

# Compositional Equivalence of Insect-Protected Glyphosate-Tolerant Soybean MON 87701 × MON 89788 to Conventional Soybean Extends across Different World Regions and Multiple Growing Seasons

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**S** Supporting Information

**ABSTRACT:** The soybean product MON 87701 × MON 89788 expresses both the *cry1Ac* gene derived from *Bacillus thuringiensis* and the *cp4 epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene derived from *Agrobacterium* sp. strain CP4. Each biotechnology-derived trait confers specific benefits of insect resistance and glyphosate tolerance, respectively. The purpose of this study was to compare the composition of seed and forage from this combined-trait product to those of conventional soybean grown in geographically and climatically distinct regions. Field trials were conducted in the United States during the 2007 growing season, in Argentina during the 2007–2008 growing season, and in the northern and southern soybean regions of Brazil during the 2007–2008 and 2008–2009 growing seasons. Results demonstrated that the compositional equivalence of MON 87701 × MON 89788 to the conventional soybean extended across all regions and growing seasons. Further evaluation of the data showed that natural variation (region and growing season) contributed more to compositional variability in soybean, particularly for such components as isoflavones, fatty acids, and vitamin E, than transgene insertion.

**KEYWORDS:** soybean (*Glycine max*), insect-resistant, glyphosate-tolerant, biotechnology, composition

## INTRODUCTION

Two Monsanto Co. biotechnology-derived soybean products are designed to offer benefits in weed and pest management. These are second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701. MON 89788 contains the *cp4 epsps* (5-enolpyruvylshikimate-3-phosphate synthase) gene derived from *Agrobacterium* sp. strain CP4. MON 87701 contains the *cry1Ac* gene derived from *Bacillus thuringiensis*, and expression of the Cry1Ac protein provides protection from feeding damage caused by certain lepidopteran insect pests. MON 87701 × MON 89788 contains both *cry1Ac* and *cp4 epsps* genes, and this combination of the two traits in a single soybean offers simplified pest management control.

Compositional assessments of the single-trait products, MON 89788 and MON 87701, have been conducted previously.<sup>1,2</sup> The purpose of this study was to compare the composition of seed and forage from MON 87701 × MON 89788 to those of a near-isogenic conventional soybean grown in four geographically and climatically distinct regions. Field trials were conducted in the United States during the 2007 growing season, in Argentina during the 2007–2008 growing season, and in the northern and southern soybean regions of Brazil during the 2007–2008 and 2008–2009 growing seasons. All field trials included a range of commercially available soybean varieties to complement information on compositional variability currently in the scientific literature. Compositional analyses included measurement of essential macro- and micronutrients, known toxicants and antinutrients, and selected secondary metabolites in harvested seed, as well as

measurement of proximates in both forage and harvested seed. These components were selected on the basis of internationally accepted guidelines proposed by the Organisation of Economic Cooperation and Development (OECD) for the assessment of new crops (see refs 1–3).

## MATERIALS AND METHODS

**Soybean Samples for Compositional Analyses.** Forage and seed samples were collected from MON 87701 × MON 89788, conventional controls, and commercial soybean varieties (called reference substances) grown in the United States during the 2007 growing season, in Argentina during the 2007–2008 growing season, and in the northern and southern soybean regions of Brazil, respectively, for maturity group 8 and 5 materials during the 2007–2008 and 2008–2009 growing seasons. The geographic regions selected for the United States, Argentina, and Brazil field trials were representative of major commercial growing areas in both the northern and southern hemispheres. The U.S. field production comprised five replicated sites: these were in Baldwin County, Alabama; Jackson County, Arkansas; Clarke County, Georgia; Jackson County, Illinois; and Wayne County, North Carolina. The Argentinean 2007–2008 field trial included three replicated sites in the province of Buenos Aires (Tacuari, Gahan, and Berdier), and one replicated site in each of the provinces of Córdoba (Alejo Ledesma) and Santa Fe

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**Table 1. Protein and Amino Acid Composition of Seed from MON 87701 × MON 89788 (Test) Grown in the United States and Argentina**

component <sup>a</sup>	USA (2007)			Argentina (2007–2008)		
	test	control	commercial	test	control	commercial
	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>
protein	39.75 (0.86) <sup>d</sup>	37.80 (0.86)		38.42 (0.38)	37.89 (0.38)	
	37.29–43.02	32.29–41.87	38.14–42.66	36.74–39.94	36.05–39.20	34.70–42.19
alanine	1.74 (0.029) <sup>d</sup>	1.69 (0.029)		1.68 (0.020)	1.62 (0.021)	
	1.68–1.92	1.59–1.82	1.66–1.93	1.59–1.81	1.43–1.75	1.43–1.87
arginine	2.67 (0.069)	2.58 (0.069)		2.76 (0.061)	2.67 (0.062)	
	2.38–2.91	2.37–2.89	2.54–2.99	2.50–3.36	2.34–2.97	2.15–3.05
aspartic acid	5.02 (0.10) <sup>d</sup>	4.85 (0.10)		4.99 (0.093)	4.75 (0.095)	
	4.73–5.59	4.46–5.34	4.74–5.50	4.63–5.54	4.01–5.18	4.18–5.72
cystine/cysteine	0.63 (0.014)	0.61 (0.014)		0.60 (0.017)	0.57 (0.018)	
	0.49–0.74	0.56–0.69	0.53–0.68	0.52–0.68	0.46–0.69	0.41–0.71
glutamic acid	7.82 (0.15) <sup>d</sup>	7.53 (0.15)		7.61 (0.16) <sup>d</sup>	7.19 (0.16)	
	7.42–8.74	6.89–8.26	7.53–8.72	7.09–8.49	5.49–7.82	6.20–8.62
glycine	1.75 (0.026) <sup>d</sup>	1.70 (0.026)		1.68 (0.026)	1.63 (0.027)	
	1.64–1.91	1.64–1.85	1.67–1.99	1.57–1.95	1.42–1.77	1.41–1.88
histidine	1.11 (0.014) <sup>d</sup>	1.08 (0.015)		1.06 (0.020)	1.04 (0.021)	
	1.04–1.20	1.03–1.15	1.04–1.24	0.95–1.26	0.90–1.19	0.86–1.16
isoleucine	1.82 (0.037) <sup>d</sup>	1.76 (0.037)		1.76 (0.029)	1.69 (0.030)	
	1.70–2.03	1.64–1.96	1.73–2.02	1.66–1.96	1.41–1.84	1.49–1.92
leucine	3.05 (0.065) <sup>d</sup>	2.94 (0.066)		2.91 (0.042) <sup>d</sup>	2.81 (0.043)	
	2.84–3.36	2.73–3.29	2.93–3.32 <sup>c</sup>	2.73–3.23	2.41–3.01	2.39–3.15
lysine	2.75 (0.059) <sup>d</sup>	2.62 (0.060)		2.58 (0.044)	2.49 (0.045)	
	2.51–3.04	2.42–2.91	2.35–3.15	2.33–2.87	2.22–2.75	2.19–3.00
methionine	0.54 (0.011)	0.53 (0.012)		0.52 (0.013)	0.50 (0.014)	
	0.45–0.63	0.47–0.59	0.49–0.62	0.44–0.66	0.42–0.59	0.39–0.65
phenylalanine	2.13 (0.056)	2.04 (0.056)		1.96 (0.037)	1.90 (0.038)	
	1.91–2.37	1.91–2.38	1.97–2.44	1.82–2.26	1.63–2.10	1.62–2.14
proline	2.02 (0.035) <sup>d</sup>	1.96 (0.035)		2.00 (0.029)	1.93 (0.029)	
	1.88–2.22	1.85–2.12	1.83–2.19	1.87–2.23	1.71–2.06	1.63–2.18
serine	2.04 (0.032) <sup>d</sup>	1.96 (0.032)		1.96 (0.032)	1.89 (0.033)	
	1.88–2.22	1.87–2.13	1.95–2.27	1.85–2.26	1.51–2.06	1.63–2.18
threonine	1.60 (0.020) <sup>d</sup>	1.55 (0.020)		1.52 (0.024)	1.47 (0.024)	
	1.51–1.71	1.49–1.68	1.49–1.71	1.41–1.76	1.23–1.60	1.28–1.72
tryptophan	0.51 (0.0067)	0.50 (0.0068)		0.51 (0.0057)	0.49 (0.0058)	
	0.47–0.55	0.46–0.53	0.39–0.48	0.47–0.52	0.41–0.51	0.45–0.56
tyrosine	1.11 (0.034)	1.10 (0.034)		1.03 (0.032)	1.01 (0.033)	
	1.01, 1.25	0.98, 1.22	1.04–1.31	0.88–1.24	0.74–1.22	0.79–1.25
valine	1.92 (0.032) <sup>d</sup>	1.86 (0.032)		1.88 (0.033)	1.81 (0.034)	
	1.81–2.09	1.76–2.04	1.83–2.13	1.77–2.11	1.50–1.94	1.57–2.03

