JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Compositional Equivalence of DAS-444Ø6-6 (AAD-12 + 2mEPSPS + PAT) Herbicide-Tolerant Soybean and Nontransgenic Soybean

Miles D. Lepping,*,† Rod A. Herman,† and Brian L. Potts‡

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

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

ABSTRACT: Soybeans from transgenic event DAS-444Ø6-6 are the first to express three proteins that provide tolerance to broad-spectrum herbicides. DAS-444Ø6-6 soybean expresses the aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme from the soil bacterium *Delftia acidovorans*, which provides tolerance to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D); the double-mutant 5-enolpyruvylshikimate-3-phosphate synthase (2mEPSPS) enzyme encoded by a modified version of the *epsps* gene from maize (*Zea mays*), which provides tolerance to the herbicide glyphosate; and the phosphinothricin acetyltransferase (PAT) enzyme from *Streptomyces viridochromogenes*, which provides tolerance to the herbicide glufosinate. The purpose of this study was to determine if the nutrient and antinutrient composition of forage and grain from DAS-444Ø6-6 soybean are similar to those of nontransgenic soybean. Forage was analyzed for proximates, fiber, and minerals; grain analyses further included vitamins, amino acid and fatty acid profiles, and antinutrients and bioactive components (lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, and isoflavones). Results indicate that DAS-444Ø6-6 soybean is compositionally equivalent to nontransgenic soybean. Findings are consistent with similar studies for other input traits, as endogenous plant metabolic pathways that influence composition are expected to be less affected by transgenesis compared with traditional plant-breeding methods.

KEYWORDS: *soybean (Glycine max), crop composition, substantial equivalence, transgenic, 2,4-D, glyphosate, glufosinate*

INTRODUCTION

To inform the safety assessment of transgenic plants intended for human and/or livestock consumption, the nutrient and antinutrient composition of the transformed crop are compared with the composition of nontransgenic varieties.[1] Compositional data for nontransgenic varieties may be obtained from near-isoline(s) of the host used for gene introgression, as well as commercially and publicly available varieties reported in the scientific literature and/or produced concurrently with the transgenic variety under study.[2,3] Although composition studies are currently required to support assessments for transgenic crops, their utility has been questioned following 20 years of data demonstrating only minor and expected variation in transgenic crop composition as compared with variation imparted through traditional breeding practices.[4]

Dow AgroSciences LLC and MS Technologies LLC have developed the first three-gene herbicide-tolerant soybean event. Event DAS-444Ø6-6 soybean expresses three herbicide-tolerant (HT) proteins: (1) the aryloxyalkanoate dioxygenase-12 (AAD-12) enzyme from the soil bacterium *Delftia acidovorans*, which provides tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D);[5] (2) the double-mutant 5-enolpyruvylshikimate-3-phosphate synthase (2mEPSPS) enzyme encoded by a modified version of the *epsps* gene from maize (*Zea mays*), which provides tolerance to glyphosate;[6] and (3) the phosphinothricin acetyltransferase (PAT) enzyme from *Streptomyces viridochromogenes*, which provides tolerance to glufosinate herbicides.[7] A single vector containing the *aad-12*, *2mepsps*, and *pat* gene expression cassettes was introduced into the publicly available soybean line Maverick via *Agrobacterium tumefaciens*-mediated transformation to produce transgenic event DAS-444Ø6-6. Through tolerance to multiple, broad-spectrum herbicides, DAS-444Ø6-6 soybean is expected to provide growers with a powerful integrated weed management tool for managing herbicide-resistant weeds in soybean production systems.[8]

Compositional equivalence with nontransgenic soybean has been demonstrated for a different soybean event (DAS-68416-4) expressing the AAD-12 and PAT proteins.[9] In the present study, DAS-444Ø6-6 soybean was similarly examined through compositional analysis of crop products (forage and grain) harvested from a series of replicated field trials established in soybean-growing regions of the United States.

MATERIALS AND METHODS

Field Phase. Ten field experiments were conducted in 2010 to produce soybean forage and grain for compositional analysis. Field sites were located in Sycamore, GA; Richland and Bagley, IA; Carlyle and Wyoming, IL; Sheridan, IN; Deerfield, MI; Fisk, MO; and Brunswick and York, NE. Soybean treatments included DAS-444Ø6-6 soybean in a Maverick-variety genetic background, a nontransgenic near-isogenic Maverick line (isoline), and six nontransgenic commercial lines [Dairyland (DSR) 75213-72, DSR 98860-71, DSR 99914N, DSR 99915, Porter 75148, and Williams 82] as reference varieties.

Experiments at each site contained five entries of DAS-444Ø6-6 soybean, with each entry receiving a different herbicide treatment. Herbicide treatments included unsprayed (not treated with 2,4-D, glufosinate, or glyphosate) or sprayed with 2,4-D, glufosinate, glyphosate, or a combination of all three herbicides (2,4-D + glufosinate + glyphosate). The herbicides 2,4-D and glyphosate were applied as one pre-emergence application and two postemergence

Received: August 25, 2013
Revised: October 12, 2013
Accepted: October 15, 2013
Published: November 5, 2013

Table 1. Proximate, Fiber, and Mineral Composition of Forage from DAS-444Ø6-6 Soybean

component	DAS-444Ø6-6 mean (min−max)[a]	DAS-444Ø6-6 + 2,4-D mean (min−max)[a]	DAS-444Ø6-6 + glufosinate mean (min−max)[a]	DAS-444Ø6-6 + glyphosate mean (min−max)[a]	DAS-444Ø6-6 + three herbicides mean (min−max)[a]	isoline (Maverick) mean (min−max)[a]	reference min−max[b]	literature min−max[c]
Proximate (% dwt)[d]								
moisture (% fwt)[d]	77.6[e]	77.6[e]	77.6[e]	77.2[e]	77.4[e]	78.7		
	(71.8−80.5)	(71−81)	(69.9−81.1)	(69.1−80.7)	(69.8−81.1)	(75.6−82.3)	70.9−81.4	32.05−84.60
ash	9.4	9.2	8.9	9.7	8.9	8.9		
	(7.13−28.3)	(5.96−31)	(6.14−19)	(6.57−24)	(6.42−21.4)	(6.85−18.7)	5.86−36.6	4.68−10.78
carbohydrate	68.4	68.9	68.2	68.8	68.8	68.0		
	(49.8−76.3)	(59.1−73)	(53.8−74.4)	(56.3−74.4)	(59.5−73.8)	(55.8−74.3)	48.8−74.7	59.8−80.18
protein	19.7	19.4	19.2	19.6	19.7	19.7		
	(16−25.3)	(15.5−23.4)	(14.8−24.1)	(14.5−23.9)	(15.2−25.2)	(13.7−23.4)	13−29.1	11.2−24.71
fat	2.75	2.81	2.85	2.68	2.68	2.87		
	(0.769−4.01)	(1.6−3.88)	(1.14−3.79)	(1.91−3.52)	(1.47−3.69)	(1.95−4.11)	1.69−4.63	1.01−9.87
Fiber (% dwt)[d]								
ADF	31.5	31.7	31.7	32.3	30.6	31.6		
	(24.3−44.2)	(23−42.2)	(25−44.7)	(26−43.6)	(23.7−39.3)	(22.9−38.7)	21.5−57.2	22.72−59.03
NDF	37.4	38.1	37.3	37.5	37.6	37.6		
	(27.2−51.3)	(27.3−50)	(28.4−50.4)	(24.7−50)	(21.8−52.3)	(29.1−46.5)	24.9−63.1	19.61−73.05
Minerals (mg/100 g dwt)[d]								
calcium	1236	1208	1211	1227	1263	1240		
	(652−1590)	(817−1560)	(650−1540)	(858−1600)	(762−1760)	(880−1770)	695−1860	NR[d]
phosphorus	266	266	264	265	265	271		
	(177−381)	(197−374)	(186−385)	(197−399)	(170−394)	(190−384)	175−427	NR[d]

