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Compositional Safety of Herbicide-Tolerant DAS-81910-7 Cotton

Rod A. Herman,*[,†] Brandon J. Fast,[†] Tempest Y. Johnson,[†] Jane Sabbatini,[‡] and Gary W. Rudgers[†]

[†]Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268, United States

[‡]Covance Laboratories Inc., 3301 Kinsman Boulevard Madison, Wisconsin 53704, United States

ABSTRACT: DAS-81910-7 cotton is a transgenic event that was transformed to contain the *aad-12* and *pat* genes. These genes code for the AAD-12 and PAT proteins, which confer tolerance to the herbicides 2,4-D and glufosinate, respectively. Crop composition studies were conducted with DAS-81910-7 cotton (both nonsprayed and sprayed with 2,4-D and glufosinate) to comply with requirements of regulatory authorities responsible for evaluating crop safety. Results indicate compositional equivalence between DAS-81910-7 cottonseed and nontransgenic cottonseed and between sprayed and nonsprayed DAS-81910-7 cottonseed. This study builds on the results from many prior studies which support the conclusion that transgenesis is less likely to unexpectedly alter the composition of crops as compared with traditional breeding.

KEYWORDS: *composition, DAS-81910-7, cotton, safety*

INTRODUCTION

DAS-81910-7 cotton was transformed to contain the *aad-12* and *pat* genes. These genes code for the AAD-12 and PAT proteins, which confer tolerance to the herbicides 2,4-D and glufosinate, respectively.[1,2] Although crop composition studies (e.g., analysis of nutrient and antinutrient levels) are almost universally required by regulatory authorities to support the safety assessment of transgenic crops, their value has been questioned on the basis of the substantial literature generated over the past two decades indicating that transgenesis is less impactful on composition compared with traditional breeding.[3,4] Crop composition studies represent the single most costly regulatory study in support of genetically modified crops (typically in excess of U.S. $1 million per study), and the current body of literature, although extensive, has not yet obviated this regulatory requirement.[4] This high regulatory cost, in part, discourages public sector scientists from pursuing transgenic approaches intended to improve agriculture in the developing world. Therefore, it is imperative to continue to augment the peer-reviewed literature with results from such studies. Here we report results from a study designed to investigate the compositional equivalence between DAS-81910-7 and nontransgenic cottonseed.

Composition studies for herbicide-tolerant crops explicitly require the inclusion of herbicide-sprayed and nonsprayed entries in some regulatory jurisdictions.[5] Herbicides have been used widely on nontransgenic crops for many decades without concern for adverse effects on crop composition and without reports of any adverse health effects related to altered crop composition. The herbicide 2,4-D has been used commercially on nontransgenic crops for over 60 years and has been reported to act as a growth regulator when applied to nontransgenic crops;[1] therefore, one might expect some alteration of crop composition due to application of 2,4-D to DAS-81910-7 cotton, even though such changes would be expected to be minor and not adverse on the basis of 2,4-D's history of safe use on nontransgenic crops. Here we report the composition of DAS-81910-7 cottonseed compared with nontransgenic cottonseed and the effects of spraying 2,4-D (and glufosinate) on DAS-81910-7 cottonseed composition.

MATERIALS AND METHODS

The composition of cottonseed from event DAS-81910-7, a near-isogenic nontransgenic comparator (isoline), and six nontransgenic commercial varieties (ALL-TEX 1203, ALL-TEX A102, ALL-TEX LA122, Paymaster HS 200, Rader 271, and Speed) was investigated for samples collected from field plots grown at eight sites in the United States (subexperiment 1). Transgenic and isoline entries were grown at all eight field sites, and three of the six commercial varieties were grown at each site to comply with European Food Safety Authority (EFSA) guidance.[6] To evaluate potential compositional differences between DAS-81910-7 cotton with and without application of 2,4-D and glufosinate, a second subexperiment (subexperiment 2) was conducted at each trial location, which contained two entries, DAS-81910-7 sprayed with a tank mix of 2,4-D and glufosinate and a nonsprayed DAS-81910-7 entry. Subexperiment 2 (in which 2,4-D and glufosinate were applied) was spatially separated from subexperiment 1 (in which no 2,4-D or glufosinate was applied) to exclude the potential for 2,4-D to affect the composition of non-2,4-D-tolerant entries in the first subexperiment (isoline and nontransgenic commercial varieties).

Experiments were located near Tallassee, AL; Sycamore, GA; Washington, LA; Fisk, MO; Greenville, MS; Mebane, NC; Groom, TX; and East Bernard, TX, USA. Four replicate plots of each entry were established at each site, with each plot consisting of four rows that were 7.6 m long. The seed spacing was approximately 7.6 cm, and the row spacing was approximately 76 cm. Each four-row plot was separated from adjacent plots by two border rows. The entries in each subexperiment were arranged in a randomized complete block design, and the two subexperiments were separated by 30.5 m. Standard commercial agronomic practices (e.g., insect, weed, and disease control) were implemented at each field site (uniformly across all entries in the trial) to produce a commercially acceptable crop.

For the herbicide-treated DAS-81910-7 entry in subexperiment 2, the spray volume was approximately 187 L/ha, and all applications included 2% v/v ammonium sulfate. The 2,4-D (GF-2654 choline formulation), at 1120 g ae/ha, and glufosinate (Ignite 280 SL), at 596 g ai/ha, were applied in a tank mixture as two broadcast applications (at the 3- and 6-node stages).

