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Stability in the Composition Equivalence of Grain from Insect-Protected Maize and Seed from Glyphosate-Tolerant Soybean to Conventional Counterparts over Multiple Seasons, Locations, and Breeding Germplasms

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S *Supporting Information*

ABSTRACT: Insect-protected maize MON 810 and Roundup Ready soybean 40-3-2 represent major milestones in the adoption of genetically modified (GM) crops to enhance agricultural productivity. This study provides an assessment of the compositional stability of these products over multiple seasons, multiple germplasms, and diverse geographies encompassing North, Central, and South America and Europe. The compositional assessment evaluated levels of proximates in MON 810 and proximates, antinutrients, and isoflavones in 40-3-2. The means and range values for component levels in the GM crops and their conventional comparators were consistently similar to each other within each corresponding year from 2000 to 2009. To our knowledge, this study represents the first meta-analysis of comparative composition assessments of GM products. This approach, combined with graphical approaches, provided an effective summary of the overall data set and confirmed the continued compositional equivalence of these important crops to their conventional counterparts over time.

KEYWORDS: Soybean (*Glycine max*), maize (*Zea mays*), genetically modified, composition, statistical analysis

INTRODUCTION

The development of insect-protected maize through the incorporation of *Bacillus thuringiensis*-derived proteins and the development of herbicide-tolerant soybean through the incorporation of glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. have been key milestones in the continued widespread adoption of genetically modified (GM) technology. The Cry1Ab-containing product MON 810 was introduced in 1998, and although it was superseded in many countries by newer *B. thuringiensis* and multitrait products, MON 810 remains the only GM maize crop grown in Europe. However, within Europe, it has also been the subject of national bans inspiring much debate over the scientific rationale for such actions.[1]

Roundup Ready Soybean (40-3-2) was introduced in 1996 and now represents over 90% of soybean grown in the United States and South America. The European Food Safety Authority recently approved continued authorization for marketing of 40-3-2. As with MON 810, Roundup Ready 40-3-2 has also been the subject of extensive scientific and policy debate.[2]

Earlier studies have shown MON 810[3] and 40-3-2[4−6] to be compositionally equivalent to their conventional counterparts. Since at least 2000, Monsanto has maintained annual compositional analysis programs for MON 810 and 40-3-2. These programs have involved measurement of key components from different MON 810 hybrids and 40-3-2 varieties grown across diverse geographic regions. This report presents compositional data from a total of 112 maize hybrids and 74 soybean varieties grown over at least nine growing seasons in geographic areas encompassing the United States, Canada, South America, and Europe. The report further utilized meta-analysis techniques to summarize results from these compositional analyses. Meta-analysis has been applied previously to GM related studies, for example, on the effects of *B. thuringiensis* maize (and cotton) on invertebrate pests.[7] There has also been a compositional meta-analysis on the effect of environmental stresses on soybean composition,[8] but to our knowledge, this study represents the first meta-analysis of comparative composition assessments of GM products.

MATERIALS AND METHODS

Soybean 40-3-2. The test substances were a total of 112 unique commercial Roundup Ready 40-3-2 varieties that were grown from 2000 to 2009. Test substances were grown in Canada in 2002 and 2005. References substances were conventional commercial soybean varieties grown from 2000 to 2009 in the United States. Reference substances were grown in Canada in 2002 and 2004. A total of 25 40-3-2 and conventional varieties each were grown in 2000 and 2001, 16 were grown in 2002, and 12 were grown from 2003 to 2008. Eleven 40-3-2 and 10 conventional varieties were grown in 2009. Normal agronomic practices were followed. Glyphosate treatments of the 40-3-2 samples were at commercially relevant rates. Material names and site locations are provided in the Supporting Information (Table 1).

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Table 1. Proximate Content of Seed from Roundup Ready (40-3-2) and Conventional Soybean Grown from 2000 to 2009