[a]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single-replicate plots. [b]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [c]See Materials and Methods for reference citations. [d]fwt = fresh weight, dwt = dry weight, NR = not reported. [e]Statistically significant difference with isoline ($P < 0.05$).

Table 2. Proximate, Fiber, and Mineral Composition of Grain from DAS-444Ø6-6 Soybean

component	DAS-444Ø6-6 mean (min−max)[a]	DAS-444Ø6-6 + 2,4-D mean (min−max)[a]	DAS-444Ø6-6 + glufosinate mean (min−max)[a]	DAS-444Ø6-6 + glyphosate mean (min−max)[a]	DAS-444Ø6-6 + three herbicides mean (min−max)[a]	isoline (Maverick) mean (min−max)[a]	reference min−max[b]	literature min−max[c]
Proximate (% dwt)[d]								
moisture (% fwt)[d]	10.2	10.0	9.9	9.9	9.9	10.6		
	(7.1−22.1)	(7.19−13.8)	(6.54−14.1)	(7.13−14.5)	(6.87−12.9)	(7.58−20.4)	7.26−17.2	4.7−34.4
ash	5.23	5.24	5.24	5.21	5.22	5.15		
	(4.66−6.34)	(4.59−5.99)	(4.55−6.87)	(4.49−5.78)	(4.48−6.42)	(4.49−5.86)	4.45−6.3	3.885−6.994
carbohydrate	37.22[e]	37.11[e]	37.21[e]	37.38[e]	37.04[e]	38.13		
	(32.5−40.8)	(31.3−41.3)	(31.2−40.6)	(32.1−41.5)	(32.2−40.7)	(32.6−47.7)	28.7−43	29.3−50.2
protein	38.0	38.5	38.2	38.2	38.6[e]	37.8		
	(35.6−39.7)	(37.1−40.4)	(34−40.6)	(36−40.9)	(36.1−42.5)	(29.7−40.3)	35.1−44.9	32−48.4
fat	19.5	19.2	19.3	19.2	19.1	18.9		
	(16.9−23.5)	(15.8−23.4)	(16.7−23.4)	(16.5−23.1)	(16.2−22.6)	(13.6−23.5)	15.3−22.9	8.104−24.7
Fiber (% dwt)[d]								
ADF	15.2	15.5	15.0	15.6	14.9	15.5		
	(7.68−20.7)	(8.71−18.7)	(10.3−18.2)	(11.7−19.6)	(11.3−20.3)	(9.84−24.1)	8.02−20.9	7.81−26.26
NDF	17.0	17.1	16.7	17.3	16.5[e]	17.7		
	(9.41−20.9)	(10.9−21.4)	(13.1−20.1)	(13.2−22)	(13.4−19.7)	(14.6−24.1)	13.3−22.2	8.53−23.90
total dietary fiber	21.6	21.7	21.5	21.9	21.4	22.4		
	(18−25.4)	(17.9−26.1)	(17.3−26.5)	(16.9−25.8)	(16−25.6)	(16.7−27.8)	16.2−27.7	NR[d]
Minerals (mg/100 g dwt)[d]								
calcium	324[e]	318[e]	304	320[e]	306	301		
	(261−425)	(252−404)	(241−398)	(249−413)	(243−404)	(235−403)	174−383	116.55−510
copper	1.34	1.35	1.32	1.35	1.33	1.32		
	(1.01−1.71)	(1.03−1.93)	(1.04−1.74)	(1.15−1.7)	(1.09−1.71)	(0.995−1.68)	0.91−1.77	0.632−1.092
iron	8.3	7.7	8.6	7.8	8.5	8.2		
	(6.33−26.2)	(6.18−9.64)	(6.55−41.9)	(6.42−12.3)	(6.54−24.5)	(6.51−14.2)	5.35−87.9	3.734−10.954
magnesium	231	230	227	230	229	229		
	(205−283)	(206−287)	(202−276)	(203−279)	(200−284)	(207−279)	195−317	219.40−312.84
manganese	3.10	3.27	3.09	3.18	3.14	2.99		
	(1.69−8.27)	(1.78−10.4)	(2.03−8.57)	(1.89−10.8)	(2.08−9.46)	(2.11−7.83)	1.9−9.53	2.52−3.876
phosphorus	561	558	554	558	557	557		
	(394−661)	(384−681)	(377−657)	(403−645)	(388−660)	(400−640)	360−659	506.74−935.24
potassium	1780[e]	1790[e]	1770[e]	1770[e]	1770[e]	1730		
	(1610−1930)	(1640−1940)	(1630−1930)	(1620−1890)	(1610−1940)	(1580−1850)	1530−2030	1868.01−2510
selenium (ppb)[d]	438	489	389	420	469	451		
	(77.7−1770)	(63.8−2320)	(77−1770)	(84−1670)	(72.3−2360)	(66.7−1980)	59.5−3380	NR[d]
zinc	4.34	4.34	4.25	4.3	4.3	4.17		
	(3.45−5.02)	(3.53−5.59)	(3.62−5.01)	(3.56−5.05)	(3.67−6.01)	(3.62−4.7)	3.34−5.82	4.98−7.578

[a]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single replicate plots. [b]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [c]See Materials and Methods for reference citations. [d]fwt = fresh weight, dwt = dry weight, NR = not reported, ppb = parts per billion. [e]Statistically significant difference with isoline ($P < 0.05$).