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Cottonseed samples were collected at maturity from each plot, acid delinted, and shipped to Covance Laboratories Inc. (Madison, WI, USA) for compositional analysis. Analyses included proximates (moisture, carbohydrates, ash, crude fat, and protein), fiber [crude fiber, total dietary fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF)], minerals (calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium, sulfur, and zinc), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine), fatty acids (8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 16:0 palmitic, 16:1 palmitoleic, 17:0 heptadecanoic, 17:1 heptadecenoic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 18:3 γ-linolenic, 20:0 arachidic, 20:1 eicosenoic, 20:2 eicosadienoic, 20:3 eicosatrienoic, 20:4 arachidonic, and 22:0 behenic acids), vitamins [β-carotene, thiamin hydrochloride (thiamine HCl), riboflavin, niacin, pyridoxine hydrochloride (pyridoxine HCl), folic acid, and α-tocopherol], and antinutrients (dihydrosterculic acid, malvalic acid, sterculic acid, free gossypol, and total gossypol).

Samples were received frozen and remained frozen for the duration of the analytical phase until being removed for preparation or analysis. Samples were cryogenically ground to a homogeneous state using a blender and liquid nitrogen prior to assay. Methods of analysis have been published previously[7] with the following exceptions.

Crude fiber was quantitated as the loss on ignition of dried residue remaining after digestion of the samples with 1.25% sulfuric acid and 1.25% sodium hydroxide solutions under specific conditions.[8] For molybdenum and sulfur the samples were wet-ashed with nitric acid using microwave digestion. Using inductively coupled plasma mass spectrometry, the amount of each element was determined by comparing the counts generated by the unknowns with those generated by standard solutions of known concentrations.[9] For selenium, the samples were closed-vessel microwave digested with nitric acid and water. After digestion, the solutions were brought to a final volume with water. To normalize the organic contribution between samples and standards, a dilution was prepared for analysis that contained methanol. The selenium concentration was determined with Se78 using an inductively coupled plasma−mass spectrometer with a dynamic reaction cell by comparing the counts generated by standard solutions.[10] For fatty acids, the lipid was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantification.[11,12] For thiamin HCl, the samples were subjected to acid hydrolysis to denature matrix and free bound thiamin analogues. The treated sample was brought to volume, filtered, and injected onto a reversed phase column using a high-performance liquid chromatography system with a postcolumn derivatization reaction coil and detected via a fluorescence detector. As thiamin monophosphate is not completely reacted, thiamin and thiamin monophosphate were both quantitated separately. Final results are the sum of the two components converted to thiamin HCl form.[13]

For cyclopropanoid fatty acids, the total lipid fraction was extracted from the samples using chloroform and methanol. A portion of the lipid fraction was then saponified with a mild alkaline hydrolysis. The free fatty acids were extracted with ethyl ether and hexane. The free fatty acids were then converted to their phenacyl derivatives with 2-bromoacetophenone. The derivatized extracts were injected on a high-performance liquid chromatography system equipped with an ultraviolet detector. The relative percent of total fatty acids for each peak was calculated from peak areas.[14]

For free gossypol, the samples were extracted with an aqueous acetone solution and filtered. Duplicate aliquots were made, and the active aliquot was reacted with aniline with heat applied in a water bath. Active and inactive aliquots were brought to volume with an aqueous isopropyl alcohol solution and read on a spectrophotometer at 440 nm. The absorbance difference was then compared with a linear curve calculated from standards that were aliquoted, reacted, and read in the same fashion as the samples.[15] Total gossypol defines gossypol and gossypol derivatives, both free and bound, in cottonseed products that are capable of reacting with 3-amino-1-propanol in dimethylformamide solution to form a diaminopropanol complex, which then reacts with aniline to form dianilinogossypol under the conditions of the method. Gossypol, gossypol analogues, and gossypol derivatives having an available aldehyde moiety were measured by using the method.[16]

Analysis of variance (ANOVA) was conducted across test sites for each subexperiment using a mixed model.[17] Entry was considered a fixed effect; location, block within location, and location-by-entry were designated random effects. Paired contrasts between the nonsprayed DAS-81910-7 entry and the isoline in subexperiment 1 and between nonsprayed and sprayed DAS-81910-7 entries in subexperiment 2 were made using t tests. Multiplicity was addressed by applying a false-discovery-rate adjustment to the P values, and differences were considered significant at the 95% confidence level.[18,19] Analytes were excluded from the statistical analysis when >50% of the data were less than the limit of quantitation (<LOQ). These analytes were not suitable to include in the ANOVA because data sets in which most points are excluded or set to a given nominal value have substantially up-biased data or have improperly underestimated variability, respectively. The range of values for the nontransgenic commercial cottonseed reference varieties was determined to put any statistically significant differences into context. In addition, literature ranges for nontransgenic cottonseed composition were assembled for further contextualization.[20−26]

RESULTS AND DISCUSSION

The composition of DAS-81910-7 cottonseed was compared with that of a near-isogenic nontransgenic line (isoline) and six nontransgenic commercial lines grown at eight field sites (subexperiment 1). A separate experiment was conducted at the same eight field sites (subexperiment 2) to determine if the composition of DAS-81910-7 cottonseed was affected when plants were sprayed with 2,4-D and glufosinate. Samples were evaluated for 73 analytes including proximates and fiber, minerals, amino acids, fatty acids, vitamins, and antinutrients. The following analytes were excluded from the statistical analyses because >50% of the data were less than the method LOQ: 8:0 caprylic, 10:0 capric, 12:0 lauric, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 16:1 palmitoleic, 17:0 heptadecanoic, 17:1 heptadecenoic, 18:3 γ-linolenic, 20:2 eicosadienoic, 20:3 eicosatrienoic, 20:4 arachidonic, and β-carotene. Selenium was the only analyte included in the statistical analysis for which <LOQ values were present. Approximately 27% of the data for both the transgenic entries and isoline entry were observed to be <LOQ, and these data were excluded from the analysis due to the nearly equal distribution among entries.