year	substance[a]	ash mean (range)	carbohydrate by calculation mean (range)	fat mean (range)	moisture mean (range)	protein mean (range)
2000	RR 40-3-2	5.25 (4.89−5.52)	36.59 (33.72−39.05)	18.47 (15.77−21.20)	5.36 (5.06−5.73)	39.69 (36.98−43.00)
	conventional	5.36 (5.04−5.78)	35.95 (32.61−39.75)	18.91 (15.52−22.26)	5.34 (5.08−5.59)	39.79 (36.36−42.64)
2001	RR 40-3-2	5.59 (4.90−6.02)	38.38 (35.31−42.98)	18.24 (14.58−20.01)	6.58 (6.27−6.89)	37.80 (33.44−41.46)
	conventional	5.50 (5.03−5.97)	38.34 (34.87−42.03)	17.88 (14.37−20.86)	6.58 (6.34−6.94)	38.28 (34.39−40.61)
2002	RR 40-3-2	5.61 (5.02−6.06)	33.79 (31.53−35.98)	20.43 (19.45−21.25)	5.99 (5.17−7.53)	40.18 (38.02−42.36)
	conventional	5.57 (4.98−6.00)	33.53 (30.97−36.45)	19.70 (17.77−22.14)	6.28 (5.27−8.80)	41.21 (37.65−45.33)
2003	RR 40-3-2	5.19 (4.52−5.65)	39.32 (36.14−44.13)	17.82 (14.87−20.56)	5.29 (4.31−6.20)	37.68 (32.78−40.38)
	conventional	5.27 (4.71−5.67)	39.26 (35.95−44.21)	17.17 (12.95−20.75)	5.59 (4.72−9.90)	38.31 (36.42−39.84)
2004	RR 40-3-2	5.30 (5.01−5.57)	36.93 (33.22−41.05)	20.25 (17.89−21.48)	6.42 (5.85−7.11)	37.52 (32.58−40.99)
	conventional	5.37 (5.06−5.64)	35.50 (31.10−37.87)	19.52 (17.32−22.02)	7.37 (5.75−9.92)	39.64 (34.57−42.47)
2005	RR 40-3-2	5.00 (4.68−5.35)	37.73 (35.62−41.41)	18.46 (15.61−21.04)	5.72 (5.32−6.07)	38.79 (37.47−40.36)
	conventional	5.20 (4.56−5.54)	37.70 (33.41−40.45)	18.39 (14.61−19.80)	5.81 (5.44−6.43)	38.72 (36.55−42.01)
2006	RR 40-3-2	4.97 (4.68−5.40)	37.40 (33.15−40.61)	17.74 (15.59−21.58)	8.43 (7.75−9.26)	39.89 (37.15−43.64)
	conventional	4.81 (4.42−5.18)	37.57 (34.85−41.61)	17.17 (12.75−19.48)	8.50 (8.07−9.04)	40.46 (39.02−42.30)
2007	RR 40-3-2	5.58 (5.19−6.13)	34.95 (31.69−37.26)	20.60 (18.06−23.41)	8.54 (6.94−12.25)	38.87 (35.73−42.86)
	conventional	5.81 (5.38−6.33)	35.44 (33.00−38.45)	19.90 (17.54−22.23)	7.72 (5.85−10.90)	38.84 (37.66−40.34)
2008	RR 40-3-2	5.19 (4.84−5.58)	36.23 (33.36−38.66)	19.78 (17.82−22.76)	7.16 (5.32−9.07)	38.81 (35.75−40.56)
	conventional	5.27 (4.92−5.79)	35.92 (33.29−37.27)	20.26 (17.66−23.08)	7.08 (5.45−8.77)	38.56 (36.66−40.57)
2009	RR 40-3-2	5.00 (4.63−5.41)	37.21 (33.01−39.91)	19.02 (17.31−21.68)	7.22 (6.10−8.75)	38.76 (35.56−42.81)
	conventional	4.67 (4.22−4.96)	35.85 (32.52−40.20)	19.69 (16.82−22.15)	6.91 (6.22−7.78)	39.81 (38.05−42.80)
combined	**RR 40-3-2**	**5.31 (4.52−6.13)**	**36.88 (31.53−44.13)**	**18.99 (14.58−23.41)**	**6.53 (4.31−12.25)**	**38.83 (32.58−43.64)**
	conventional	**5.32 (4.22−6.33)**	**36.56 (30.97−44.21)**	**18.77 (12.75−23.08)**	**6.56 (4.72−10.90)**	**39.36 (34.39−45.33)**

[a] Units in % dry weight except moisture, which is in % fresh weight. Values for 2000 and 2001 are based on 25 RR 40-3-2 and conventional varieties, and 2002 and 2003−2008 values are based on 16 and 12 varieties. Values for 2009 are based on 11 RR 40-3-2 and 10 conventional varieties.

Maize MON 810. The test substances were a total of 74 unique commercial MON 810 hybrids that were grown from 2000 to 2009. Test substances were also grown in Honduras in 2007, Europe in 2008, and Brazil in 2009. Reference substances were conventional commercial hybrids grown from 2000 to 2007 in the United States. References were also grown in Mexico in 2007, Europe in 2008, and Brazil in 2009. A total of 12 MON 810 and commercial hybrids each were grown in 2000−2001, 2004, and 2007−2009, seven were grown in 2005, and six were grown in 2006. With the exceptions of the 2001 and 2002 studies and in part for 2007, all MON 810 and the corresponding conventional comparator were grown at the same location. Normal agronomic practices were followed. Material names and site locations are provided in the Supporting Information (Table 2).