dx.doi.org/10.1021/jf403775d | J. Agric. Food Chem. 2013, 61, 11180−11190

applications during V3 (three-trifoliate stage) and R2 (full flowering). The herbicides 2,4-D and glyphosate were applied as a tank mixture in the combination entry. The 2,4-D and glyphosate application rates were 1.12 and 1.26 kg acid equivalent (ae)/ha, respectively, at each application timing. Glufosinate was applied as two postemergence applications during V5 (five-trifoliate stage) and R1 (beginning flowering) at rates of 0.37 and 0.45 kg active ingredient (ai)/ha, respectively. Herbicides were applied in a carrier volume of 187 L/ha, and the spray solution for each application contained ammonium sulfate (AMS) at a rate of 2% v/v. The commercial formulations of 2,4-D, glyphosate, glufosinate, and AMS adjuvant used in this study were Weedar 64 (454 g ae/L, Nufarm, Inc.), Durango DMA (trademark of Dow AgroSciences LLC) (490 g ae/L, Dow AgroSciences LLC), Liberty (200 g ai/L, Bayer CropScience), and N-Pak AMS Liquid (Winfield Solutions LLC), respectively. In addition to structured herbicide treatments applied to specific entries, all entries, including the nontransgenic isoline, received identical, site-specific maintenance applications of insecticides, fungicides, and conventional soybean herbicides (excluding 2,4-D, glyphosate, and glufosinate-containing products) as necessary to promote optimal crop health.

The commercial reference varieties were arranged across sites in a balanced incomplete-block design, where three of the six commercial lines were randomized at each site. Within each site, the five DAS-444Ø6-6 entries, the isoline, and the three assigned commercial lines were then arranged as a randomized complete-block design with four replicate blocks. Plots were four rows wide (76 cm apart) by 7.6 m long with an in-row seed spacing of approximately 6 cm. Two nontransgenic soybean border rows flanked each plot, and a minimum of four border rows surrounded the entire trial at each site. Soybean forage (300 g) and grain samples (500 g) were collected from the center two rows of each plot during the R3 (beginning pod) and R8 (full maturity) growth stages, respectively. Samples were shipped frozen to the analytical laboratory (Covance Laboratories Inc., Madison, WI, USA) for compositional analysis.

Compositional Analyses. Soybean forage and grain were assayed for compositional components (Covance Laboratories), using methods previously described by Herman et al.[9] Forage was quantitatively analyzed for proximate (moisture, ash, crude protein, and crude fat), fiber [acid detergent fiber (ADF) and neutral detergent fiber (NDF)], and mineral (calcium and phosphorus) content. Grain was analyzed for the same components as forage with the addition of the following: total dietary fiber; minerals (copper, iron, magnesium, manganese, potassium, selenium, sodium, and zinc); amino acid profile; fatty acid profile; vitamins [β-carotene, thiamin hydrochloride, riboflavin, niacin, pantothenic acid, pyridoxine hydrochloride, folic acid, ascorbic acid, and tocopherols (α, β, γ, δ, and total)]; and antinutrients and bioactive components (lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, and isoflavones). Carbohydrate content for forage and grain was calculated from the proximate by difference [i.e., % carbohydrate = 100% − (% protein + % fat + % ash + % moisture)] using fresh weight results, followed by conversion to a dry weight measure. In addition to the Herman et al.[9] analyte list and associated methods, the present study includes the mineral selenium. A brief summary of the method for selenium analysis is presented below. Selenium samples were wet-ashed with nitric acid using microwave digestion. Using inductively coupled plasma mass spectrometry, the level of selenium was determined by comparing the counts generated by the field samples to those generated by standard solutions of known concentrations.

Statistical Analysis and Data Interpretation. Analytes for which >50% of samples in the isoline and transgenic entries were less than the limit of quantitation (LOQ) were excluded from statistical analysis. Grain analytes satisfying the criteria for exclusion were fatty acids (8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 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, and 20:4 arachidonic); vitamins [vitamin A (β-carotene), β-tocopherol], and the mineral sodium. These analytes are generally not observed in significant or detectable quantities in soybean, which is supported by the relatively few reports of detection from soybean in the International Life Sciences Institute's Crop Composition Database (ILSI-CCDB):[10] www.cropcomposition.org/.

The primary method used to compare and interpret compositional results was through examination of across-site means within the context of the range reported for the nontransgenic crop.[10−20] Site means and literature ranges were also plotted to visualize results at the local level, where values below the limit of quantitation were plotted as zero. Additionally, data were subjected to a mixed-model analysis of variance (ANOVA) using PROC MIXED in SAS/STAT version 9.2.[21] Entry was defined as a fixed effect; site, replicate block within site, and the site-by-entry interaction were defined as random effects. Paired contrasts (t-tests) were conducted between the isoline and each DAS-444Ø6-6 entry. Following ANOVA, a false-discovery rate (FDR) procedure was used to adjust P values for multiplicity,[22,23] and differences were considered to be statistically significant at $P < 0.05$. These procedures have been applied in previous studies examining the composition of transgenic crops.[2,9,24]

RESULTS AND DISCUSSION

To assess the safety of raw commodities produced by a novel transgenic crop, the nutrient and antinutrient composition of forage and grain from event DAS-444Ø6-6 soybean generated in a replicated U.S. field study were compared with the composition of nontransgenic soybean. Results are presented in Tables 1−6 and discussed below. The available literature describing the concentrations of some soybean analytes is relatively sparse, resulting in the narrow ranges reported here or the absence of literature values for those analytes (e.g., some minerals and vitamins). The absence of some literature ranges is expected because soybean has not been traditionally considered to be a dietary source for all analytes that may be quantitatively assessed.

Forage Composition. Proximate, fiber, and mineral content in forage samples was similar between DAS-444Ø6-6 entries and the isoline, with the exception of slightly lower moisture levels for DAS-444Ø6-6 entries [77.2−77.6% fresh weight (fwt)] compared with the isoline (78.7% fwt). All forage end points for DAS-444Ø6-6, including moisture content, were within ranges observed for nontransgenic soybean (Table 1).

Grain Composition. Proximate levels for grain from the DAS-444Ø6-6 entries were also similar to those of the isoline, with the exception of small differences for carbohydrate and isolated differences for protein and fat, where some DAS-444Ø6-6 entries contained more protein [sprayed with either 2,4-D [38.5% dry weight (dwt) or all herbicides (38.6% dwt)] or fat [unsprayed (19.5% dwt) or sprayed with glufosinate (19.3% dwt)] and less carbohydrate (all entries; 37.0−37.4% dwt) than the isoline (protein, 37.8%; fat, 18.9%; carbohydrate, 38.1% dwt). Because carbohydrate content is calculated from the proximate by difference (see Materials and Methods), the presence of more protein, fat, or other proximate components is expected to result in a partial decrease in carbohydrates. Fiber levels for grain were statistically indistinguishable from the isoline (17.7% dwt) with the exception of lower neutral detergent fiber levels for one of five DAS-444Ø6-6 entries (all herbicides applied; 16.5% dwt). For both proximate and fiber, analyte values for DAS-444Ø6-6 entries were within ranges observed for nontransgenic soybean (Table 2).