Nonsprayed DAS-81910-7 Cottonseed. Of the 59 compositional analytes included in the statistical analysis, 7 analytes differed significantly between DAS-81910-7 cottonseed and the near-isogenic nontransgenic cottonseed in subexperiment 1 (Tables 1−6). The manganese level was 15% lower in the transgenic entry compared with the isoline (Table 2). It is clear from Figure 1 that manganese levels within the transgenic entries were actually more similar to most of the nontransgenic commercial varieties than were those from the isoline, indicating that manganese levels within the transgenic entries were normal for the crop. The total gossypol level was 12% lower in the transgenic entry compared with the isoline (Table 6). Gossypol is an antinutrient, so a marginal decrease in this analyte, if it were to occur across varieties, would not represent a safety concern. However, total gossypol levels were normal for the

Table 1. Proximates and Fiber

analytical component % dry wt	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
ash	0.185	4.29 ± 0.11	4.17 ± 0.11	3.53−5.21	0.945	4.31 ± 0.13	4.28 ± 0.13	3.7−5.342
		3.67−5.14	3.62−4.76			3.69−5.08	3.79−5.02	
carbohydrates	0.05	47.5 ± 0.9	49.1 ± 0.9	42.3−54.2	0.945	50.2 ± 1.1	50.5 ± 1.1	39.0−53.62
		44.5−53.6	44.7−57.9			46.9−56.6	45.7−58.6	
crude fat	0.642	21.7 ± 0.7	21.3 ± 0.7	15.8−27.9	0.945	20.9 ± 0.9	20.5 ± 0.9	14.4−27.292
		18.3−25.2	15.6−26.2			17.3−25.9	15.1−26.6	
protein	0.055	26.4 ± 0.9	25.4 ± 0.9	21.5−32.3	0.945	24.6 ± 1.0	24.7 ± 1.0	12−32.97
		22.3−31.5	22.2−31.1			19.2−29.7	19.2−29.7	
moisture[e]	0.815	8.16 ± 0.24	8.01 ± 0.24	6.37−10.2	0.998	8.16 ± 0.20	8.17 ± 0.20	2.25−15.9
		6.64−9.74	6.56−9.39			6.95−9.20	6.92−9.13	
ADF	0.642	25.9 ± 0.5	25.3 ± 0.5	20.4−29.4	0.945	26.0 ± 0.5	25.7 ± 0.5	19.74−40.5
		23.0−28.6	21.3−28.6			22.1−29.8	22.4−30.0	
crude fiber	0.815	18.1 ± 0.3	17.9 ± 0.3	15.1−23.5	0.945	18.6 ± 0.3	17.8 ± 0.3	13.45−23.10
		16.0−21.3	14.8−22.7			13.7−23.6	15.6−19.8	
NDF	0.815	34.0 ± 0.6	33.8 ± 0.6	27.2−38.2	0.945	35.2 ± 0.6	34.8 ± 0.6	25.56−53.6
		30.7−40.3	28.3−39.7			30.6−39.3	31.3−39.3	
total dietary fiber	0.591	44.8 ± 1.0	45.7 ± 1.0	37.6−51.3	0.945	46.6 ± 1.1	47.2 ± 1.1	33.69−47.55
		40.3−53.5	40.7−56.5			40.0−54.0	41.7−54.1	

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate. [e]Percent fresh weight.

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Table 2. Minerals

analytical component mg/100 g dry wt	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
calcium	0.059	124 ± 7	133 ± 7	78.9−204	0.998	136 ± 7	136 ± 7	100−330
		88.1−164	98.0−172			113−178	114−178	
copper	0.096	0.900 ± 0.074	0.859 ± 0.074	0.466−1.44	0.998	0.864 ± 0.077	0.863 ± 0.077	0.313−2.457
		0.525−1.37	0.532−1.30			0.446−1.32	0.426−1.41	
iron	0.747	4.20 ± 0.24	4.25 ± 0.24	3.43−6.45	0.945	4.25 ± 0.21	4.30 ± 0.21	3.671−31.838
		3.24−6.26	3.45−5.76			3.28−5.71	3.43−5.57	
magnesium	0.679	387 ± 18	384 ± 18	285−470	0.945	391 ± 19	388 ± 19	340−493.12
		291−487	282−488			250−494	283−498	
manganese	0.021	1.59 ± 0.10	1.35 ± 0.10	0.983−2.28	0.99	1.31 ± 0.08	1.31 ± 0.08	1.069−2.216
		1.17−2.36	0.968−2.20			0.985−1.93	1.01−2.04	
molybdenum	0.702	0.0385 ± 0.0123	0.0363 ± 0.0123	0.00326−0.122	0.945	0.0360 ± 0.0106	0.0344 ± 0.0106	no range
		0.00412−0.107	0.00427−0.106			0.00557−0.0980	0.00539−0.0999	
phosphorus	0.259	652 ± 42	633 ± 42	460−901	0.945	655 ± 45	649 ± 45	482.54−991.57
		491−924	469−921			412−936	472−933	
potassium	0.331	1078 ± 23	1055 ± 23	938−1290	0.945	1074 ± 23	1070 ± 23	960−1448.35
		958−1230	937−1190			939−1200	934−1190	
selenium[e]	0.642	110 ± 30	122 ± 30	<LOQ−676	0.945	194 ± 95	180 ± 95	no range
		<LOQ−382	<LOQ−378			<LOQ−2010	<LOQ−1070	
sodium	0.577	123 ± 8	111 ± 8	73.9−192	0.945	128 ± 9	136 ± 9	5.4−740
		77.5−178	64.7−188			78.7−207	75.3−192	
sulfur	0.747	495 ± 67	535 ± 67	331−847	0.945	520 ± 74	499 ± 74	no range
		305−952	311−1850			279−1430	303−1180	
zinc	0.591	3.36 ± 0.13	3.47 ± 0.13	2.77−4.26	0.998	3.51 ± 0.21	3.48 ± 0.21	2.70−5.95
		2.63−3.93	2.47−5.29			2.33−5.53	2.23−5.94	