<sup>a</sup> Percent dry weight. <sup>b</sup> The mean, standard error, and range of 15 values (three replicates from each of five field sites). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown at each respective production. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.

(San Francisco). The control variety was A5547, a conventional line that has a genetic background similar to that of MON 87701 × MON 89788 in the U.S. and Argentinean field productions. Commercial soybean varieties included in both U.S. and Argentinean field trials were A5843, A5959, CMA 5804AOC, UA 4805, Ozark, Anand, Hornbeck C5894, A5560, CMC 5901COC, LEE 74, A5403, A4922, H4994, H5218, A5427, DP 5989, Hutcheson, USG 5601T, and Fowler. H6686 was included in the U.S. field

trials, and USG 5002T was included in the Argentinean field trials. Four different commercial varieties were included at each site. At each site, starting seeds were planted in a randomized complete block design with three replicates for each substance. Compositional analysis was conducted on only one biological replicate of the reference substances from the U.S. production.

For the Brazilian production, Monsoy 8329 (maturity group 8) served as the control for MON 87701 × MON 89788 grown in the

Table 2. Protein and Amino Acid Composition of Seed from MON 87701 × MON 89788 (Test) Grown in Brazil

component <sup>a</sup>	Brazil (2007–2008)				Brazil (2008–2009)							
	northern region		southern region		both regions		northern regions		southern regions		both regions	
	test	control	test	control	commercial	test	control	test	control	test	control	commercial
protein	39.71 (0.70) <sup>d</sup>	38.59 (0.70)	38.01 (0.70)	37.72 (0.70)	37.61 (0.55)	37.37 (0.55)	38.17 (0.55) <sup>d</sup>	36.70 (0.55)	37.25–39.26	36.70 (0.55)	34.78–38.46	35.46–41.18
alanine	38.42–40.50	36.86–40.42	37.16–38.93	35.50–39.36	35.95–39.06	36.56–38.51	37.25–39.26	34.78–38.46	1.64 (0.020) <sup>d</sup>	1.66 (0.020) <sup>d</sup>	1.57 (0.020)	
arginine	1.73 (0.026)	1.72 (0.026)	1.70 (0.026)	1.68 (0.026)	1.64 (0.020)	1.65 (0.020)	1.66 (0.020) <sup>d</sup>	1.57 (0.020)	1.59–1.73	1.62–1.68	1.51–1.63	1.55–1.77
aspartic acid	1.67–1.79	1.66–1.85	1.65–1.73	1.59–1.73	1.59–1.72	1.62–1.68	1.62–1.70	1.51–1.63	2.69 (0.061) <sup>d</sup>	2.70 (0.054)	2.61 (0.054)	2.40–3.00
cysteine/cysteine	2.50–2.92	2.44–2.68	2.39–2.61	2.52 (0.062)	2.47–2.88	2.51–2.86	2.45–2.92	2.35–2.78	4.88 (0.074)	4.81 (0.10)	4.50 (0.10)	4.32–5.60
glutamic acid	4.57–5.05	4.57–5.24	4.57–4.89	4.71 (0.076)	4.88 (0.10)	4.81 (0.10)	4.88 (0.10) <sup>d</sup>	4.50 (0.10)	0.57 (0.018)	0.64 (0.046)	0.66 (0.046)	0.45–0.84
glycine	0.50–0.63	0.52–0.61	0.62 (0.018)	0.59 (0.019)	0.61 (0.046)	0.64 (0.046)	0.70 (0.046)	0.66 (0.046)	0.50–0.63	0.52–0.61	0.53–0.80	6.67–8.85
histidine	8.30 (0.12)	8.15 (0.12)	8.04 (0.12)	7.98 (0.13)	7.61 (0.14)	7.53 (0.14)	7.64 (0.14) <sup>d</sup>	7.09 (0.14)	7.91–8.58	7.90–8.28	7.08–8.03	1.64–2.03
isoleucine	1.79 (0.024)	1.75 (0.024)	1.66 (0.024)	1.65 (0.025)	1.82 (0.023)	1.81 (0.023)	1.81 (0.023) <sup>d</sup>	1.72 (0.023)	1.69–1.88	1.73–1.88	1.57–1.80	0.98–1.32
leucine	1.69–1.88	1.66–1.78	1.62–1.71	1.54–1.75	1.75–0.84	1.73–1.88	1.69–1.91	1.57–1.80	1.15 (0.015)	1.12 (0.015)	1.09 (0.019) <sup>d</sup>	1.63–1.98