Eight of the 10 minerals analyzed in grain were found to be present in the isoline and DAS-444Ø6-6 entries at similar levels (Table 2). Calcium was present in slightly higher concentrations in three of five DAS-444Ø6-6 entries [unsprayed (324 mg/100 g dwt) and sprayed with either 2,4-D (318 mg/100 g

Table 3. Amino Acid Composition of Grain from DAS-444Ø6-6 Soybean

component (% dwt)[a]	DAS-444Ø6-6 mean (min−max)[b]	DAS-444Ø6-6 + 2,4-D mean (min−max)[b]	DAS-444Ø6-6 + glufosinate mean (min−max)[b]	DAS-444Ø6-6 + glyphosate mean (min−max)[b]	DAS-444Ø6-6 + three herbicides mean (min−max)[b]	isoline (Maverick) mean (min−max)[b]	references min−max[c]	literature min−max[d]
alanine	1.67	1.67	1.68	1.67	1.68	1.68		
	(1.58−1.76)	(1.6−1.74)	(1.6−1.74)	(1.6−1.81)	(1.59−1.88)	(1.56−1.76)	1.55−1.9	1.43−2.10
arginine	2.71	2.75	2.74	2.74	2.75	2.74		
	(2.46−2.88)	(2.6−2.95)	(2.6−2.99)	(2.55−3)	(2.58−3.16)	(2.55−2.94)	2.59−3.45	2.15−3.46
aspartic acid	4.19	4.23	4.22	4.22	4.24	4.18		
	(3.9−4.4)	(4.03−4.49)	(4.02−4.59)	(3.94−4.64)	(3.89−4.82)	(3.85−4.45)	3.58−4.94	3.81−6.04
cystine	0.556[e]	0.547	0.56[e]	0.549	0.56[e]	0.532		
	(0.493−0.661)	(0.474−0.645)	(0.489−0.672)	(0.482−0.652)	(0.487−0.66)	(0.458−0.647)	0.429−0.71	0.37−0.81
glutamic acid	6.22	6.26	6.27	6.26	6.28	6.26		
	(5.65−6.78)	(5.82−6.76)	(5.8−6.95)	(5.85−6.92)	(5.74−7.18)	(5.75−6.74)	5.85−7.77	5.84−9.15
glycine	1.63	1.65	1.64	1.65	1.65	1.64		
	(1.51−1.73)	(1.58−1.73)	(1.55−1.75)	(1.56−1.84)	(1.54−1.87)	(1.53−1.75)	1.55−1.89	1.41−2.00
histidine	1.02	1.04	1.02	1.03	1.03	1.02		
	(0.91−1.1)	(0.957−1.11)	(0.964−1.13)	(0.935−1.17)	(0.946−1.19)	(0.943−1.1)	0.197−1.18	0.86−1.24
isoleucine	1.81	1.83	1.81	1.83	1.82	1.81		
	(1.64−1.94)	(1.67−2.02)	(1.58−1.93)	(1.68−2.12)	(1.64−2.06)	(1.66−1.94)	1.68−2.18	1.49−2.08
leucine	2.83	2.87	2.85	2.86	2.86	2.84		
	(2.62−2.99)	(2.72−3.06)	(2.67−3.06)	(2.69−3.2)	(2.67−3.25)	(2.65−3.02)	2.68−3.32	2.2−4.0
lysine	2.48	2.53	2.48	2.49	2.49	2.46		
	(2.11−2.76)	(2.14−2.76)	(2.22−2.8)	(2.06−2.97)	(2.12−3.03)	(2.1−2.77)	2−3.04	2.19−3.32
methionine	0.502	0.505	0.501	0.499	0.507	0.504		
	(0.435−0.595)	(0.449−0.567)	(0.456−0.555)	(0.445−0.564)	(0.442−0.621)	(0.447−0.577)	0.418−0.596	0.39−0.68
phenylalanine	1.89	1.91	1.9	1.91	1.91	1.9		
	(1.72−2.02)	(1.8−2.04)	(1.77−2.05)	(1.77−2.13)	(1.77−2.18)	(1.76−2.04)	1.8−2.28	1.6−2.44
proline	1.95	1.95	1.95	1.96	1.95	1.96		
	(1.81−2.13)	(1.84−2.09)	(1.67−2.12)	(1.76−2.19)	(1.77−2.34)	(1.77−2.12)	1.78−2.41	1.63−2.28
serine	1.80	1.79	1.82	1.82	1.83	1.79		
	(1.58−1.96)	(1.62−1.97)	(1.62−2)	(1.64−1.97)	(1.6−2.1)	(1.62−1.98)	1.56−2.2	1.11−2.48
threonine	1.51	1.52	1.52	1.53	1.53	1.52		
	(1.38−1.59)	(1.42−1.59)	(1.43−1.6)	(1.46−1.6)	(1.42−1.75)	(1.43−1.58)	1.4−1.75	1.14−1.89
tryptophan	0.588	0.589	0.575	0.583	0.583	0.574		
	(0.521−0.739)	(0.515−0.676)	(0.512−0.641)	(0.505−0.699)	(0.517−0.645)	(0.512−0.667)	0.495−0.704	0.30−0.67
tyrosine	1.44	1.46	1.45	1.45	1.45	1.44		
	(1.35−1.52)	(1.39−1.54)	(1.38−1.55)	(1.38−1.61)	(1.35−1.62)	(1.36−1.51)	1.34−1.64	0.79−1.61
valine	1.85	1.86	1.85	1.86	1.85	1.85		
	(1.71−1.96)	(1.68−2.03)	(1.61−1.96)	(1.71−2.14)	(1.7−2.01)	(1.72−1.98)	1.71−2.16	1.5−2.44

[a]dwt = dry weight. [b]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single replicate plots. [c]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [d]See Materials and Methods for reference citations. [e]Statistically significant difference with isoline ($P < 0.05$).