Compositional Analysis. For both soybean and maize, the analysis of fat, ash, and moisture was conducted using AOAC methods. The protein content was determined using a Kjeldahl technique. Carbohydrates by calculation were determined using fresh weight-derived data and the formula carbohydrates by calculation (%) = 100 − moisture (%) − ash (%) − fat (%) − protein (%). For soybean, levels of isoflavones were determined by high-performance liquid chromatography analysis;[4,5] the lectin content was determined using hematocrit of lyophilized rabbit blood, and trypsin inhibitors were measured using an AOCS method. Method details can be found in refs 4 and 5. Composition analyses were performed singly on ground soybean seed samples from 2000 to 2005 and in duplicate on ground soybean seed samples from 2006 to 2009 by Covance Laboratories Inc. (WI). For MON 810, duplicate analyses were conducted in 2001 by Covance Laboratories Inc.; in 2002−2008, duplicate analyses were conducted by EPL-BAS (Harristown, IL); and in 2009, duplicate analyses were conducted by TECAM (Sao Paulo, Brazil). For all samples, compositional analyses were conducted within 3−9 months of harvest. Ground samples were stored at −20 °C.

Graphical Presentations. Box plots were generated on JMP (Version 8.02, Copyright 2009 by SAS Institute Inc., Cary NC). The top and bottom lines represent a measure of variability based upon the interquartile range, and the horizontal line within the box indicates the median. The box contains the observed values from the lower quartile (25%) to the upper quartile (75%) of the distribution and includes 50% of the observed values.

Statistical Meta-Analyses. Meta-analyses were performed using MetaEasy, a Microsoft Excel add-in.[9] All of the required summary statistics, including test substance group size, reference substance group size, standard deviations of the test and reference substances, and means differences between test and reference substances, were generated by the SAS MEANS procedure [SAS Software Release 9.2 (TS1M0), Copyright 2002−2008 by SAS Institute Inc., Cary, NC]. For each component, the effect and range of values from the test and reference samples across studies were summarized using a fixed-effects model. Heterogeneity was checked using Cochran's Q. The resultant graph is a forest plot where the confidence interval (CI) for each study is represented by a horizontal line and the point estimate is represented by a square. The size of the square corresponds to the weight of the study in the meta-analysis and is reversely proportional to the within-sample variance in each year.

RESULTS AND DISCUSSION

Tabular and Graphical Presentation. Tables 1 and 2 summarize the proximate, antinutrient, and isoflavone contents of harvested seed from 40-3-2 and conventional soybean grown from 2000 to 2009. The means and range values for 40-3-2 and conventional soybean analyte levels were consistently similar to each other within each corresponding year from 2000 to 2009. This is reflected in results shown for the combined means. For example, in the case of proximates, protein mean values for

Table 2. Antinutrient and Isoflavone Content of Seed from Roundup Ready (40-3-2) and Conventional Soybean Grown from 2000 to 2009