lysine	1.06–1.27	1.06–1.17	1.03–1.16	1.00–1.11	1.13 (0.019)	1.13 (0.019)	1.16 (0.019) <sup>d</sup>	1.09 (0.019)	1.80–2.23	1.81 (0.023) <sup>d</sup>	1.73 (0.023)	2.75–3.24
methionine	1.89 (0.024)	1.84 (0.024)	1.78 (0.024)	1.76 (0.025)	1.82 (0.023)	1.80 (0.023)	1.81 (0.023) <sup>d</sup>	1.73 (0.023)	2.99 (0.038)	2.94 (0.038)	2.87 (0.038)	2.26–2.77
phenylalanine	1.80–1.95	1.74–1.92	1.70–1.82	1.68–1.85	1.75–1.93	1.76–1.85	1.72–1.90	1.61–1.82	2.85–3.17	2.88–3.04	2.66–3.00	0.34–0.69
proline	3.02–3.17	2.90–3.09	2.91–3.00	2.93 (0.039)	2.99 (0.038)	2.94 (0.038)	3.00 (0.038) <sup>d</sup>	2.87 (0.038)	2.51 (0.047)	2.49 (0.047)	2.35 (0.047)	1.86–2.44
serine	2.93 (0.066)	2.96 (0.066)	2.78 (0.066)	2.77 (0.070)	2.85–3.18	2.88–3.04	2.85–3.17	2.66–3.00	2.36–2.76	2.42–2.56	2.25–2.44	1.90–2.24
threonine	2.61–3.22	2.62–3.32	2.54–2.89	2.59–3.13	2.36–2.76	2.42–2.56	2.34–2.69	2.25–2.44	0.55 (0.023)	0.55 (0.023)	0.54 (0.023)	1.86–2.44
tyrosine	0.49–0.62	0.52–0.59	0.51–0.59	0.47–0.59	0.45–0.63	0.47–0.60	0.47–0.63	0.34–0.69	2.15 (0.034) <sup>d</sup>	2.12 (0.034)	2.05 (0.034)	1.90–2.24
valine	2.10 (0.033)	2.01 (0.033)	1.93 (0.033)	1.93 (0.034)	2.15 (0.034)	2.12 (0.034)	2.15 (0.034) <sup>d</sup>	2.05 (0.034)	2.02–2.23	2.02–2.25	1.97 (0.036)	1.84–2.26
	2.00–2.25	1.90–2.14	1.87–1.98	1.78–2.05	2.00–2.23	2.02–2.25	1.98–2.27	1.83–2.18	1.91–2.18	1.87–2.07	1.80–2.13	1.90–2.24
	2.06 (0.027) <sup>d</sup>	1.99 (0.027)	2.01 (0.027)	1.99 (0.027)	2.05 (0.030)	2.01 (0.030)	2.06 (0.030) <sup>d</sup>	1.95 (0.030)	2.02 (0.036)	1.94 (0.036)	1.97 (0.036)	1.84–2.26
	1.97–2.21	1.93–2.10	1.94–2.03	1.84–2.08	1.96–2.15	1.92–2.09	1.94–2.18	1.77–2.03	1.96–2.15	1.92–2.09	1.94–2.18	1.47–1.77
	1.61 (0.020)	1.58 (0.020)	1.56 (0.020)	1.54 (0.021)	1.60 (0.017)	1.59 (0.017)	1.62 (0.017) <sup>d</sup>	1.54 (0.017)	1.57–1.66	1.51–1.66	1.44–1.59	0.30–0.53
	1.54–1.69	1.54–1.62	1.52–1.60	1.46–1.63	1.57–1.66	1.51–1.66	1.55–1.69	1.44–1.59	0.42 (0.014)	0.44 (0.014)	0.45 (0.014)	0.93–1.50
	0.47 (0.012)	0.47 (0.012)	0.49 (0.012)	0.48 (0.013)	0.42 (0.014)	0.44 (0.014)	0.45 (0.014)	0.30–0.49	0.29–0.46	0.39–0.46	0.36–0.49	1.79–2.18
	0.35–0.51	0.45–0.49	0.48–0.50	0.39–0.51	0.29–0.46	0.39–0.46	0.31–0.51	0.30–0.49	1.30 (0.042)	1.22 (0.042)	1.16 (0.042)	1.90–2.00
	1.15 (0.025)	1.11 (0.025)	1.04 (0.025)	1.02 (0.026)	1.20–1.40	0.99–1.41	1.17 (0.042)	0.93–1.50	1.20–1.40	0.99–1.41	0.99–1.26	
	1.03–1.28	1.03–1.17	1.01–1.11	0.92–1.12	1.20–1.40	0.99–1.41	1.04–1.33	0.93–1.50	1.92 (0.038) <sup>d</sup>	1.87 (0.038)	1.86 (0.038)	
	1.94 (0.025) <sup>d</sup>	1.90 (0.025)	1.89 (0.025)	1.87 (0.025)	1.83–2.03	1.81–1.96	1.90–2.08	1.71–2.01	1.83–2.03	1.81–1.96	1.71–2.01	
	1.90–2.00	1.85–1.97	1.84–1.94	1.75–1.96	1.83–2.03	1.81–1.96	1.90–2.08	1.71–2.01				

<sup>a</sup> Percent dry weight. <sup>b</sup> The mean, standard error, and range of eight values (four replicates from each of two field sites in the northern or southern soybean region). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown across both soybean regions. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.

**Table 3. Composition of Fat, Fatty Acid, and Vitamin E from Seed of MON 87701 × MON 89788 (Test) Grown in the United States and Argentina**