Table 4. Fatty Acid Composition of Grain from DAS-444Ø6-6 Soybean

component (% total fatty acid)	DAS-444Ø6-6 mean (min−max)[a]	DAS-444Ø6-6 + 2,4-D mean (min−max)[a]	DAS-444Ø6-6 + glufosinate mean (min−max)[a]	DAS-444Ø6-6 + glyphosate mean (min−max)[a]	DAS-444Ø6-6 + three herbicides mean (min−max)[a]	isoline (Maverick) mean (min−max)[a]	reference min−max[b]	literature min−max[c]
16:0 palmitic	10.7[d]	10.7[d]	10.7[d]	10.7[d]	10.6[d]	10.9		
	(10.21−11.02)	(10.24−11.11)	(10.07−11.06)	(10.25−11.2)	(10.05−11.03)	(10.4−12.55)	9.5−11.31	1.40−15.77
18:0 stearic	4.47	4.47	4.48	4.48	4.52	4.51		
	(3.96−4.93)	(4.05−4.89)	(4.07−5.04)	(4.08−4.96)	(4.11−4.95)	(3.88−5)	3.28−4.98	0.50−5.88
18:1 oleic	21.4[d]	21.5[d]	21.8[d]	21.7[d]	21.9[d]	23.5		
	(18.4−23.7)	(18.8−25.9)	(18.9−26)	(19.1−26.3)	(19.2−26.3)	(20.8−28.3)	18.1−27.9	2.60−45.68
18:2 linoleic	54.8[d]	54.6[d]	54.4[d]	54.5[d]	54.2[d]	53.0		
	(53.4−56.8)	(52.6−56.5)	(52.5−56.1)	(52.1−56.2)	(51.7−56.2)	(50.9−54.5)	50.1−56.7	7.58−58.8
18:3 linolenic	7.77[d]	7.79[d]	7.86[d]	7.76[d]	7.92[d]	7.32		
	(5.56−9.38)	(5.33−9.29)	(5.48−9.47)	(5.46−9.42)	(5.38−9.48)	(5.03−8.88)	4.83−9.82	1.27−12.52
20:0 arachidic	0.323	0.323	0.325	0.323	0.327	0.328		
	(0.29−0.353)	(0.293−0.358)	(0.289−0.366)	(0.29−0.359)	(0.296−0.357)	(0.298−0.39)	0.254−0.427	0.038−2.09
20:1 eicosenoic	0.171	0.171	0.171	0.172	0.168	0.169		
	(0−0.239)	(0−0.254)	(0−0.247)	(0−0.24)	(0−0.239)	(0−0.254)	0−0.272	0.024−0.350
22:0 behenic	0.332	0.331	0.332	0.328	0.335[d]	0.326		
	(0.303−0.368)	(0.298−0.371)	(0.299−0.367)	(0.294−0.365)	(0.309−0.371)	(0.273−0.365)	0.29−0.454	0.043−0.595

[a]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single replicate plots. [b]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [c]See Materials and Methods for reference citations. [d]Statistically significant difference with isoline ($P < 0.05$).

Table 5. Vitamin Composition of Grain from DAS-444Ø6-6 Soybean

component (mg/kg)	DAS-444Ø6-6 mean (min−max)[a]	DAS-444Ø6-6 + 2,4-D mean (min−max)[a]	DAS-444Ø6-6 + glufosinate mean (min−max)[a]	DAS-444Ø6-6 + glyphosate mean (min−max)[a]	DAS-444Ø6-6 + three herbicides mean (min−max)[a]	isoline (Maverick) mean (min−max)[a]	reference min−max[b]	literature min−max[c]
vitamin B_1	3.42	3.46	3.32	3.38	3.55	3.64		
thiamin	(2.2−4.75)	(2.35−4.5)	(1.14−5.12)	(2.03−5.01)	(2.13−5.54)	(2.28−5.87)	1.65−5.48	1.01−2.54
vitamin B_2	3.90	3.88	3.99	3.88	3.77	3.99		
riboflavin	(2.99−4.81)	(3.03−4.91)	(2.98−4.71)	(2.64−5.1)	(2.32−4.88)	(3.04−4.97)	2.72−4.76	1.90−3.21
vitamin B_3	26.2	26.5	26.3	26.6	26.4	26.5		
niacin	(19.2−32.8)	(22.9−34.3)	(21.8−34.1)	(22.9−36.8)	(22.6−35.4)	(22.5−33.8)	20.1−33	NR[d]
vitamin B_5	15.9	15.6	15.3	15.6	15.8	15.4		
pantothenic acid	(12.3−20.5)	(8.29−20.4)	(13−19.8)	(12.3−21.2)	(12.9−20.3)	(12.5−20.1)	9.55−18.1	NR[d]
vitamin B_6	4.85	4.86	4.79	4.94	4.93	4.89		
pyridoxine	(3.68−5.7)	(3.98−5.95)	(4.19−5.85)	(4.15−5.88)	(4.08−6.19)	(3.95−5.81)	2.77−6.2	NR[d]
vitamin B_9	4.09	4.03	4.02	4.07	3.92[e]	4.29		
folic acid	(2.72−5.46)	(2.63−5.85)	(2.5−5.84)	(2.88−6.02)	(2.57−5.28)	(2.7−5.78)	2.35−5.98	2.386−4.709
vitamin C	107.8	111.1	112.1	112.8	113.6	121.4		
ascorbic acid	(16.5−181)	(17.6−194)	(16.8−173)	(23.8−193)	(25.3−171)	(0−198)	0−141	NR[d]
α-tocopherol	22.2	22.4	22.2	21.9	22.2	18.6		
	(10.9−69)	(11−56.8)	(10.1−106)	(10.9−55.7)	(8.85−76.2)	(10.5−46)	6.43−49.9	0.108−61.693
γ-tocopherol	185[e]	184[e]	179	183[e]	181	174		
	(157−224)	(154−220)	(99−214)	(153−217)	(116−227)	(88.4−208)	116−215	NR[d]
δ-tocopherol	72.0	71.5	73.8	72.1	72.2	73.3		
	(40.4−94.3)	(35.5−94.6)	(40.5−99.6)	(40−98.2)	(31.5−96.1)	(22.5−96.8)	40−114	NR[d]
total tocopherol	279[e]	278[e]	275	277[e]	276	266		
	(244−316)	(252−306)	(161−375)	(248−308)	(196−330)	(132−305)	199−321	NR[d]

[a]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single replicate plots. [b]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [c]See Materials and Methods for reference citations. [d]NR = not reported. [e]Statistically significant difference with isoline ($P < 0.05$).

dx.doi.org/10.1021/jf403775d | J. Agric. Food Chem. 2013, 61, 11180−11190

Table 6. Antinutrient and Isoflavone Composition of Grain from DAS-444Ø6-6 Soybean