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate. [e]PPB.

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Table 3. Amino Acids

analytical component % of total amino acids	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
alanine	0.815	4.44 ± 0.03	4.45 ± 0.03	4.18−4.66	0.945	4.48 ± 0.05	4.49 ± 0.05	4.08−5.30
		4.29−4.60	4.22−4.74			4.265−4.734	4.25−4.746	
arginine	0.096	12.6 ± 0.2	12.5 ± 0.2	11.6−13.7	0.945	12.4 ± 0.2	12.4 ± 0.2	10.83−15.18
		11.9−13.7	11.5−13.7			11.3−13.5	11.3−13.8	
aspartic acid	0.642	10.1 ± 0.2	10.3 ± 0.2	9.59−11.4	0.945	10.3 ± 0.2	10.6 ± 0.2	9.00−12.37
		9.67−11.0	9.75−12.0			9.69−11.7	9.70−12.3	
cystine	0.427	1.79 ± 0.04	1.83 ± 0.04	1.59−2.34	0.945	1.80 ± 0.03	1.79 ± 0.03	1.53−2.35
		1.56−2.13	1.53−2.21			1.56−2.24	1.54−2.17	
glutamic acid	0.815	20.1 ± 0.1	20.1 ± 0.1	19.4−21.2	0.945	19.9 ± 0.2	19.8 ± 0.2	20.24−22.90
		19.2−20.7	18.6−20.7			18.9−20.7	18.3−20.7	
glycine	0.969	4.42 ± 0.04	4.41 ± 0.04	4.10−4.59	0.945	4.45 ± 0.05	4.44 ± 0.05	4.29−5.72
		4.14−4.61	4.08−4.55			4.05−4.63	4.07−4.74	
histidine	0.583	2.84 ± 0.02	2.87 ± 0.02	2.65−3.08	0.945	2.84 ± 0.02	2.83 ± 0.02	2.91−3.88
		2.70−3.05	2.60−3.00			2.69 − 3.00	2.61−2.98	
isoleucine	0.847	3.62 ± 0.02	3.61 ± 0.02	3.18−3.82	0.945	3.63 ± 0.03	3.62 ± 0.03	3.10−4.46
		3.28−3.79	3.39−3.80			3.27−3.88	3.33−3.88	
leucine	0.815	6.30 ± 0.02	6.31 ± 0.02	6.04−6.54	0.945	6.34 ± 0.04	6.32 ± 0.04	6.03−8.11
		6.15−6.49	6.18−6.45			6.15−6.60	6.14−6.60	
lysine	0.577	4.69 ± 0.06	4.73 ± 0.06	4.27−5.03	0.945	4.72 ± 0.07	4.74 ± 0.07	4.62−6.60
		4.36−5.09	4.44−5.107			4.40−5.21	4.39−5.15	
methionine	0.702	1.64 ± 0.02	1.63 ± 0.02	1.34−1.79	0.945	1.65 ± 0.03	1.65 ± 0.03	1.27−2.28
		1.41−1.80	1.46−1.77			1.43−1.99	1.45−1.91	
phenylalanine	0.726	5.70 ± 0.06	5.68 ± 0.06	5.44−6.02	0.945	5.68 ± 0.06	5.63 ± 0.06	5.44−7.23
		5.49−6.12	5.20−6.01			5.35−5.88	5.22−5.97	
proline	0.983	4.04 ± 0.01	4.04 ± 0.01	3.78−4.19	0.998	4.04 ± 0.02	4.05 ± 0.02	3.81−5.30
		3.91−4.12	3.90−4.19			3.90−4.20	3.92−4.16	
serine	0.925	4.63 ± 0.04	4.63 ± 0.04	4.26−5.05	0.998	4.63 ± 0.03	4.63 ± 0.03	4.15−5.87
		4.35−4.88	4.32−4.88			4.35−4.92	4.29−4.93	
threonine	0.911	3.54 ± 0.03	3.54 ± 0.03	3.19−3.76	0.945	3.57 ± 0.04	3.58 ± 0.04	2.67−4.26
		3.31−3.71	3.29−3.68			3.26−3.80	3.34−3.80	
tryptophan	0.747	1.43 ± 0.02	1.42 ± 0.02	1.30−1.68	0.998	1.42 ± 0.02	1.42 ± 0.02	0.91−1.40
		1.24−1.59	1.27−1.65			1.25−1.60	1.20−1.65	
tyrosine	0.436	3.34 ± 0.02	3.31 ± 0.02	3.19−3.46	0.945	3.33 ± 0.02	3.32 ± 0.02	2.63−3.46
		3.20−3.46	3.18−3.42			3.19−3.49	3.16−3.47	
valine	0.679	4.75 ± 0.02	4.74 ± 0.02	4.36−5.02	0.945	4.76 ± 0.04	4.74 ± 0.04	4.49−6.24
		4.43−4.96	4.50−4.92			4.36−5.02	4.45−5.06	

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate.