component <sup>a</sup>	USA (2007)			Argentina (2007–2008)		
	test	control	commercial	test	control	commercial
	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>
total fat	19.98 (0.77)	20.12 (0.77)		18.13 (0.22)	18.41 (0.23)	
	16.66–22.31	17.24–22.55	17.90–23.56	17.30–19.09	17.59–19.28	15.10–20.28
capric	0.20 (0.014)	0.21 (0.014)				
	0.13–0.27	0.16–0.26	0.15–0.27			
myristic	0.09 (0.0031)	0.09 (0.0031)		0.08 (0.0016)	0.08 (0.0017)	
	0.08–0.10	0.08–0.11	0.06–0.10	0.08–0.10	0.07–0.10	0.06–0.10
palmitic	11.53 (0.12) <sup>d</sup>	11.88 (0.12)		11.29 (0.12)	11.43 (0.12)	
	11.16–11.19	11.50–12.13	9.80–12.38	10.63–11.84	10.80–12.04	9.90–12.63
palmitoleic	0.09 (0.0032)	0.09 (0.0033)		0.087 (0.0032)	0.087 (0.0033)	
	0.08–0.11	0.08–0.11	0.07–0.14	0.063–0.099	0.070–0.10	0.05–0.12
heptadecanoic	0.090 (0.0021)	0.093 (0.0021)		0.11 (0.0025)	0.11 (0.0025)	
	0.08–0.10	0.08–0.10	0.08–0.10	0.09–0.12	0.10–0.11	0.09–0.13
heptadecenoic	0.04 (0.0031)	0.04 (0.0032)				
	0.02–0.05	0.02–0.05	0.02–0.06			
stearic	4.26 (0.22) <sup>d</sup>	4.70 (0.22)		4.71 (0.11) <sup>d</sup>	4.98 (0.11)	
	3.46–5.21	4.03–5.36	3.21–5.24	4.41–5.26	4.59–5.63	3.81–5.50
oleic	23.09 (1.28)	22.71 (1.28)		18.45 (0.32)	18.73 (0.32)	
	19.66–29.38	20.34–28.78	16.69–35.16	17.31–19.43	17.69–19.99	17.22–22.96
linoleic	52.51 (0.94)	51.76 (0.95)		55.29 (0.33) <sup>d</sup>	54.51 (0.33)	
	48.35–55.13	47.18–54.07	44.17–57.72	53.85–56.44	53.20–55.53	51.51–56.73
linolenic	6.81 (0.45) <sup>d</sup>	7.11 (0.45)		8.93 (0.20)	8.97 (0.20)	
	5.04–7.82	5.34–8.26	4.27–8.81	8.26–9.73	8.32–9.90	7.59–9.60
arachidic	0.46 (0.025) <sup>d</sup>	0.51 (0.025)		0.43 (0.012) <sup>d</sup>	0.46 (0.012)	
	0.37–0.55	0.41–0.64	0.36–0.55	0.38–0.49	0.42–0.55	0.35–0.52
eicosenoic	0.24 (0.012)	0.23 (0.012)		0.15 (0.0040)	0.15 (0.0040)	
	0.18–0.30	0.18–0.28	0.21–0.30	0.13–0.19	0.14–0.16	0.13–0.22
eicosadienoic	0.04 (0.0030)	0.04 (0.0030)		0.05 (0.0039)	0.04 (0.0040)	
	0.02–0.05	0.02–0.05	0.02–0.05	0.02–0.08	0.02–0.07	0.02–0.06
behenic	0.54 (0.028)	0.54 (0.028)		0.42 (0.0093)	0.44 (0.0095)	
	0.44–0.62	0.45–0.65	0.38–0.59	0.38–0.47	0.39–0.49	0.35–0.50
vitamin E	5.65 (0.52)	6.24 (0.52)		2.87 (0.18) <sup>d</sup>	3.42 (0.18)	
	3.80–7.56	4.88–7.94	1.65–8.08	2.39–3.41	2.87–4.11	1.12–6.94

<sup>a</sup> Percent dry weight for total fat; % total FA for fatty acids; mg/100 g dry weight for vitamin E. <sup>b</sup> The mean, standard error, and range of 15 values (three replicates from each of five field sites). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown at the each respective production. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.

northern soybean region and A5547 (maturity group 5) was the control for the southern soybean region production. The trial was conducted at four replicated sites: Cachoeira Dourada, Minas Gerais, and Sorriso, Mato Grosso, in the northern soybean region of Brazil and Não-Me-Toque, Rio Grande do Sul, and Rolândia, Paraná, in the southern soybean region of Brazil. For 2007–2008, commercial soybean varieties in the Brazilian production included Monsoy 8352, Monsoy 8360, Monsoy 8757, TMG 103, TMG 115, BRS Valiosa, BRS Favorita, and BRS Conquista in the northern soybean region and CD-214, CD-213, V-Max, and CD 215 in the southern soybean region. For 2008–2009, the production included Monsoy 7908, Monsoy 8360, Monsoy 8757, TMG 103, TMG 115, BRS Valiosa, and BRS Go Luziana in the northern soybean region and Apollo, Impacto, CD-226, Fundacep 53, Magna, CD-215, V-Max, and CD 226

in the southern soybean region. Four different commercial varieties were included at each site. At each site, starting seeds were planted in a randomized complete block design with four replicates per block for each substance. For all productions, normal agronomic practices were followed for each growing region including the application of registered non-glyphosate-containing maintenance agrochemicals as required for optimal growth. Forage material was collected at approximately the R6 growth stage (full seed) from at least six plants from each plot by cutting at the base and compositing the six individual plants into one sample per plot. The forage samples were transferred to dry ice or a  $-70\text{ }^{\circ}\text{C}$  ultra-freezer within 30 min after sampling. Seed was harvested at maturity and stored at ambient temperature. Forage samples were shipped frozen on dry ice, and seeds were shipped at ambient temperature. At Monsanto

Table 4. Fat, Fatty Acid, and Vitamin E Composition of Seed from MON 87701 × MON 89788 (Test) Grown in Brazil