component	DAS-444Ø6-6 mean (min−max)[a]	DAS-444Ø6-6 + 2,4-D mean (min−max)[a]	DAS-444Ø6-6 + glufosinate mean (min−max)[a]	DAS-444Ø6-6 + glyphosate mean (min−max)[a]	DAS-444Ø6-6 + three herbicides mean (min−max)[a]	isoline (Maverick) mean (min−max)[a]	reference min−max[b]	literature min−max[c]
lectin	107[e]	94	94	92	99[e]	79		
(HU[d]/mg protein)	(0.6−228)	(39.64−188)	(56.3−146)	(44.8−151)	(31−196)	(27.9−153)	18.5−144	37−323
phytic acid	1.18	1.19	1.18	1.18	1.19	1.19		
(% dwt[d])	(0.679−1.53)	(0.65−1.59)	(0.603−1.51)	(0.707−1.46)	(0.651−1.55)	(0.513−1.53)	0.55−1.54	0.41−2.74
raffinose	0.80	0.80	0.80	0.80	0.79	0.82		
(% dwt)	(0.556−1.22)	(0.581−1.18)	(0.569−1.22)	(0.438−1.3)	(0.478−1.23)	(0.497−1.29)	0.569−1.4	0.212−1.62
stachyose	3.88	3.87	3.86	3.83	3.89	3.88		
(% dwt)	(3.38−4.11)	(3.21−4.26)	(2.95−4.29)	(2.77−4.18)	(3.08−4.38)	(2.98−4.22)	2.92−4.48	1.21−6.1
trypsin inhibitor	35.0	35.5[e]	36.6[e]	33.6	34.2	30.8		
(TIU[d]/mg)	(19−56)	(17.4−71)	(21.1−78.9)	(21.3−51.7)	(21.8−62.6)	(18.4−54.6)	15.6−59.7	18.14−118.68
daidzein	777	799	800	771	781	809		
(μg/g)	(175−1420)	(179−1470)	(182−1510)	(124−1490)	(149−1430)	(186−1450)	153−1710	25−2453.5
genistein	863	870	877	848	855	890		
(μg/g)	(300−1730)	(251−1690)	(264−1720)	(215−1770)	(186−1700)	(267−1670)	205−1980	28−2837.2
glycitein	459	465	452	448	443	453		
(μg/g)	(237−1250)	(223−1290)	(223−1340)	(197−1250)	(212−1270)	(222−1300)	85.2−1630	15.3−349.19

[a]Mean across 10 sites with four replicates at each site; minimum (min) and maximum (max) values represent values from single replicate plots. [b]Minimum and maximum values observed from six commercially available varieties planted in a balanced incomplete-block design across sites with three varieties at each site. [c]See Materials and Methods for reference citations. [d]HU = hemagglutinating units, TIU = trypsin inhibitor units, dwt = dry weight. [e]Statistically significant difference with isoline ($P < 0.05$).

dwt) or glyphosate (320 mg/100 g dwt)], and potassium was present in slightly higher concentrations in all DAS-444Ø6-6 entries (1770−1790 mg/100 g dwt) than in the isoline (calcium, 301 mg/100 g dwt; potassium, 1730 mg/100 g dwt). However, values for calcium and potassium were similar to those observed for nontransgenic soybean (Table 2).

Of the 18 amino acids examined in the present study, only cystine levels in grain from DAS-444Ø6-6 entries (unsprayed and sprayed with glufosinate or all herbicides applied) differed statistically from the isoline (Table 3). Cystine levels were slightly higher in these DAS-444Ø6-6 entries (0.6% dwt each) compared with the isoline (0.5% dwt); however, all entries were similar to levels observed for nontransgenic soybean. Marginally higher amino acid levels and, consequently, higher crude protein levels have been observed for transgenic maize,[2] which likely resulted from event selection processes following transformation, rather than transformation itself. Plants that are derived from single plant selections of soybean, as are transgenic events during event selection, can differ markedly from parent lines in basic agronomic traits due to normal intracultivar variation.[25]

Fatty acid levels for grain were similar between DAS-444Ø6-6 entries and the isoline for all fatty acids examined, although palmitic [10.6−10.7% total fatty acid (TFA)] and oleic acid (21−22% TFA) were slightly lower and linoleic (54−55% TFA) and linolenic acid (8% TFA) were slightly higher in DAS-444Ø6-6 entries compared with the isoline (palmitic, 10.9%; oleic, 24%; linoleic, 53%; linolenic, 7% TFA) (Table 4). Differences between DAS-444Ø6-6 entries and the isoline across these four major soybean fatty acids were approximately 5%, indicating a small amount of variation between transgenic and nontransgenic entries. An isolated difference was also observed for behenic acid, for which values were slightly higher in DAS-444Ø6-6 plots to which all herbicides were applied (0.34% TFA) compared with the isoline (0.33% TFA). Despite small differences, fatty acid levels for DAS-444Ø6-6 entries and the isoline were similar to those observed for nontransgenic soybean (Table 4).

The majority of the vitamin and tocopherol levels examined for grain were found to be similar between DAS-444Ø6-6 entries and the isoline (Table 5). An isolated exception was observed for vitamin B_9 (folic acid), which was found to be slightly lower in DAS-444Ø6-6 plots to which all herbicides were applied (3.9 mg/kg dwt) compared with the isoline (4.3 mg/kg dwt). The observation was not supported by the remaining four DAS-444Ø6-6 entries, and mean values were similar to those observed for nontransgenic soybean, suggesting differences were not due to genotype or herbicide treatments. Among the tocopherols, γ-tocopherol levels were slightly higher in DAS-444Ø6-6 entries (179−185 mg/kg dwt) compared with the isoline (174 mg/kg dwt), which is reflected in the trend observed for total tocopherol (Table 5), for which values for DAS-444Ø6-6 entries were similar to those from commercial soybean lines within the present study and similar to commercial soybean varieties examined in previous years.[9]

Levels for antinutrients and bioactive compounds in grain were similar between DAS-444Ø6-6 entries and the isoline for each of the analytes examined (lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor) (Table 6). Statistically higher values were observed for lectin in two of five DAS-444Ø6-6 entries [unsprayed, 107 hemagglutination units (HU)/mg protein; all herbicides applied, 99 HU/mg protein] compared with the isoline (79 HU/mg protein) and for trypsin inhibitor in two of five DAS-444Ø6-6 entries [sprayed with 2,4-D, 36 trypsin inhibitor units (TIU)/mg dwt; sprayed with glufosinate, 37 TIU/mg dwt] compared with the isoline (31 TIU/mg dwt). The observations for higher lectin and trypsin inhibitor content were not supported by the remaining three DAS-444Ø6-6 entries in each case, and mean values were similar to those observed for commercial lines within the study and within the literature range for nontransgenic soybean. Additionally, bioactive components including lectin and trypsin inhibitor are inactivated during standard processing of soybean grain prior to consumption.[13,14,26,27]

Isoflavone content (total daidzein, genistein, and glycitein equivalents) in grain was similar between DAS-444Ø6-6 entries and the isoline; no statistical differences were observed, and values for DAS-444Ø6-6 entries were similar to those observed for nontransgenic soybean (Table 6). Isoflavones, in particular, exhibit greater variation than many compositional end points.[19] Variation across geographies was well illustrated by the site-specific stratification of values for each isoflavone (Figure 1). For total glycitein content, 2 of the 10 sites exhibited values that significantly broaden the literature range for this analyte. These higher levels were consistent across all entries planted at each site; therefore, values were not deemed to be outliers. To facilitate figure interpretation, it is noteworthy to consider that as part of the balanced incomplete-block design, each commercial, nontransgenic reference entry received only half the replication of the remaining entries across all sites. The lower level of replication for the reference lines is expected to limit the distribution of plotted values compared with the transgenic and isoline entries.