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Table 4. Fatty Acids

analytical component % of total fatty acids	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
8:0 caprylic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
10:0 capric		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
12:0 lauric		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
14:0 myristic	0.002	0.720 ± 0.041 0.545−0.907	0.657 ± 0.041 0.489−0.872	0.4324−1.05	0.945	0.651 ± 0.041 0.508−0.871	0.648 ± 0.041 0.506−0.866	0.455−2.40
14:1 myristoleic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
15:0 pentadecanoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ−0.481
15:1 pentadecenoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
16:0 palmitic	0.114	22.55 ± 0.58 20.37−25.67	22.26 ± 0.58 20.04−26.12	18.76−26.07	0.945	22.26 ± 0.66 19.71−26.14	22.16 ± 0.66 19.53−25.99	15.11−28.10
16:1 palmitoleic	0.003	0.494 ± 0.022 0.411−0.639	0.460 ± 0.022 0.392−0.588	0.379−0.636	0.945	0.454 ± 0.023 0.378−0.587	0.451 ± 0.023 0.373−0.592	0.464−1.190
17:0 heptadecanoic		<LOQ−0.0990	<LOQ−0.105	<LOQ−0.109	0.103	<LOQ−0.104	<LOQ−0.103	<LOQ−0.119
17:1 heptadecenoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
18:0 stearic	0.642	2.311 ± 0.068 1.951−2.685	2.334 ± 0.068 1.943−2.645	1.801−2.962	0.945	2.341 ± 0.073 1.959−2.628	2.313 ± 0.073 1.875−2.612	0.20−3.11
18:1 oleic	0.001	14.84 ± 0.35 13.74−17.16	13.95 ± 0.35 12.76−16.50	12.93−17.09	0.945	13.82 ± 0.34 12.54−16.50	13.80 ± 0.34 12.64−16.08	12.8−25.3
18:2 linoleic	0.003	58.5 ± 0.8 54.4−61.1	59.7 ± 0.8 54.9−62.5	52.4−63.9	0.945	59.9 ± 0.9 54.5−62.9	60.0 ± 0.9 54.7−62.9	46.00−59.4
18:3 linolenic	0.577	0.2032 ± 0.0083 0.1733−0.2412	0.2117 ± 0.0083 0.1782−0.2991	0.1460−0.2567	0.945	0.2126 ± 0.0090 0.1829−0.2761	0.2150 ± 0.0090 0.1828−0.2688	0.11−0.35
18:3 γ-linolenic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ−0.232
20:0 arachidic	0.722	0.2509 ± 0.0105 0.2088−0.2949	0.2492 ± 0.0105 0.2029−0.3063	0.1855−0.3242	0.945	0.2530 ± 0.0111 0.2010−0.3113	0.2521 ± 0.0111 0.2067−0.2991	0.186−0.414
20:1 eicosenoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ−0.098
20:2 eicosadienoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ
20:3 eicosatrienoic		<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ

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Table 4. continued

analytical component % of total fatty acids	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
20:4 arachidonic				<LOQ				<LOQ
		<LOQ	<LOQ			<LOQ	<LOQ	
22:0 behenic	0.464	0.1373 ± 0.0060	0.1341 ± 0.0060	0.1035−0.1749	0.945	0.1358 ± 0.0071	0.1386 ± 0.0071	<LOQ−0.295
		0.1119−0.1692	0.1057−0.1693			0.1101−0.1757	0.1092−0.1726	

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate.

Table 5. Vitamins

analytical component mg/kg dry wt	subexperiment 1				subexperiment 2			
	P value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	literature range min−max[c]
α-tocopherol (vitamin E)	0.803	89.7 ± 11.0	88.4 ± 11.0	31.1−151	0.945	90.3 ± 9.9	88.7 ± 9.9	70.825−197.243
		30.3−134	26.7−136			20.4−135	27.6−129	
vitamin A (β-carotene)				<LOQ−0.267				no range
		<LOQ−0.264	<LOQ−0.323			<LOQ−0.249	<LOQ−0.238	
vitamin B1 (thiamin HCl)	0.969	10.3 ± 0.4	10.3 ± 0.4	5.54−14.7	0.998	10.4 ± 0.5	10.4 ± 0.5	no range
		7.52−12.6	8.28−13.2			6.14−13.4	7.42−15.4	
vitamin B2 (riboflavin)	0.888	6.24 ± 0.45	6.34 ± 0.45	3.44−9.52	0.998	6.65 ± 0.47	6.65 ± 0.47	no range
		3.52−9.95	3.82−9.82			3.69−12.5	3.97−10.0	
vitamin B3 (niacin)	0.62	27.8 ± 1.2	28.5 ± 1.2	20.4−36.8	0.998	28.2 ± 1.1	28.1 ± 1.1	no range
		21.7−35.6	22.7−35.3			21.3−37.7	23.5−34.3	
vitamin B6 (pyridoxine HCl)	0.464	3.83 ± 0.09	3.72 ± 0.09	2.84−5.12	0.945	3.70 ± 0.13	3.77 ± 0.13	no range
		3.15−4.49	3.10−4.54			3.18−4.72	3.08−5.28	
vitamin B9 (folic acid)	0.888	1.66 ± 0.07	1.67 ± 0.07	1.10−2.40	0.945	1.56 ± 0.09	1.64 ± 0.09	no range
		1.14−2.38	1.17−2.26			0.872−2.31	1.15−2.29	

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate.