component <sup>a</sup>	Brazil (2007–2008)				Brazil (2008–2009)					
	northern region		southern region		northern region		southern region		both regions	
	test	control	test	control	test	control	test	control	test	control
	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>	mean (SE) <sup>b</sup>
	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>	range <sup>b</sup>
	commercial	commercial	commercial	commercial	commercial	commercial	commercial	commercial	commercial	commercial
	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>	range <sup>c</sup>
total fat	20.33 (0.69)	20.65 (0.69)	19.48 (0.69)	19.29 (0.69)	21.22 (0.57)	21.18 (0.57)	19.14 (0.57)	20.04 (0.57)	19.14 (0.57)	20.04 (0.57)
	19.74–21.46	19.35–21.55	18.55–20.61	17.77–20.74	20.59–21.96	19.58–22.46	17.46–20.70	18.92–21.42	17.46–20.70	18.92–21.42
myristic	0.07 (0.0041)	0.07 (0.0041)	0.09 (0.0041)	0.09 (0.0041)	0.07 (0.0046)	0.07 (0.0046)	0.09 (0.0046) <sup>d</sup>	0.10 (0.0046)	0.09 (0.0046) <sup>d</sup>	0.10 (0.0046)
	0.06–0.08	0.06–0.08	0.08–0.09	0.09–0.09	0.07–0.08	0.07–0.08	0.08–0.10	0.09–0.1	0.08–0.10	0.09–0.1
palmitic <sup>d</sup>	10.36 (0.27)	10.28 (0.27)	11.47 (0.27)	11.66 (0.27)	11.08 (0.13)	10.98 (0.13)	11.48 (0.13) <sup>d</sup>	12.00 (0.13)	11.48 (0.13) <sup>d</sup>	12.00 (0.13)
	9.86–10.91	9.87–10.62	11.30–11.66	11.23–12.08	10.90–11.28	10.53–11.27	11.33–11.64	11.74–13.12	11.33–11.64	11.74–13.12
palmitoleic	0.11 (0.0023) <sup>d</sup>	0.12 (0.0023)	0.09 (0.0023)	0.10 (0.0024)	0.11 (0.0027)	0.12 (0.0027)	0.097 (0.0027)	0.10 (0.0027)	0.097 (0.0027)	0.10 (0.0027)
	0.10–0.12	0.11–0.12	0.09–0.09	0.09–0.10	0.11–0.12	0.11–0.13	0.09–0.10	0.09–0.12	0.09–0.10	0.09–0.12
heptadecanoic	0.08 (0.0018) <sup>d</sup>	0.08 (0.0018)	0.10 (0.0018)	0.10 (0.0018)	0.09 (0.0013) <sup>d</sup>	0.08 (0.0013)	0.10 (0.0013)	0.10 (0.0013)	0.10 (0.0013)	0.10 (0.0013)
	0.08–0.09	0.08–0.08	0.09–0.10	0.09–0.11	0.08–0.09	0.08–0.09	0.09–0.10	0.09–0.11	0.09–0.10	0.09–0.11
heptadecenoic										
stearic	2.99 (0.18)	2.92 (0.18)	4.07 (0.18) <sup>d</sup>	4.25 (0.18)	0.06–0.07	0.06–0.07	0.02–0.06	0.05–0.06	0.02–0.06	0.02–0.07
	2.77–3.25	2.68–3.21	3.82–4.28	4.07–4.58	3.12 (0.25)	3.23 (0.25)	4.03 (0.25) <sup>d</sup>	4.33 (0.25)	4.03 (0.25) <sup>d</sup>	4.33 (0.25)
oleic	37.54 (3.36)	40.43 (3.36)	21.83 (3.36)	22.60 (3.36)	2.88–3.33	2.74–3.73	3.65–4.50	3.98–4.68	3.65–4.50	3.98–4.68
	29.98–44.60	36.87–45.68	20.27–23.57	19.33–25.94	30.05 (1.95)	31.58 (1.95)	22.89 (1.95)	21.85 (1.95)	22.89 (1.95)	21.85 (1.95)
linoleic	42.29 (2.65)	39.73 (2.65)	53.49 (2.65)	52.23 (2.66)	26.76–33.71	28.85–36.99	20.14–27.53	18.91–24.87	20.14–27.53	18.91–24.87
	36.46–48.46	35.36–42.48	52.56–54.19	49.98–54.09	48.61 (1.62)	47.35 (1.62)	52.90 (1.62)	52.78 (1.62)	48.61 (1.62)	52.78 (1.62)
linolenic	5.42 (0.48)	5.20 (0.48)	7.80 (0.48)	7.91 (0.48)	44.93–51.69	43.37–49.02	49.56–54.94	45.99–59.66	44.93–51.69	49.56–54.94
	4.86–6.14	4.87–5.41	7.12–8.49	6.98–9.04	5.73 (0.42)	5.44 (0.42)	7.27 (0.42)	7.58 (0.42)	5.73 (0.42)	7.58 (0.42)
arachidic	0.35 (0.010)	0.35 (0.010)	0.39 (0.010) <sup>d</sup>	0.42 (0.010)	5.45–5.94	4.96–5.89	6.46–7.95	5.17–8.72	5.45–5.94	6.46–7.95
	0.34–0.36	0.33–0.39	0.39–0.40	0.39–0.43	0.35 (0.022)	0.35 (0.022)	0.40 (0.022) <sup>d</sup>	0.43 (0.022)	0.35 (0.022)	0.43 (0.022)
eicosenoic	0.30 (0.017)	0.31 (0.017)	0.19 (0.017)	0.18 (0.017)	0.31–0.39	0.31–0.38	0.38–0.42	0.30–0.50	0.31–0.39	0.38–0.42
	0.24–0.33	0.26–0.35	0.16–0.23	0.16–0.20	0.27 (0.016)	0.28 (0.016)	0.22 (0.016)	0.30–0.50	0.27 (0.016)	0.22 (0.016)
eicosadienoic	0.03 (0.0046)	0.03 (0.0046)	0.04 (0.0046)	0.03 (0.0049)	0.26–0.29	0.25–0.31	0.18–0.28	0.16–0.30	0.26–0.29	0.18–0.28
	0.02–0.04	0.02–0.05	0.02–0.05	0.02–0.05						
behenic	0.48 (0.0083)	0.48 (0.0083)	0.44 (0.0083)	0.44 (0.0085)	0.45 (0.019)	0.45 (0.019)	0.46 (0.019)	0.46 (0.019)	0.45 (0.019)	0.46 (0.019)
	0.46–0.50	0.46–0.50	0.43–0.45	0.41–0.47	0.41–0.49	0.42–0.48	0.44–0.48	0.39–0.56	0.41–0.49	0.42–0.48
vitamin E	4.54 (0.80)	4.84 (0.80)	4.31 (0.80)	4.93 (0.80)	4.55 (0.89)	5.50 (0.89)	5.80 (0.89) <sup>d</sup>	7.19 (0.89)	4.55 (0.89)	5.80 (0.89) <sup>d</sup>
	4.20–4.85	4.59–5.12	3.33–5.43	3.40–6.29	2.57–5.37	4.88–6.39	4.62–7.08	1.57–5.52	2.57–5.37	4.62–7.08

<sup>a</sup> Percent dry weight for total fat; % total FA for fatty acids; mg/100 g dry weight for vitamin E. <sup>b</sup> The mean, standard error, and range of 8 values (four replicates from each of two field sites in the northern or southern soybean region). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown across both soybean regions. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.



Co. forage and seed samples were homogenized by grinding with dry ice to a fine powder and stored frozen at approximately  $-20\text{ }^{\circ}\text{C}$ .

**Compositional Analyses.** Components assessed in forage samples included proximates (ash, fat, moisture, and protein), carbohydrates by calculation, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Components assessed in seed samples included proximates (ash, fat, moisture, and protein), carbohydrates by calculation, ADF, NDF, total amino acids, fatty acids (FA), lectins, phytic acid, raffinose, stachyose, trypsin inhibitors, isoflavones (daidzein, genistein, and glycitein), and vitamin E ( $\alpha$ -tocopherol). Compositional analyses were conducted in compliance with Good Laboratory Practice (GLP) requirements at EPL-BAS Laboratories, in Niantic, IL. Brief descriptions of the methods utilized for the analyses are described in refs 1 and 2.