The similar composition of event DAS-444Ø6-6 and commercial nontransgenic soybean support a conclusion of substantial equivalence of DAS-444Ø6-6 with conventional soybean. Cultivation of DAS-444Ø6-6 soybean under management practices including the application of conventional soybean pesticides (fungicides, insecticides, selective herbicides) and broad-spectrum herbicides designed for use with DAS-444Ø6-6 is expected to have no impact on the compositional safety of soybean products.

In the present study, the influence of environment is well illustrated through the variation in isoflavone values between sites, despite the finding of no differences across sites (Figure 1). The levels for minerals, vitamins, and isoflavones reported from the nontransgenic crop demonstrate that current literature ranges could benefit from a broader sampling of environments and germplasm. Composition studies have thoroughly demonstrated that effects due to geographic region, growing season, germplasm/variety selection, and plant stress factors are distinctly stronger than those due to transgene insertion and related breeding processes.[2,3,9,19,28−30]

The detection of some statistically significant, but small-magnitude, differences between the near-isoline and transgenic entries attests to the power of the experimental design and analytical methods. The absence of consistent differences across transgenic entries, the negligible magnitude of these differences, and the finding that results for DAS-444Ø6-6 fall within the range of values reported for nontransgenic varieties indicate no biologically relevant compositional differences between DAS-444Ø6-6 and nontransgenic soybean. The common observation of random differences and/or biologically nonrelevant differences in composition studies is further supported for DAS-444Ø6-6 through a cross-examination of the results presented here with those of a different soybean event (DAS-

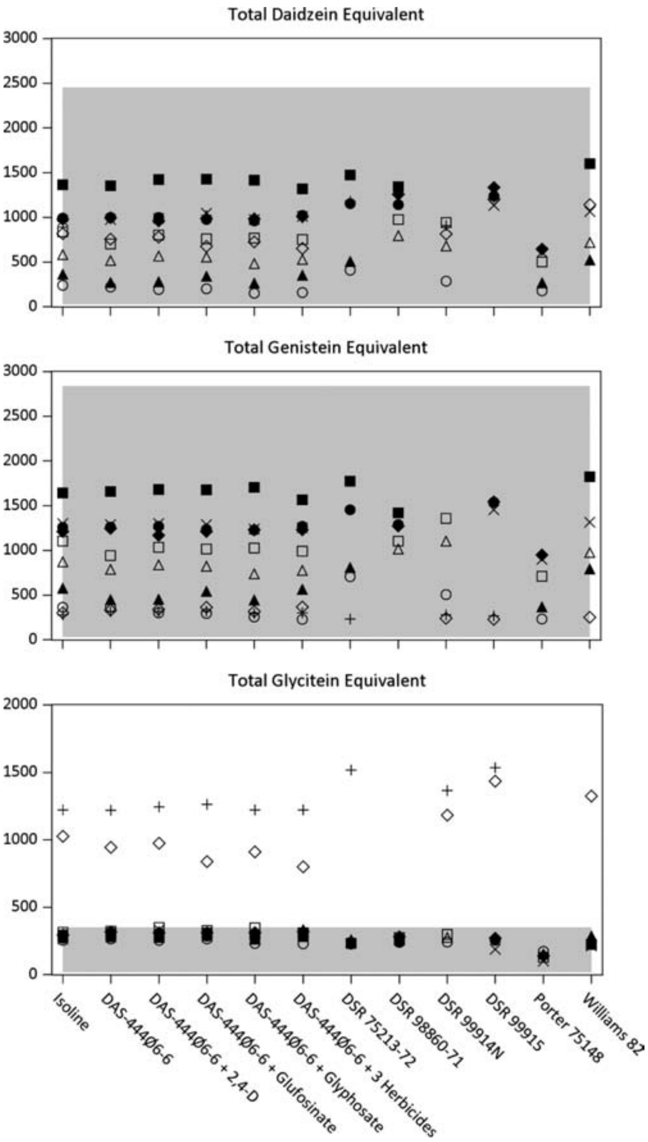

Figure 1. Site-specific isoflavone levels in soybean grain observed for a nontransgenic near-isogenic variety (Maverick), unsprayed DAS-444Ø6-6 (AAD-12 + 2mEPSPS + PAT), 2,4-D-treated DAS-444Ø6-6, glufosinate-treated DAS-444Ø6-6, glyphosate-treated DAS-444Ø6-6, 2,4-D + glufosinate + glyphosate-treated DAS-444Ø6-6, and commercially available varieties [Dairyland (DSR) 75213-72, DSR 98860-71, DSR 99914N, DSR 99915, Porter 75148, and Williams 82]. Commercial varieties were planted at half the number of sites as the remaining entries. The shaded area spans the published values for each analyte, and levels are expressed as micrograms per gram dry weight. Symbols in the plots are as follows: ○, Sycamore (GA); ×, Richland (IA); +, Bagley (IA); △, Carlyle (IL); □, Wyoming (IL); ◇, Sheridan (IN); ●, Deerfield (MI); ▲, Fisk (MO); ■, Brunswick (NE); ◆, York (NE).

68416-4) expressing the AAD-12 and PAT proteins,[9] in which no consistent trends in compositional differences were found using the same nontransgenic, near-isogenic variety (Maverick) used in the present study. The value of conducting composition studies to detect unintended effects due to transgene insertion is diminished in light of the overwhelming evidence that such effects are often small in magnitude, especially when compared to traditional breeding, and contribute little biologically relevant information to inform the risk assessment for novel crop traits.

AUTHOR INFORMATION

Corresponding Author

*(M.D.L.) Phone: (317) 337-4587. Fax: (317) 337-7055. E-mail: mdlepping@dow.com.

Notes

The authors declare the following competing financial interest(s): MDL & RAH are employees of Dow AgroSciences LLC, a wholly owned subsidiary of The Dow Chemical Company, which develops transgenic crops and produces insecticides, herbicides, and fungicides for agricultural applications and residential pest control. BLP is employed by Covance Laboratories Inc. which performed the compositional analysis under contract from Dow AgroSciences LLC.

ACKNOWLEDGMENTS

We thank Jason Dority, Rebecca Hall, Tempest Johnson, and Michelle Mayes for study management; the field teams led by principal investigators David Bennett (Bennett Agricultural Research Corp.), Fritz Koppatschek (ABG Ag Services), Dan Easton (Easton Agri-Consulting, Inc.), Nathan Goldschmidt (Shoffner Farm Research, Inc.), Matt Krause, Eric Ehlers, Sue Dorsey, Tim Boeker, Chris Cromer, and Chad Harris (SGS North America) for sample production; the analytical teams of Covance Laboratories for composition analysis; the Quality Assurance groups of Covance Laboratories and Dow AgroSciences; and Alyssa Brune, John Cuffe, Brandon Fast, Mark Krieger, Siva Kumpatla, Barry Schafer, Guomin Shan, Monica Sorribas, Nicholas Storer, and Laura Tagliani for reviewing a draft of the paper.