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Table 6. Antinutrients

	subexperiment 1				subexperiment 2			
analytical component % of total fatty acids	*P* value[a]	isoline mean ± SE[b] min−max[c]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	ref range min−max[c]	*P* value[a]	DAS-81910-7 nonsprayed mean ± SE[b] min−max[c]	DAS-81910-7 sprayed[d] mean ± SE[b] min−max[c]	lit. range min−max[c]
dihydrosterculic acid	0.259	0.205 ± 0.007 0.150−0.247	0.214 ± 0.007 0.174−0.261	0.153−0.325	0.945	0.216 ± 0.006 0.175−0.269	0.214 ± 0.006 0.181−0.254	<LOQ−0.310
malvalic acid	0.029	0.524 ± 0.023 0.403−0.645	0.577 ± 0.023 0.426−0.762	0.402−0.854	0.945	0.591 ± 0.026 0.474−0.722	0.612 ± 0.026 0.450−0.806	0.17−0.759
sterculic acid	0.259	0.275 ± 0.011 0.173−0.447	0.297 ± 0.011 0.209−0.358	0.196−0.440	0.998	0.301 ± 0.011 0.215−0.366	0.301 ± 0.011 0.196−0.391	0.13−0.56
free gossypol[e]	0.059	0.959 ± 0.057 0.593−1.36	0.833 ± 0.057 0.556−1.17	0.492−1.28	0.945	0.817 ± 0.061 0.479−1.20	0.806 ± 0.061 0.498−1.13	0.454−1.399
total gossypol[e]	0.01	1.08 ± 0.05 0.829−1.44	0.946 ± 0.049 0.719−1.19	0.551−1.41	0.998	0.919 ± 0.049 0.519−1.31	0.925 ± 0.049 0.624−1.32	0.547−1.522

[a]*P* value for *t* test (false discovery rate adjusted). [b]Pooled standard error. [c]Minimum−maximum plot value. [d]Sprayed with 2,4-D plus glufosinate. [e]Percent dry weight.

Figure 1. Site means for entries in subexperiments 1 and 2 for manganese (mg/100 g dry weight), total gossypol (percent dry weight), and malvalic acid (percent of total fatty acids). Shaded area represents the literature range for each analyte. Locations are represented by the following symbols: open circle = AL; solid circle = Groom, TX; + = LA; × = GA; open triangle = MO; solid triangle = East Bernard, TX; open square = MS; open diamond = NC. 1910 = DAS-81910-7. Note that each nontransgenic commercial variety was included, on average, at only half of the sites, so the spread of data for these entries is expected to be less than that for the control and transgenic entries, which were present at all eight sites.