year	substance[a]	trypsin inhibitor mean (range)	lectin mean (range)	daidzein mean (range)	genistein mean (range)	glycitein mean (range)
2000	RR 40-3-2	45.89 (24.61−64.56)				
	conventional	44.42 (30.05−65.78)				
2001	RR 40-3-2	41.81 (23.73−72.95)	2.83 (1.14−6.46)	676.53 (238.1−1796.6)	875.08 (399.06−1572.0)	155.74 (84.7−363.02)
	conventional	41.69 (27.91−75.52)	3.38 (1.05−9.03)	600.35 (218.2−1530.3)	805.40 (390.4−1476.8)	154.44 (90.3−308.6)
2002	RR 40-3-2	50.99 (36.09−90.59)	0.79 (0.15−1.69)	541.99 (188.9−927.7)	629.62 (413.6−997.8)	136.61 (68.4−203.5)
	conventional	50.51 (34.73−74.95)	1.82 (0.57−6.13)	537.66 (174.8−1235.9)	639.96 (323.2−918.86)	129.83 (44.80−228.07)
2003	RR 40-3-2	35.63 (26.93−48.71)	1.82 (0.97−2.49)	670.68 (400.0−1146.6)	793.69 (544.4−1086.8)	157.13 (74.5−331.4)
	conventional	33.39 (23.68−53.02)	1.91 (0.94−2.90)	670.68 (293.2−1168.4)	814.51 (424.72−1098.7)	141.26 (53.1−226.7)
2004	RR 40-3-2	32.35 (24.63−55.03)	0.86 (0.26−1.34)	698.12 (386.1−1051.7)	949.41 (505.9−1335.9)	189.57 (74.3−343.8)
	conventional	31.52 (25.09−39.28)	0.87 (0.14−2.01)	884.27 (417.4−1424.3)	1011.26 (608.1−1660.5)	161.27 (110.5−296.6)
2005	RR 40-3-2	29.80 (20.89−45.35)	1.55 (1.03−2.90)	763.10 (383.4−1741.3)	707.05 (532.4−1348.4)	148.35 (49.8−322.9)
	conventional	26.35 (18.96−38.58)	1.75 (0.59−4.21)	625.69 (228.1−1084.4)	693.24 (313.9−900.1)	146.51 (61.8−286.4)
2006	RR 40-3-2	29.63 (21.06−60.09)	3.33 (0.81−7.91)	1008.64 (214.1−2054.0)	938.47 (271.2−1636.7)	176.25 (105.7−264.5)
	conventional	26.44 (18.52−40.75)	3.33 (1.80−7.36)	1018.41 (336.9−1837.5)	960.10 (505.6−1328.9)	142.09 (89.6−211.5)
2007	RR 40-3-2	31.62 (25.61−49.45)	2.82 (0.31−9.96)	559.55 (258.0−1296.6)	631.53 (255.8−1307.7)	129.07 (56.4−234.5)
	conventional	36.26 (23.18−56.20)	2.88 (0.42−11.40)	773.39 (128.5−1424.8)	732.49 (180.5−1145.7)	149.75 (56.4−276.1)
2008	RR 40-3-2	36.89 (27.47−64.11)	3.59 (1.07−9.19)	978.00 (361.0−1601.6)	982.47 (544.9−1226.7)	149.95 (82.0−220.4)
	conventional	36.17 (24.26−50.23)	2.00 (1.09−3.77)	1007.63 (594.9−1529.1)	992.18 (778.9−1156.4)	152.18 (89.7−287.4)
2009	RR 40-3-2	23.99 (19.66−30.41)	1.66 (0.42−3.72)	875.46 (337.0−1449.5)	1002.67 (552.5−1272.3)	133.81 (84.9−212.7)
	conventional	28.05 (17.23−38.11)	2.26 (0.67−3.83)	825.62 (347.6−1504.8)	962.71 (577.6−1319.7)	150.48 (63.2−288.9)
combined	**RR 40-3-2**	**37.74 (19.66−90.59)**	**2.17 (0.15−9.96)**	**736.71 (188.9−2054.0)**	**830.74 (255.8−1636.7)**	**152.86 (49.8−363.0)**
	conventional	**37.33 (17.23−75.52)**	**2.36 (0.14−11.40)**	**740.60 (128.5−1837.5)**	**830.27 (180.5−1660.5)**	**147.57 (44.80−308.58)**

[a] Trypsin inhibitor units are TIU/mg dry weight, lectins are hemagglutinating unit/dry weight, and isoflavones are ug/g dry weight. Values for 2000 and 2001 are based on 25 RR 40-3-2 and conventional varieties, and 2002 and 2003−2008 are based on 16 and 12 varieties. Values for 2009 are based on 11 RR 40-3-2 and 10 conventional varieties.

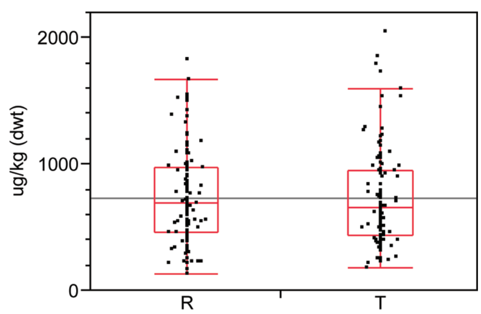

Figure 1. Box plot of daidzein values from analysis of soybean 40-3-2 (T) and conventional comparators (R) from a total of nine growing seasons (2001−2009). Individual data points are shown in jiggered format. The histograms show the distribution of a total of 245 data points (121 R and 124 T) and also highlight the similarity in range and distribution.

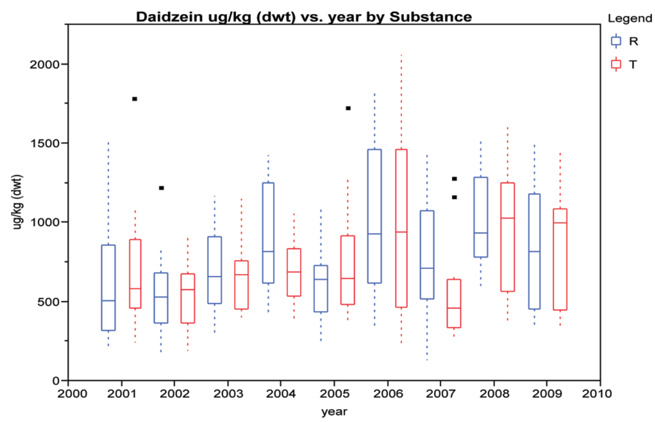

Figure 2. Box plots of daidzein values from analysis of soybean 40-3-2 (T) and conventional comparators (R) showing results from nine growing seasons (2001−2009).