**Statistical Analysis of Composition Data.** The following analytes had  $>50\%$  of the observations below the assay limit of quantitation (LOQ) from all field productions and, as a result, were excluded from the statistical analysis: caprylic acid, lauric acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid,  $\gamma$ -linolenic acid, eicosatrienoic acid, arachidonic acid, and erucic acid. Capric acid was excluded from the Argentinean and both Brazilian field productions, heptadecenoic acid was excluded from the Argentinean and Brazilian 2007–2008 field productions, and eicosadienoic acid was excluded from the Brazilian 2008–2009 field production. For individual measurements below the LOQ, a value equal to half the LOQ was assigned prior to statistical analyses. Eicosadienoic acid from the U.S., Argentinean, and Brazilian 2007–2008 field productions and heptadecenoic acid from U.S. and Brazilian 2008–2009 field productions were assigned a value of half the LOQ value.

A studentized PRESS residual test was applied to each data set to identify outliers. For forage, one moisture value for one reference variety from the U.S. field production and one fat value for one reference variety from the Argentina field trial were outside the  $\pm 6$  studentized PRESS residual range and were excluded from statistical analyses. For seed, there were no components identified as outliers.

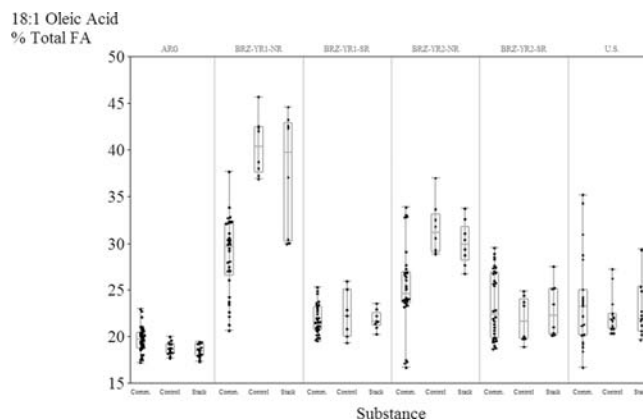
For each production, data across all sites were combined for statistical analyses. The combined site analyses for all four productions used the model

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where  $Y_{ijk}$  = unique individual observation,  $U$  = overall mean,  $T_i$  = substance effect,  $L_j$  = random location effect,  $B(L)_{jk}$  = random block within location effect,  $LT_{ij}$  = random location by substance interaction effect, and  $e_{ijk}$  = residual error. All productions included two to three additional test substances that were not part of this specific study but included in the analysis of variance model. Differences between mean values for MON 87701  $\times$  MON 89788 and control components were statistically significant using a predetermined  $\alpha$  level of 5%. Statistical analyses were conducted on SAS (SAS Software Release 9.1 and 9.2 (TS1M0); Copyright 2002–2008 by SAS Institute Inc., Cary NC).

## RESULTS AND DISCUSSION

**Protein and Amino Acid Composition in Seed.** Mean values for seed protein were slightly higher in MON 87701  $\times$  MON 89788 when compared to the control (Tables 1 and 2). Mean differences ranged from 0.24% dw (northern Brazil, 2008–2009) to 1.95% dw (USA, 2007). Relative magnitudes of difference, with respect to the control, ranged from 0.64 to 5.16%, and statistical significance ( $p < 0.05$ ) was observed at only three of the six regions. Protein levels in soybean seed are influenced by both genotype and environment including location, water stress, temperature, and/or nitrogen supply;<sup>4</sup> however, mean protein values for MON 87701  $\times$  MON 89788 and the control did not



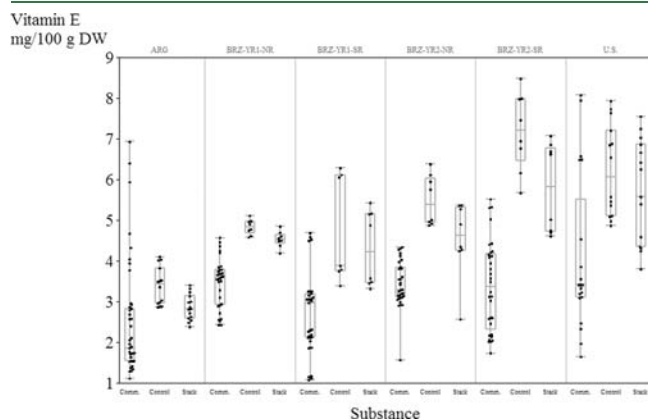
**Figure 1.** Ranges of values of oleic acid in soybean seed harvested from all productions (ARG, Argentina, 2007–2008; BRZ-YR1-NR, Brazil northern region 2007–2008; BRZ-YR1-SR, Brazil southern region 2007–2008; BRZ-YR2-NR, Brazil northern region 2008–2009; BRZ-YR2-SR, Brazil southern region 2008–2009; U.S., United States 2007).

substantially differ across the different productions of this study. Rather, an extensive range of values for the individual replicates of both MON 87701  $\times$  MON 89788 and the control was observed within each production. This range was generally of the order  $\sim 2$ –6% dw, although the replicate values for the control from the U.S. 2007 trial ranged from 32.29 to 41.87% dw, a difference of 9.58% dw. The contribution of genetics and environment to variation in protein levels from each production was also evident in the values observed for the commercial references (Tables 1 and 2). Overall, for all productions, the range of values of seed protein for MON 87701  $\times$  MON 89788 and the control overlapped extensively and magnitudes of differences observed between MON 87701  $\times$  MON 89788 and the control protein mean values were small. Mean differences between the proteinogenic amino acids MON 87701  $\times$  MON 89788 and the control were also of correspondingly small magnitude with extensive overlap of test, control, and commercial values (Tables 1 and 2).

**Fat, Fatty Acid, and Vitamin E ( $\alpha$ -Tocopherol) Composition in Seed.** There were no consistent differences across all productions between mean values of seed fat of MON 87701  $\times$  MON 89788 and the control (Tables 3 and 4). Mean differences ranged from a decrease in MON 87701  $\times$  MON 89788 of 0.90% dw (southern Brazil, 2008–2009) to an increase of 0.17% dw (southern Brazil, 2007–2008). Relative magnitudes of difference, with respect to the control, ranged from a decrease of 4.44% to an increase of 0.88%. Statistical significance ( $p < 0.05$ ) was not observed for any of the productions. Overall, the range of values of seed fat for MON 87701  $\times$  MON 89788 and the control overlapped extensively, and any observed differences between MON 87701  $\times$  MON 89788 and the control fat mean values were small. As for protein, fat levels were characterized by a large range in replicate values within productions. This was particularly true for the U.S. 2007 trial, in which MON 87701  $\times$  MON 89788 and control values ranged from 16.66 to 22.31% dw and from 17.24 to 22.55% dw, respectively (Table 3). As for protein, the contribution of genetics and environment to variation in fat levels from each production was also evident in the values observed for the commercial references (Tables 3 and 4).

