
| 18A-1987 | 17NV3028-3 | AD1  | 16.67       |
| 18A-1937 | 17C1298-4  | AD1 × AD2 | 19.70 |
| 18A-1955 | 17C1126-W  | AD1 × AD2 | 3.33 |
| 18A-1992 | 17NV4021-B2| AD1 × AD2 | 20.00 |
| 18A-1995 | 17NV4029-B2| AD1 × AD2 | 10.00 |
| 18A-1939 | 17C1299-4  | AD1 × AD2 | 3.33 |
| 18A-1942 | 17C1302-1  | AD1 × AD2 | 3.33 |
| 18A-1950 | 17C1337-10 | AD1 × AD2 | 0          |
| 18A-1927 | 17C1283-W  | AD2  | 0           |
| 18A-1928 | 17C1285-W  | AD2  | 0           |
| 18A-1929 | 17C1288-W  | AD2  | 0           |
| 18A-1930 | 17C1289-W  | AD2  | 0           |
| 18A-1931 | 17C1290-W  | AD2  | 0           |
| 18A-1932 | 17C1296-1  | AD2  | 0           |
| 18A-1933 | 17C1296-W  | AD2  | 0           |
| 18A-1934 | 17C1297-W  | AD2  | 0           |
| 18A-1935 | 17C1297-1  | AD2  | 0           |
| 18A-1936 | 17C1298-1,2| AD2  | 0           |
| 18A-1938 | 17C1299-1,2,3 | AD2  | 0           |
| 18A-1941 | 17C1299-W  | AD2  | 0           |
| 18A-1943 | 17C1303-W  | AD2  | 0           |
| 18A-1944 | 17C1304-W  | AD2  | 0           |
| 18A-1946 | 17C1307-W  | AD2  | 0           |
| 18A-1947 | 17C1309-W  | AD2  | 0           |
| 18A-1948 | 17C1310-1,2| AD2  | 0           |
| 18A-1949 | 17C1336-9  | AD2  | 0           |
| 18A-1951 | 17C1344-W4 | AD2  | 0           |
| 18A-1952 | 17C11362-W2| AD2  | 0           |
| 18A-1953 | 17C1362-W  | AD2  | 0           |
| 18A-1954 | 17C1362-1  | AD2  | 0           |
| 18A-1956 | 17C1363-W2 | AD2  | 0           |
| 18A-1957 | 17NV1016-1 | AD2  | 0           |
| 18A-1958 | 17NV1016-B | AD2  | 0           |
| 18A-1959 | 17NV1016-B2| AD2  | 0           |
| 18A-1960 | 17NV1021-1 | AD2  | 0           |
| 18A-1961 | 17NV1021-2,3,4 | AD2  | 0           |
| 18A-1962 | 17NV1021-5 | AD2  | 0           |
| 18A-1963 | 17NV1021-8 | AD2  | 0           |
| 18A-1964 | 17NV1025-B | AD2  | 0           |
| 18A-1965 | 17NV1026-1 | AD2  | 0           |
| 18A-1966 | 17NV1026-3 | AD2  | 0           |
| 18A-1967 | 17NV1026-B | AD2  | 0           |
| 18A-1968 | 17NV2016-1 | AD2  | 0           |
| 18A-1969 | 17NV2016-2,3,4 | AD2  | 0           |
| 18A-1970 | 17NV2024-1,2,3,4 | 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.

### 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. 2011).
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 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                   |
| LA 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 135-03  | 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                   |
of tolerance to the herbicide. On the other 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 may 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 sensitivity. 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

| 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 | 16.67                   |
| 17S1003   | New Mexico State University | 18PYT07 | 10.00                   |
| 17S1008   | New Mexico State University | 18PYT08 | 16.67                   |
| 17S1012   | New Mexico State University | 18PYT09 | 3.00                    |
| 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 | 10.02  |
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 Thys sen et al. (2018) were consistent. It will be interesting to study the genetic basis of these three types of cotton in response to trifloxysulfuron. Thys sen 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

\[ \text{Table 6} \quad \text{Mean crop injury rating caused by herbicide trifloxysulfuron in test 18HQ with 32 Upland cotton genotypes, Las Cruces, NM, US, June 2018} \]

| 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 | 21.04 |
| NM 16W1094 | New Mexico State University | Glanded, non-GE | 24.27 |
| NM 17T1069 | New Mexico State University | Glanded, non-GE | 10.45 |
| NM 17T1363 | New Mexico State University | Glanded, non-GE | 17.17 |
| NM 17T1364 | New Mexico State University | Glanded, non-GE | 11.85 |
| NM 13P1125 | New Mexico State University | Glanded, non-GE | 18.42 |
| Acala 1517-18 GLS | New Mexico State University | Glanded, 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)
accumulate enough protein over time to metabolize 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 gossypol production in cotton may 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 compared to trifloxysulfuron similarly to gossypol in cotton. However, in this study, glandless cotton responded to trifloxysulfuron 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.

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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Declarations

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Not applicable.

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

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