40-3-2 and conventional soybean were 38.83 and 39.36% dw, respectively; corresponding values for fat were 18.99 and 18.77% dw (Table 1). For all proximates, the combined data showed that differences in relative magnitudes in mean values, with respect to the control, were small and ranged from 0.2 (ash) to 1.35% (protein). In the case of antinutrient and isoflavone contents, mean values for 40-3-2 and conventional soybean were again very similar; for example, trypsin inhibitor mean values for 40-3-2 and conventional soybean were 37.74 and 37.33 trypsin inhibitor unit (TIU)/mg dw, respectively; corresponding values for daidzein were 736.71 and 740.60 ug/g dw. The combined data showed that differences in relative magnitudes in mean values, with respect to the control, were small and ranged from 0.06 (genistein) to 8.1% (lectin). The similarity in the distribution of values is shown graphically for the major isoflavone, daidzein; Figure 1 shows a box plot of values from an analysis across all nine growing seasons, and Figure 2 shows results from each growing season separately. The corresponding figures for all other soybean analytes are shown in the Supporting Information.

Table 3. Proximate Analyses of Grain from MON 810 and Conventional Maize Hybrids Harvested from 2001 to 2009

year	substance[a]	ash mean (range)	carbohydrates by calculation mean (range)	fat mean (range)	moisture mean (range)	protein mean (range)
2001	MON 810	1.39 (1.16−1.62)	86.22 (83.65−87.90)	3.69 (3.03−4.54)	11.58 (9.57−17.40)	8.70 (7.60−10.30)
	conventional	1.48 (1.34−1.71)	84.11 (79.80−85.55)	3.66 (2.99−4.73)	9.14 (7.86−10.40)	10.76 (9.42−13.70)
2002	MON 810	1.23 (1.07−1.40)	86.97 (85.72−88.81)	2.93 (1.97−3.75)	11.56 (6.98−15.10)	8.85 (6.98−15.10)
	conventional	1.42 (1.26−1.56)	84.80 (82.13−86.50)	3.32 (2.76−4.30)	9.01 (7.66−10.40)	10.46 (9.15−12.06)
2003	MON 810	1.24 (1.04−1.45)	85.97 (85.25−86.95)	3.97 (3.02−4.88)	11.16 (9.50−12.60)	8.82 (7.62−10.06)
	conventional	1.20 (1.05−1.46)	86.32 (85.21−87.68)	3.79 (3.31−4.40)	10.91 (8.82−12.95)	8.69 (7.33−9.78)
2004	MON 810	1.22 (1.03−1.41)	86.56 (85.26−87.57)	3.96 (3.43−4.35)	8.50 (7.29−9.69)	8.27 (7.17−9.01)
	conventional	1.17 (1.01−1.36)	86.93 (85.73−88.18)	3.77 (3.27−4.44)	8.27 (7.56−9.13)	8.14 (7.40−9.03)
2005	MON 810	1.46 (1.31−1.64)	86.70 (85.27−88.15)	3.80 (3.40−4.25)	10.96 (8.88−12.45)	8.04 (7.02−8.83)
	conventional	1.45 (1.31−1.61)	86.84 (85.64−87.57)	4.06 (3.66−4.47)	11.19 (9.16−12.80)	7.63 (6.79−8.33)
2006	MON 810	1.25 (1.12−1.38)	85.79 (84.61−86.41)	3.64 (3.17−4.51)	8.38 (7.60−9.15)	9.31 (8.62−10.31)
	conventional	1.18 (1.07−1.26)	86.67 (85.38−87.71)	3.21 (2.66−4.48)	8.33 (7.54−9.38)	8.92 (8.33−10.04)
2007	MON 810	1.44 (1.01−1.84)	84.34 (81.04−88.21)	4.72 (3.31−5.90)	9.28 (7.50−11.45)	9.49 (7.41−11.65)
	conventional	1.31 (1.06−1.46)	85.12 (82.46−87.75)	4.33 (2.99−5.51)	9.65 (7.53−13.10)	9.22 (7.64−10.75)
2008	MON 810	1.26 (1.06−1.44)	86.22 (81.85−87.84)	3.63 (3.18−4.90)	12.74 (11.94−13.25)	8.88 (7.54−11.88)
	conventional	1.33 (1.20−1.43)	86.39 (83.24−88.64)	3.59 (2.77−4.82)	13.01 (12.38−14.03)	8.69 (6.91−11.46)
2009	MON 810	1.27 (1.07−1.42)	84.81 (83.36−86.05)	3.96 (3.05−4.97)	13.36 (12.06−15.52)	9.96 (8.33−11.34)
	conventional	1.26 (1.05−1.37)	84.94 (82.97−86.56)	4.00 (3.11−4.74)	13.59 (12.24−15.76)	9.80 (8.13−11.14)
combined	**MON 810**	**1.30 (1.01−1.84)**	**85.92 (81.04−88.81)**	**3.82 (1.97−5.90)**	**10.98 (6.98−17.40)**	**8.95 (6.98−15.10)**
	conventional	**1.31 (1.01−1.71)**	**85.68 (79.80−88.64)**	**3.77 (2.66−5.51)**	**10.42 (7.53−15.76)**	**9.24 (6.79−13.70)**