The major fatty acids of soybean seed are palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. As shown in Tables 3 and 4, there were no consistent differences between the

fatty acid profiles of MON 87701 × MON 89788 and the control across the U.S., Argentina, and Brazil productions. Strikingly, there were greater differences in the levels of fatty acids in the harvested seed of the conventional controls grown for the different productions than there were between MON 87701 × MON 89788 and the control for any production (see Figure 1 for oleic acid). For example, magnitude differences in stearic acid, oleic acid, linoleic acid, and linolenic acid between Monsoy 8329 and A5547, when expressed as a percentage of A5547, from the



**Figure 2.** Ranges of values of vitamin E in soybean seed harvested from all productions (ARG, Argentina 2007–2008; BRZ-YR1-NR, Brazil northern region 2007–2008; BRZ-YR1-SR, Brazil southern region 2007–2008; BRZ-YR2-NR, Brazil northern region 2008–2009; BRZ-YR2-SR, Brazil southern region 2008–2009; U.S., United States, 2007).

Brazil production from 2007 to 2008 were 31.3, 78.9, 23.9, and 24.0%, respectively (Table 4). No magnitude differences >10.4% for these components were observed for any of the MON 87701 × MON 89788 versus control mean comparisons at any production. Neither was statistical significance ( $p < 0.05$ ) observed for any differences at all Brazil sites for any of the fatty acids. The contribution of genetics, environment, and growing season to the compositional variability observed in the fatty acid levels of the different controls and commercial varieties from the Brazil productions are discussed in more detail in Zhou et al.<sup>5</sup>

Differences in the fatty acid profiles from the U.S. and Argentina production fields were also observed (Table 3). Thus, for example, the range of individual replicate values for control and commercial references for oleic acid in the United States were 20.34–28.78% total FA and 16.69–35.16% total FA, whereas the Argentina values were 17.69–19.99% total FA and 17.22–22.96% total FA. Corresponding values for MON 87701 × MON 89788 in the United States were 19.66–29.38% total FA and 17.31–19.43% total FA in Argentina.

The lack of consistent or meaningful differences between MON 87701 × MON 89788 and the control attests to the lack of impact of transgene insertion on fatty acid composition in this combined-trait product, whereas the wide range of values for respective fatty acids within and between productions confirms a high degree of natural variability in these components.

Mean values of seed vitamin E were slightly lower in MON 87701 × MON 89788 when compared to the control for all six productions, although statistical significance ( $p < 0.05$ ) was observed only at two (Argentina and southern Brazil, 2008–2009) (Tables 3 and 4). Mean differences ranged from 0.30 mg/100 g dw

**Table 5.** Antinutrient and Isoflavone Composition of Seed from MON 87701 × MON 89788 (Test) Grown in the United States and Argentina

component <sup>a</sup>	USA (2007)			Argentina (2007–2008)		
	test	control	commercial	test	control	commercial
	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>d</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>
lectin	1.29 (0.19) <sup>d</sup> 0.21–3.97	0.72 (0.19) 0.28–1.28	0.090–2.47	3.07 (0.42) 1.29–7.43	3.46 (0.43) 1.60–8.39	1.32–11.18
phytic acid	1.87 (0.12) 1.41–2.26	1.97 (0.12) 1.31–2.66	1.10–2.32	1.46 (0.12) 1.02–1.99	1.55 (0.12) 1.02–2.10	0.81–2.27
raffinose	1.32 (0.19) 0.69–1.90	1.34 (0.19) 0.43–1.85	0.52–1.62	1.05 (0.039) 0.77–1.38	1.15 (0.039) 0.98–1.29	0.58–1.44
stachyose	4.70 (0.63) 2.77–6.12	4.93 (0.63) 2.27–6.65	1.97–5.55	3.82 (0.11) <sup>d</sup> 3.36–4.24	4.02 (0.11) 3.14–4.38	2.91–4.84
trypsin inhibitor	27.07 (1.23) 23.43–34.78	28.57 (1.24) 22.49–34.20	20.84–37.24	27.81 (1.41) 21.43–34.37	27.21 (1.42) 23.45–30.96	18.14–42.51
daidzein	760 (108.20) <sup>d</sup> 323–946	604 (108.30) 199–831	214–1274	1057 (34.48) <sup>d</sup> 931–1239	935 (34.70) 827–1095	361–1458
genistein	712 (88.42) <sup>d</sup> 414–919	595 (88.52) 245–761	148–1024	941 (29.36) <sup>d</sup> 820–1037	859 (29.58) 757–976	506–1096
glycitein	162 (21.17) 100–201	157 (21.23) 61–227	32–208	176 (8.24) 134–222	185 (8.44) 137–217	49–256

<sup>a</sup> Isoflavones in  $\mu\text{g/g}$  dry weight; lectin in H.U./mg fresh weight; phytic acid, raffinose, stachyose and proximates in percent dry weight; trypsin inhibitor in TIU/mg dry weight. <sup>b</sup> The mean, standard error, and range of 15 values (three replicates from each of five field sites). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown at the each respective production. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.