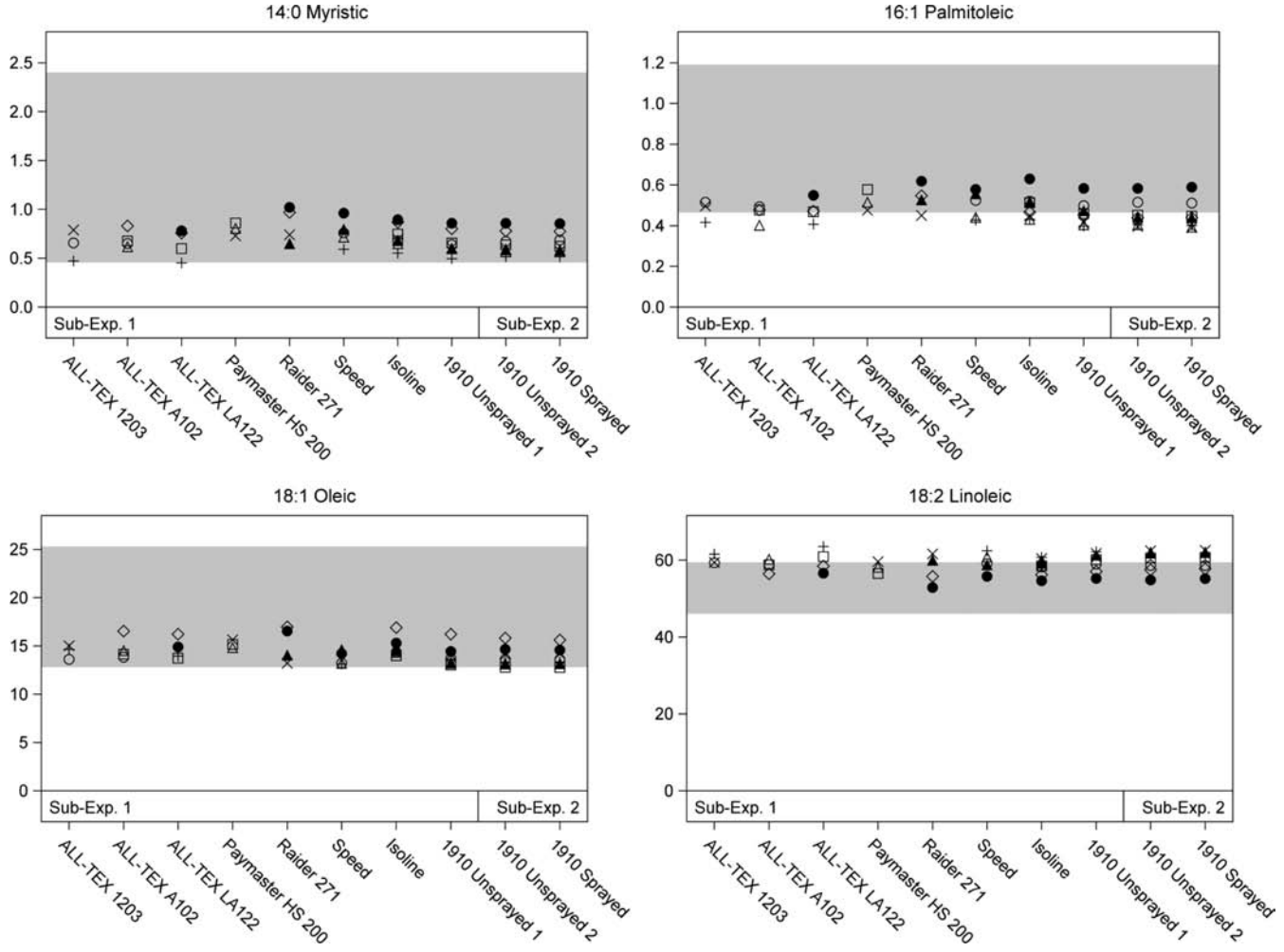

Figure 2. Site means for entries in subexperiments 1 and 2 for 14:0 myristic acid, 16:1 palmitoleic acid, 18:1 oleic acid, and 18:2 linoleic acid (percent of total fatty acids). Shaded area represents the literature range for each analyte. Locations are represented by the following symbols: open circle = AL; solid circle = Groom, TX; + = LA; × = GA; open triangle = MO; solid triangle = East Bernard, TX; open square = MS; open diamond = NC. 1910 = DAS-81910-7. Note that each nontransgenic commercial variety was included, on average, at only half of the sites, so the spread of data for these entries is expected to be less than that for the control and transgenic entries, which were present at all eight sites.

transgenic entries compared with other nontransgenic cotton varieties (Figure 1). The malvalic acid level for the transgenic entry was 10% higher compared with the isoline (Table 6); however, levels were, once again, normal for nontransgenic cotton as a whole (Figure 1).

Four fatty acids also differed significantly between the nonsprayed DAS-81910-7 entry and the isoline entry in subexperiment 1 (Table 4). The 14:0 myristic, 16:1 palmitoleic, and 18:1 oleic acid levels for the transgenic entry were 9, 7, and 6% lower, respectively, compared with the isoline, whereas the 18:2 linoleic acid level for the transgenic entry was 2% higher compared with the isoline. In addition to the small magnitude of these differences (<10% change from isoline), examination of site means for the transgenic and nontransgenic entries in this study indicates no meaningful differences for these fatty acids compared with the commercial nontransgenic varieties included in this study (Figure 2).

It is noteworthy that, separate from any potential effect of transgenesis, normal intervarietal variation is expected to result in compositional differences between a parent line and lines generated from single-plant selections from that same line (or from single cells as in the case of tissue-culture regenerated transgenic or nontransgenic plants).[4,27] It is also clear from Figures 1 and 2 that variation among nontransgenic commercial lines was greater than differences between the isoline entry and the transgenic entries.

Effects of Herbicides. Among the 59 analytes included in the compositional analysis for subexperiment 2, there were no significant differences between the nonsprayed DAS-81910-7 entry and the DAS-81910-7 entry sprayed with 2,4-D and glufosinate (Tables 1−6). The lack of spurious results in this subexperiment may be related to the small number of entries and the resulting small size of experimental blocks in the field (decrease in heterogeneity within blocks).

Conclusions. The composition of DAS-81910-7 cottonseed was found to be normal for cotton. Spraying 2,4-D and glufosinate did not significantly affect the composition of the cottonseed derived from this transgenic event. The ability to detect small differences as statistically significant between the transgenic and isoline entries for a number of analytes demonstrates the power of the experiment to highlight differences smaller than that seen between conventional nontransgenic varieties (Figures 1 and 2). This study builds on the results from many prior studies supporting transgenesis as less likely to unexpectedly perturb the composition of crops compared with traditional breeding.[3,4]

AUTHOR INFORMATION

Corresponding Author

*(R.A.H.) Phone: (317) 337-3551. E-mail: raherman@dow.com.

Notes

The authors declare the following competing financial interest(s): RH, BF, TJ, and GR are employed by Dow AgroSciences LLC which develops and markets transgenic seed. JS is employed by Covance Laboratories Inc. which was contracted by Dow AgroSciences to conduct the composition analytical work.

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REFERENCES

(1) Wright, T. R.; Shan, G.; Walsh, T. A.; Lira, J. M.; Cui, C.; Song, P.; Zhuang, M.; Arnold, N. L.; Lin, G.; Yau, K.; Russell, S. M.; Cicchillo, R. M.; Peterson, M. A.; Simpson, D. M.; Zhou, N.; Ponsamuel, J.; Zhang, Z. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 20240−20245.