[a] Units in % dry weight except moisture, which is in % fresh weight. Values for 2000−2001, 2004, and 2007−2009 are based on 12 MON 810 and conventional hybrids, and 2005 and 2006 values are based on seven and six hybrids, respectively. With the exceptions of the 2001 and 2002 studies and in part for 2007, MON 810 and conventional hybrids were grown at the same location.

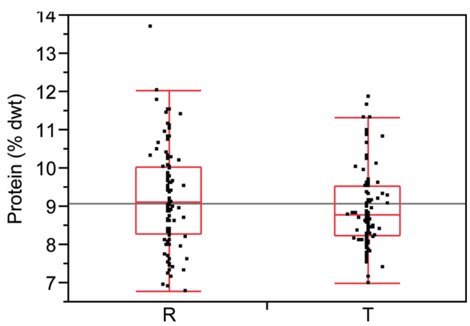

Figure 3. Box plot of protein values from analysis of MON 810 (T) and conventional comparators (R) from a total of nine growing seasons (2001−2009). Individual data points are shown in jiggered format. The histograms show the distribution of a total of 194 data points (97 R and 97 T) also highlight the similarity in range and distribution.

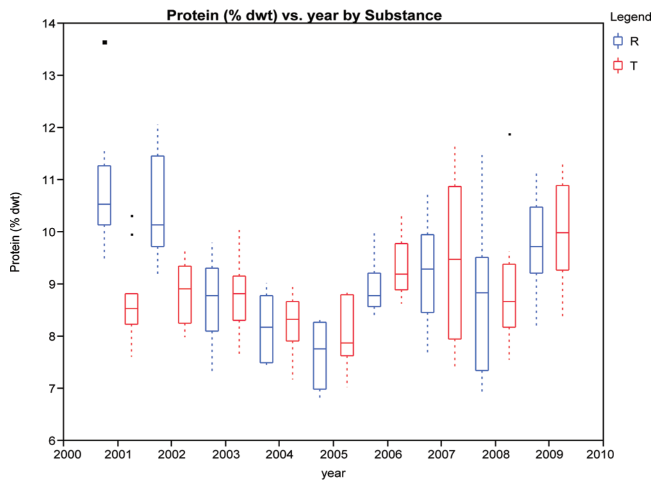

Figure 4. Box plot of protein values from analysis of MON 810 (T) and conventional comparators (R) showing results from nine growing seasons (2001−2009).

A summary of the values for ash, carbohydrate, fat, moisture, and protein obtained for MON 810 and conventional maize grain for studies conducted on samples from 2001 to 2009 is shown in Table 3. As for the case of 40-3-2, the mean and ranges for MON 810 and conventional analyte levels were consistently similar, as reflected in values for the combined results. For example, protein mean values for MON 810 and conventional maize were 8.95 and 9.24% dw, respectively; corresponding values for fat were 3.82 and 3.77% dw (Table 3). A higher protein content as well as lower carbohydrate and moisture content in conventional maize was observed in 2001 and 2002, the two growing seasons where the MON 810 and conventional hybrids were grow in different geographies (Supporting Information, Table 2).

For all components, the combined data showed that differences in relative magnitudes in mean values, with respect to the control, were small and ranged from 0.28 (carbohydrates by calculation) to 5.37% (moisture). The similarity in the distribution of values is shown graphically for protein; Figure 3 shows a box plot of values from an analysis across all nine growing seasons, and Figure 4 shows results from each growing season separately. The corresponding figures for all other maize analytes are shown in the Supporting Information.

Meta-Analysis. Meta-analysis can provide an effective summary of large amounts of data. By combining results from individual studies to generate an overall result, meta-analysis can control for between-study variation as well as increase

Table 4. Meta-Analysis of Composition of Seed from Roundup Ready (40-3-2) and Conventional Soybean Grown from 2000 to 2009[a]

study	effect	lower 95% CI	upper 95% CI	error bars
2000	0.0470	−0.5074	0.6014	0.5544
2001	0.0282	−0.5262	0.5826	0.5544
2002	0.0288	−0.6642	0.7218	0.6930
2003	−0.0569	−0.8570	0.7433	0.8002
2004	−0.1330	−0.9331	0.6672	0.8002
2005	0.0379	−0.7623	0.8381	0.8002
2006	−0.0070	−0.8072	0.7932	0.8002
2007	−0.3466	−1.1468	0.4536	0.8002
2008	0.0056	−0.8336	0.8448	0.8392
2009	−0.0895	−0.9458	0.7669	0.8564

meta-analysis	effect	lower 95% CI	upper 95% CI	var. eff
FE model	−0.0312	−0.2595	0.1971	0.0136

heterogeneity measure	value	df	*p* value
cochrane Q	0.87	9	0.9997

[a] FE, fixed-effects model; and df, degrees of freedom.