**Table 6. Antinutrient and Isoflavone Composition of Seed from MON 87701 × MON 89788 (Test) Grown in Brazil**

component <sup>a</sup>	Brazil (2007–2008)					Brazil (2008–2009)				
	northern region		southern region		both regions	northern region		southern region		both regions
	test	control	test	control		MON 87701 × MON 89788	control	MON 87701 × MON 89788	control	
	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	mean (SE) <sup>b</sup> range <sup>b</sup>	range <sup>c</sup>
lectin	4.04 (0.74) 0.64–7.52	2.32 (0.74) 0.17–7.67	3.34 (0.74) 1.37–8.11	3.68 (0.79) 0.28–5.67	0.60–8.59	5.14 (1.01) 0.72–8.52	4.78 (1.01) 1.99–8.63	4.29 (1.01) 2.60–6.11	3.71 (1.01) 1.28–8.33	0.57–8.74
phytic acid	2.12 (0.19) 1.54–2.67	2.21 (0.19) 1.84–2.68	1.97 (0.19) 1.75–2.18	2.04 (0.19) 1.40–2.60	1.17–2.46	1.39 (0.14) 0.98–1.87	1.34 (0.14) 1.11–1.53	1.59 (0.14) 1.17–2.22	1.66 (0.14) 1.32–2.22	0.96–2.00
raffinose	1.03 (0.081) <sup>d</sup> 0.91–1.18	0.84 (0.081) 0.70–1.00	0.96 (0.081) 0.90–1.04	0.97 (0.082) 0.81–1.21	0.65–1.28	1.05 (0.045) 0.90–1.20	0.96 (0.045) 0.84–1.13	1.16 (0.045) 0.98–1.27	1.24 (0.045) 1.06–1.48	0.79–1.75
stachyose	3.72 (0.18) <sup>d</sup> 3.43–3.98	4.17 (0.18) 3.75–4.55	4.06 (0.18) 3.85–4.21	3.93 (0.18) 3.65–4.20	2.09–5.02	3.76 (0.17) 3.53–4.07	4.02 (0.17) 3.77–4.27	4.21 (0.17) 3.88–5.33	4.36 (0.17) 3.84–4.87	2.88–5.55
trypsin inhibitor	37.20 (1.33) 34.15–41.01	35.46 (1.33) 32.42–39.56	30.61 (1.33) 26.43–35.70	30.09 (1.39) 27.16–35.96	23.99–41.03	36.71 (2.54) 23.58–40.89	37.58 (2.54) 32.86–43.06	32.04 (2.54) 26.43–40.46	32.44 (2.54) 27.47–35.27	23.40–45.55
daidzein	245 (157.16) 207–286	234 (157.16) 198–265	1227 (157.16) <sup>d</sup> 944–1576	1014 (157.44) 605–1544	181–2100	289 (179.79) 189–385	256 (179.79) 172–361	945 (179.79) <sup>d</sup> 646–1302	800 (179.79) 523–1204	199–1350
genistein	344 (98.77) 286–402	353 (98.77) 276–423	1011 (98.77) <sup>d</sup> 832–1209	889 (99.03) 609–1190	176–1649	410 (136.40) 223–578	394 (136.40) 250–530	772 (136.40) <sup>d</sup> 559–981	704 (136.40) 510–936	132–1324
glycitein	94 (9.89) 63–114	90 (9.89) 71–114	193 (9.89) 168–226	178 (10.28) 127–220	33–261	118 (21.18) 86–153	122 (21.18) 90–168	175 (21.18) 134–218	191 (21.18) 139–275	55–338

<sup>a</sup> Isoflavones in  $\mu\text{g/g}$  dry weight; lectin in H.U./mg fresh weight; phytic acid, raffinose, stachyose and proximates in percent dry weight; trypsin inhibitor in TIU/mg dry weight. <sup>b</sup> The mean, standard error, and range of eight values (four replicates from each of two field sites in the northern or southern soybean region). <sup>c</sup> The minimum and maximum of sample values for commercial varieties grown across both soybean regions. <sup>d</sup>  $p < 0.05$  when compared to corresponding control.

(northern Brazil, 2007–2008) to 1.39 mg/100 g dw (southern Brazil, 2008–2009). Relative magnitudes of difference, with respect to the control, were small and ranged from 0.64 to 5.16%. Vitamin E levels in soybean seed are influenced by both genotype and environment,<sup>6</sup> and this was confirmed by observed values for MON 87701 × MON 89788 and controls at the different productions as well as the wide ranges of values observed for the commercial varieties. Mean values for MON 87701 × MON 89788 ranged from 2.87 to 5.80 mg/100 g dw, and mean values for the control ranged from 3.42 to 7.19 mg/100 g dw. The range of values for the commercial varieties ranged from 1.08 to 8.08 mg/100 g dw (Tables 3 and 4; Figure 2). Zhou et al.<sup>5</sup> provide more detailed discussion on the contribution of genetics, environment, and growing season on variability in vitamin E levels at the Brazil productions.

**Antinutrient and Isoflavone Composition in Seed.** No consistent differences across the productions in mean values for the antinutrients (lectins, phytic acid, raffinose, stachyose, and trypsin inhibitors) were observed between MON 87701 × MON 89788 and the control. The mean values for the isoflavones, daidzein and genistein, were statistically significantly different ( $p < 0.05$ ) between MON 87701 × MON 89788 and the control in the U.S., Argentina, and both southern Brazil productions. Relative magnitudes of difference, however, were small, ranging from 10.6 to 25.6% for daidzein and from 9.5 to 19.7% for genistein, and were characterized by overlap of the respective individual replicate values. Indeed, extensive ranges of values for the individual replicates of both MON 87701 × MON 89788 and the control were observed within each production. This was particularly noticeable for control daidzein from the U.S. 2007 trial, in which values ranged over 4-fold, from 199  $\mu\text{g/g}$  dw to 831  $\mu\text{g/g}$  dw. There was also striking variability in isoflavone levels within and

across the Brazil productions with notable differences observed between MON 87701 × MON 89788 and control values from the northern and southern soybean regions (Table 6). The contribution of genetics and environment to variation in isoflavone levels from each production was also evident from analysis of the commercial reference varieties (Tables 5 and 6). Again, this was especially true for the Brazil productions, for which isoflavone values could vary as much as 10-fold across regions; for example, genistein values ranged from 176 to 1649  $\mu\text{g/g}$  dw in 2007–2008 and from 132 to 1324  $\mu\text{g/g}$  dw in 2008–2009. Isoflavone levels have been shown to be affected by genetic background,<sup>2</sup> geographical region and location,<sup>2,7</sup> temperature,<sup>8</sup> and stress.<sup>9,10</sup> The contribution of genetics, environment, and growing season on isoflavone levels from the Brazil productions are described in more detail in Zhou et al.<sup>5</sup>

**Proximate and Fiber Composition in Seed and Forage.** No consistent differences in mean values for proximates (excluding seed protein discussed above) and fibers in seed and forage were observed between MON 87701 × MON 89788 and the control. Summarized data are presented in Supplementary Tables 1–4 of the Supporting Information.

**Conclusion.** MON 87701 × MON 89788, a conventional control comparator, and multiple commercial varieties were grown at replicated fields in the United States during the 2007 growing season, in Argentina during the 2007–2008 growing season, and in the northern and southern soybean regions of Brazil during the 2007–2008 and 2008–2009 growing seasons. Compositional analyses showed no consistent trends in statistically significant differences ( $p < 0.05$ ) between MON 87701 × MON 89788 and the control across all productions for any components. Any reproducible differences between MON 87701 × MON 89788 and control components from each individual production were generally small in magnitude and not meaningful



from a food/feed perspective. This lack of any meaningful impact of transgene insertion on composition can be contrasted to the extensive variability in composition observed between and within studies. The contribution of the different experimental factors in this study (germplasm, production, and region) to compositional variability was particularly pronounced for components such as fatty acids, vitamin E, and isoflavones. It is concluded that forage and seed of the insect-protected, glyphosate-tolerant MON 87701 × MON 89788 are compositionally equivalent to that of conventional soybean. Furthermore, the compositional equivalence of insect-protected glyphosate-tolerant soybean, MON 87701 × MON 89788, to conventional soybean extends across different world regions and multiple growing seasons.

## ■ ASSOCIATED CONTENT

**S** **Supporting Information.** Additional tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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