(2) Herman, R. A.; Phillips, A. M.; Lepping, M. D.; Sabbatini, J. Compositional safety of DAS-68416-4 (AAD-12) herbicide-tolerant soybean. *J. Nutr. Food Sci.* **2011**, 1:2 DOI: 10.4172/215-9600.1000103.

(3) Ricroch, A. E.; Berge, J. B.; Kuntz, M. Evaluation of genetically engineered crops using transcriptomic, proteomic and metabolomic profiling techniques. *Plant Physiol.* **2011**, *155*, 1752−1761.

(4) Herman, R. A.; Price, W. D. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. *J. Agric. Food Chem.* **2013**, DOI: 10.1021/jf400135r.

(5) EFSA Panel on Genetically Modified Organisms (GMO). Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. *EFSA J.* **2011**, *9*, 2149−2169.

(6) EFSA Panel on Genetically Modified Organisms (GMO). Statistical considerations for the safety evaluation of GMOs. *EFSA J.* **2010**, *8* (1), 1250.

(7) Herman, R. A.; Phillips, A. M.; Lepping, M. D.; Fast, B. J.; Sabbatini, J. Compositional safety of event DAS-40278-9 (AAD-1) herbicide-tolerant maize. *GM Crops Food: Biotechnol. Agric.* **2010**, *1* (5), 294−311.

(8) Method 962.09. In *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Gaithersburg, MD, 2012.

(9) Method 993.14. In *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Gaithersburg, MD, 2012.

(10) Method 2011.19. In *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Gaithersburg, MD, 2012.

(11) Official method Ce 2-66. In *Official Methods and Recommended Practices of the AOCS*, 6th ed.; American Oil Chemists' Society: Champaign, IL, 2011.

(12) Official method Ce li-07. In *Official Methods and Recommended Practices of the AOCS*, 6th ed.; American Oil Chemists' Society: Champaign, IL, 2011.

(13) European Standard Determination of Vitamin B1 by HPLC. EN200314122:2003.

(14) Wood, R. High performance liquid chromatography analysis of cyclopropene fatty acid. *Biochem. Arch.* **1986**, *2*, 63−71.

(15) Official method Ba 8-78. In *Official Method and Recommended Practices of the AOCS*, 6th ed.; American Oil Chemists' Society: Champaign, IL, 2011.

(16) Official method Ba 7-58. In *Official Methods and Recommended Practices of the AOCS*, 6th ed.; American Oil Chemists' Society: Champaign, IL, 2011.

(17) SAS Institute Inc. *SAS/STAT® User's Guide*, version 9.2, 2nd ed.; SAS Institute Inc.: Cary, NC, 2009; p 5121.

(18) Curran-Everett, D. Multiple comparisons: philosophies and illustrations. *Am. J. Physiol. − Regul., Integr. Comp. Physiol.* **2000**, *279* (1), R1−R8.

(19) Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B−Methodol.* **1995**, *57* (1), 289−300.

(20) Herman, R. A.; Scherer, P. N.; Phillips, A. M.; Storer, N. P.; Krieger, M. Safe composition levels of transgenic crops assessed via a clinical medicine model. *Biotechnol. J.* **2010**, *5* (2), 172−182.

(21) Belyea, R. L.; Steevens, B. J.; Restrepo, R. J.; Clubb, A. P. Variation in composition of by-product feeds. *J. Dairy Sci.* **1989**, *72* (9), 2339−2345.

(22) Berberich, S. A.; Ream, J. E.; Jackson, T. L.; Wood, R.; Stipanovic, R.; Harvey, P.; Patzer, S.; Fuchs, R. L. The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. *J. Agric. Food Chem.* **1996**, *44* (1), 365−371.

(23) Bertrand, J. A.; Sudduth, T. Q.; Condon, A.; Jenkins, T. C.; Calhoun, M. C. Nutrient content of whole cottonseed. *J. Dairy Sci.* **2005**, *88* (4), 1470−1477.

(24) Hamilton, K. A.; Pyla, P. D.; Breeze, M.; Olson, T.; Li, M. H.; Robinson, E.; Gallagher, S. P.; Sorbet, R.; Chen, Y. Bollgard II cotton: compositional analysis and feeding studies of cottonseed from insect-protected cotton (*Gossypium hirsutum* L.) producing the Cry1Ac and Cry2Ab2 proteins. *J. Agric. Food Chem.* **2004**, *52* (23), 6969−6976.

(25) Kohel, R. J.; Glueck, J.; Rooney, L. W. Comparison of cotton germplasm collections for seed-protein content1. *Crop Sci.* **1985**, *25* (6), 961−963.

(26) Nida, D. L.; Patzer, S.; Harvey, P.; Stipanovic, R.; Wood, R.; Fuchs, R. L. Glyphosate-tolerant cotton: the composition of the cottonseed is equivalent to that of conventional cottonseed. *J. Agric. Food Chem.* **1996**, *44* (7), 1967−1974.

(27) Tokatlidis, I. S.; Tsikrikoni, C.; Tsialtas, J. T.; Lithourgidis, A. S.; Bebeli, P. J. Variability within cotton cultivars for yield, fibre quality and physiological traits. *J. Agric. Sci.* **2008**, *146* (4), 483−490.