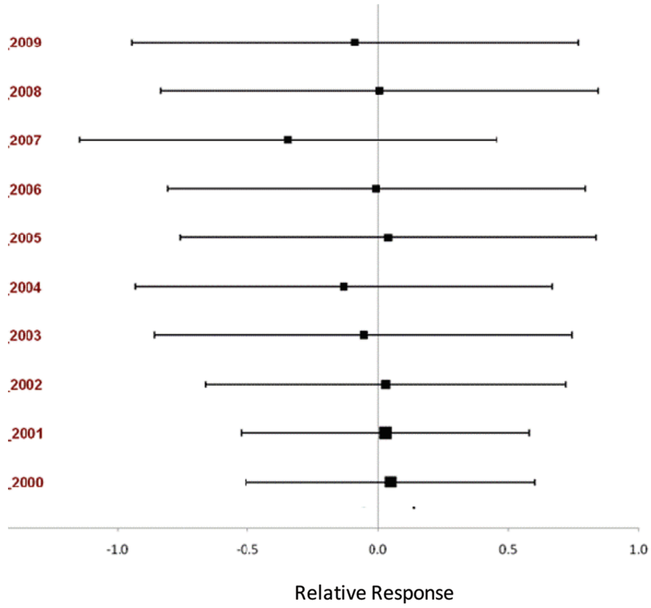

Figure 5. Forest plot of all components combined (proximate, antinutrient, and isoflavone content) of seed from soybean 40-3-2 and conventional soybean grown from 2000 to 2009.

statistical power. The meta-analysis approach was particularly appropriate here as all compositional studies assessed formed part of an annual monitoring program, and there is no possibility of selection bias, a potential criticism of some meta-analysis approaches. Furthermore, the similarity in experimental design in the individual studies for MON 810 and for 40-3-2 increased the probability that the meta-analysis would provide a meaningful summary. An assessment of the homogeneity (by measuring Cochran's Q) in the overall data set for MON 810 and for 40-3-2 confirmed that estimates of treatment effects were similar from study to study which in itself provided further evidence for the continued compositional equivalence of MON 810 and 40-3-2 to conventional comparators over multiple seasons, locations, and breeding germplasms.

Meta-analyses are intended to give a quantitative assessment of a treatment effect; in this case, the effect on transgene insertion on mean values of crop nutritional and antinutritional components. For the purposes of this study, the treatment effects for each crop component were placed on a common scale of effect size and combined to provide a composite score. This therefore reflected an effect difference on overall composition as well as complementing the tabular and graphical presentations on individual components. Table 4 shows the difference between 40-3-2 and conventional soybean means (effect) along with 95% confidence bounds for the difference for each of the individual studies. The 95% CI for the combined across study difference between 40-3-2 and conventional soybean is (−0.2595, 0.1971). Because zero is within the 95% CI, there is no significant difference between the combined study 40-3-2 and the conventional soybean means. The Table 4 results for the individual studies are presented as a forest plot in Figure 5.

Table 5 shows the difference between MON 810 and conventional means (effect) along with 95% confidence bounds for the difference for each of the nine studies. The 95% CI for the combined across study difference between MON 810 and conventional substances is (−0.2697, 0.2932). There is therefore no significant difference between the combined study MON 810

Table 5. Meta-Analysis of Composition of Grain Derived from MON 810 and Conventional Maize Grown from 2001 to 2009[a]

study	effect	lower 95% CI	upper 95% CI	error bars
2001	0.0587	−0.7414	0.8589	0.8002
2002	−0.7838	−1.5840	0.0163	0.8002
2003	0.2153	−0.5848	1.0155	0.8002
2004	0.3335	−0.4667	1.1336	0.8002
2005	−0.1725	−1.2202	0.8751	1.0477
2006	0.6747	−0.4569	1.8063	1.1316
2007	0.2179	−0.5823	1.0180	0.8002
2008	−0.0996	−0.8998	0.7005	0.8002
2009	−0.0839	−0.8841	0.7162	0.8002

meta-analysis	effect	lower 95% CI	upper 95% CI	var. eff
FE model	0.0117	−0.2697	0.2932	0.0206

heterogeneity measure	value	df	*p* value
cochrane Q	6.50	8	0.5911

[a] FE, fixed-effects model; and df, degrees of freedom.

and the conventional means. The Table 5 results for the individual studies are presented as a forest plot in Figure 6.