REFERENCES

(1) Food and Agriculture Organization (FAO)/World Health Organization (WHO). *Codex Alimentarius: Foods Derived from Biotechnology*; FAO/WHO: Rome, Italy, 2009.

(2) 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.

(3) Berman, K. H.; Harrigan, G. G.; Nemeth, M. A.; Oliveira, W. S.; Berger, G. U.; Tagliaferro, F. S. Compositional equivalence of insect-protected glyphosate-tolerant soybean MON 87701 × MON 89788 to conventional soybean extends across different world regions and multiple growing seasons. *J. Agric. Food Chem.* **2011**, *59* (21), 11643−11651.

(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) 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.

(6) Herouet-Guicheney, C.; Rouquié, D.; Freyssinet, M.; Currier, T.; Martone, A.; Zhou, J.; Bates, E. E. M.; Ferullo, J.-M.; Hendrickx, K.; Rouan, D. Safety evaluation of the double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) from maize that confers tolerance to glyphosate herbicide in transgenic plants. *Regul. Toxicol. Pharmacol.* **2009**, *54*, 143−153.

(7) Herouet, C.; Esdaile, D. J.; Mallyon, B. A.; Debruyne, E.; Schulz, A.; Currier, T.; Hendrickx, K.; Klis, R.-J. v. d.; Rouan, D. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 134−149.

(8) Green, J. M.; Castle, L. A. Transitioning from single to multiple herbicide-resistant crops. In *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*; Nandula, V. K., Ed.; Wiley: Hoboken, NJ, USA, 2010.
(9) 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*, 1−15.
(10) Ridley, W. P.; Shillito, R. D.; Coats, I.; Steiner, H.-Y.; Shawgo, M.; Phillips, A.; Dussold, P.; Kurtyka, L. Development of the International Life Sciences Institute Crop Composition Database. *J. Food Compos. Anal.* **2004**, *17*, 423−438.
(11) Iskander, F. Y. Maturation stage and mineral content in soybeans. *Food Chem.* **1987**, *24*, 29−35.
(12) Hartwig, E. E.; Kilen, T. C. Yield and composition of soybean seed from parents with different protein, similar yield. *Crop Sci.* **1991**, *31*, 290−292.
(13) Padgette, S. R.; Taylor, N. B.; Nida, D. L.; Bailey, M. R.; MacDonald, J.; Holden, L. R.; Fuchs, R. L. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J. Nutr.* **1996**, *126*, 702−716.
(14) Organization of European Cooperation and Development (OECD). *Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [Glycine max (L.) Merr.]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens*; OECD: Paris, France, 2012; series on the safety of novel foods and feeds, OECD ENV/JM/MONO (2012) 24.
(15) McCann, M. C.; Liu, K.; Trujillo, W. A.; Dobert, R. C. Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. *J. Agric. Food Chem.* **2005**, *53*, 5331−5335.
(16) Bilyeu, K. D.; Zeng, P.; Coello, P.; Zhang, Z. J.; Krishnan, H. B.; Bailey, A.; Beuselinck, P. R.; Polacco, J. C. Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant Physiol.* **2008**, *146*, 468−477.
(17) Berman, K. H.; Harrigan, G. G.; Riordan, S. G.; Nemeth, M. A.; Hanson, C.; Smith, M.; Sorbet, R.; Zhu, E.; Ridley, W. P. Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. *J. Agric. Food Chem.* **2009**, *57*, 11360−11369.
(18) Berman, K. H.; Harrigan, G. G.; Riordan, S. G.; Nemeth, M. A.; Hanson, C.; Smith, M.; Sorbet, R.; Zhu, E.; Ridley, W. P. Compositions of forage and seed from second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 from Brazil are equivalent to those of conventional soybean (*Glycine max*). *J. Agric. Food Chem.* **2010**, *58*, 6270−6276.
(19) Harrigan, G. G.; Glenna, K. C.; Ridley, W. P. Assessing the natural variability in crop composition. *Regul. Toxicol. Pharmacol.* **2010**, *58*, S13−S20.
(20) Harrigan, G. G.; Ridley, W. P.; Riordan, S. G.; Nemeth, M. A.; Sorbet, R.; Trujillo, W. A.; Breeze, M. L.; Schneider, R. W. Chemical composition of glyphosate-tolerant soybean 40-3-2 grown in Europe remains equivalent with that of conventional soybean (*Glycine max* L.). *J. Agric. Food Chem.* **2007**, *55*, 6160−6168.
(21) *SAS/STAT, version 9.2 User's Guide*, 2nd ed.; SAS Institute Inc.: Cary, NC, USA, 2009.
(22) Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B: Stat. Methodol.* **1995**, *57*, 289−300.
(23) Curran-Everett, D. Multiple comparisons: philosophies and illustrations. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2000**, *279*, R1−R8.
(24) Herman, R. A.; Storer, N. P.; Phillips, A. M.; Prochaska, L. M.; Windels, P. Compositional assessment of event DAS-59122-7 maize using substantial equivalence. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 37−47.
(25) Fasoula, V. A.; Boerma, R. Intra-cultivar variation for seed weight and other agronomic traits within three elite soybean cultivars. *Crop Sci.* **2007**, *47*, 367−373.
(26) Rackis, J. Biological and physiological factors in soybeans. *J. Am. Oil Chem. Soc.* **1974**, *51*, 161A−174A.
(27) Hammond, B. G.; Jez, J. M. Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and corn crops. *Food Chem. Toxicol.* **2011**, *49*, 711−721.
(28) Lundry, D. R.; Ridley, W. P.; Meyer, J. J.; Riordan, S. G.; Nemeth, M. A.; Trujillo, W. A.; Breeze, M. L.; Sorbet, R. Composition of grain, forage, and processed fractions from second-generation glyphosate-tolerant soybean, MON 89788, is equivalent to that of conventional soybean (*Glycine max* L.). *J. Agric. Food Chem.* **2008**, *56*, 4611−4622.
(29) Zhou, J.; Berman, K. H.; Breeze, M. L.; Nemeth, M. A.; Oliveira, W. S.; Braga, D. P. V.; Berger, G. U.; Harrigan, G. G. Compositional variability in conventional and glyphosate-tolerant soybean (*Glycine max L.*) varieties grown in different regions in Brazil. *J. Agric. Food Chem.* **2011**, *59*, 11652−11656.
(30) Lundry, D. R.; Burns, J. A.; Nemeth, M. A.; Riordan, S. G. Composition of grain and forage from insect-protected and herbicide-tolerant corn, MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax), is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2013**, *61*, 1991−1998.