The high degree of compositional parity observed between the GM crops and their conventional comparators within and across growing seasons is quite noteworthy given the known natural variability in levels of the evaluated components. For example, in soybean, protein and fat expression is a quantitative trait, and levels in mature seed are influenced by both genotype and environment. In a recent meta-analysis of environmental effects on soybean composition, Rotundo and Westgate[8] observed that water stress, temperature, and/or nitrogen supply all affected protein levels measured in mature seed. Isoflavones are especially

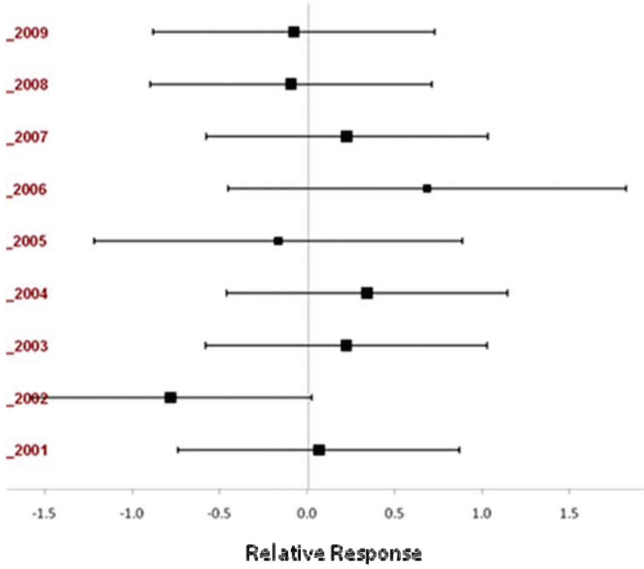

Figure 6. Forest plot on proximate content of grain with all of the components combined derived from MON 810 and conventional corn from 2001 to 2009.

variable in soybean, and one study recently concluded that,[10] "The range of values of isoflavones is overwhelming, even for homozygous genotypes growing in the same year and location, which greatly complicates genetic studies". The fact that the compositional assessment presented here highlighted no meaningful differences between 40-3-2 and the conventional comparators attests to the compositional stability of 40-3-2 and the lack of effect of transgene insertion.

Similarly, the composition in maize is known to vary according to environment. For example, changes in yield have now been clearly associated with changes in levels of grain starch and protein. These compositional changes are generally modest, and as pointed out by Scott et al.,[11] "While clear trends are observable in grain composition over the course of development of the era hybrids, the magnitude of the changes are small and on the order of magnitude of changes attributed to environmental effects". In other words, grain composition has been influenced by long-term temporal shifts in the genetic and physiologic make up of maize hybrids but is also subject to real-time environmental factors including location, biotic and abiotic stresses, and management practices. The fact that the assessment presented here highlighted no meaningful differences also attests to the compositional stability of MON 810 and the lack of effect of transgene insertion. Importantly, this observation is consistent with[12−15] proteomic and transcriptomic observations that showed near-identical protein profiles in MON 810 and comparable non-GM varieties, as well as a "lack of repeatable differential expression patterns between MON810 and comparable commercial varieties of maize". It is also consistent with data on the stability of the MON 810 transgene in maize.[16]

In conclusion, an assessment of compositional data from a total of 112 maize hybrids and 74 soybean varieties grown over at least nine growing seasons encompassing diverse geographic areas highlighted the stability in the compositional equivalence of two milestone GM crops, soybean 40-3-2, and maize MON 810. Results presented in both tabular and graphical form showed that means and ranges between the GM crops and their conventional comparators were similar for the components analyzed.

The compositional assessment further utilized meta-analysis techniques to summarize results. Specifically, meta-analysis combined the mean difference between 40-3-2 soybean and conventional soybean across 10 different studies and between the MON 810 and conventional comparators across nine studies. The meta-analysis results showed that 40-3-2 and MON 810 were not different from their conventional counterparts.

As pointed out by ref 17 in their extensive review of commercial applications of *B. thuringiensis*, "Bt transgenic crops have been overwhelmingly successful and beneficial, leading to higher yields and reducing the use of chemical pesticides and fossil fuels". Others have concluded[18] that transgenic crops support the "three traditional pillars of sustainability, i.e. economically, environmentally and socially". We have demonstrated here that these benefits have been associated with consistently maintained compositional equivalence in transgenic crops. These results presented here, as well as the application of a meta-analysis approach to GM compositional studies, therefore represent important contributions in supporting the development of agronomically important and sustainable crops.

■ ASSOCIATED CONTENT

Supporting Information. Tables of a summary of 40-3-2 and conventional soybean grown at various locations and summary of MON 810 and conventional corn hybrids harvested at various locations and figures of box plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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