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Composition of Grain and Forage from Corn Rootworm-Protected Corn Event MON 863 Is Equivalent to That of Conventional Corn (*Zea mays* L.)

CHERIAN GEORGE,*,† WILLIAM P. RIDLEY,† JANET C. OBERT,† MARGARET A. NEMETH,† MATTHEW L. BREEZE,‡ AND JAMES D. ASTWOOD†

Product Safety Center, Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, and Covance Laboratories, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin 53704

Insect-protected corn hybrids containing event MON 863 protect corn plants against feeding damage from corn rootworm (*Diabrotica*), a major North American insect pest. Corn event MON 863 contains a gene that expresses an amino acid sequence variant of the wild-type Cry3Bb1 insecticidal protein from *Bacillus thuringiensis*. The purpose of this study was to compare the composition of corn containing event MON 863 with that of conventional nontransgenic corn. Compositional analyses were conducted to measure proximates, fiber, amino acids, fatty acids, minerals, folic acid, thiamin, riboflavin, vitamin E, antinutrients, and certain secondary metabolites in grain and proximates and fiber content in forage collected from a total of eight field sites in the U.S. and Argentina. Compositional analyses demonstrated that the grain and forage of event MON 863 are comparable in their nutritional content to the control corn hybrid and conventional corn. These comparisons, together with the history of the safe use of corn as a common component of animal feed and human food, support the conclusion that corn event MON 863 is compositionally equivalent to, and as safe and nutritious as, conventional corn hybrids grown commercially today.

KEYWORDS: Corn (*Zea mays* L.); corn rootworm; insect-protected corn; composition

INTRODUCTION

Corn rootworm (CRW, *Diabrotica*) is an economically important insect pest of corn and a leading target for insecticide use in the U.S. corn belt. It is estimated that CRWs cause approximately one billion dollars worth of damage to the U.S. corn crop annually (*1*). The quantity of conventional insecticides used to control CRW annually exceeds the quantity applied to control any other targeted pest in other crops in the U.S. (*2*). Over the years, the use of these insecticides has generated a range of environmental concerns such as bioaccumulation of chlorinated hydrocarbons, avian toxicity, ground and surface water contamination, other nontarget effects, and health concerns due to worker exposure. The only alternative to pesticide use for controlling rootworms is crop rotation. However, years of crop rotation have rendered this approach less effective in certain areas of the corn belt (*1*, *3*). Corn that has been improved through biotechnology to be resistant to CRWs could be a more efficacious tool for CRW management as compared to current practices and could eliminate the potential environmental and health concerns of traditional pesticide usage.

Bacillus thuringiensis is a common microorganism that has been used as a biological pesticide for several decades. Various strains of *B. thuringiensis* are known to produce several different classes of insecticidal proteins, and more than 100 different insecticidal genes have been identified to date (*1*, *4*). The largest class consists of the δ-endotoxins, which have been expressed through biotechnology in many important crop plants including cotton, potatoes, rice, and corn (*5*−*8*). Corn plants protected from CRW feeding damage were produced by insertion of a modified gene encoding a variant *B. thuringiensis* Cry3Bb1 protein into the corn genome. Corn hybrids containing a variant *B. thuringiensis* Cry3Bb1 protein, which are commercially known as YieldGard Rootworm, are herein referred to as corn event MON 863.

For many foods, the level of food safety generally accepted by society reflects the history of their safe consumption by humans. It is recognized that in many cases the knowledge required to manage the risks associated with foods has been acquired during the course of their long history of use. Foods are generally considered safe, provided that care is taken during development, production, processing, storage, handling, and preparation. Because the absolute safety of food derived from biotechnology cannot be established by any known means, a comparison of the food derived from biotechnology to its conventional counterpart is the accepted approach to establishing

* To whom correspondence should be addressed. Tel: 314-694-3147. Fax: 314-694-8562. E-mail: cherian.george@monsanto.com.
† Monsanto Company.
‡ Covance Laboratories, Inc..

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safety (*9*). This process, known as the evaluation of substantial equivalence, has been adopted by leading international food and regulatory bodies including the World Health Organization (*10*, *11*), the United Nations Food and Agricultural Organization (*12*), the Organization for Economic Cooperation and Development (*13*−*15*), and the International Life Sciences Institute (*16*). The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather, it represents the starting point, which is used to structure the safety assessment of a new food relative to its conventional counterpart (*9*). It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety assessment of foods derived from genetically modified plants. According to this principle, if a new food or feed derived from a genetically modified crop is shown to be substantially equivalent to its conventional counterpart, then it is considered to be as safe as the food or feed from the conventional plant variety. Government authorities in Japan (*17*), Canada (*18*), the United States (*19*), the United Kingdom (*20*), the European Union (*21*), and many other countries have adopted substantial equivalence as an integral part of the basis for the safety assessment of crops developed through biotechnology and have approved a number of products using this approach.

Consistent with the established framework for safety assessment, the concept of substantial equivalence was applied to assess the safety of corn event MON 863. The food and feed safety of corn event MON 863 was confirmed by the following evaluations: (i) safety of the introduced transgene, (ii) safety of the derived protein, (iii) phenotypic and agronomic characteristics of the transgenic plant, (iv) compositional equivalence of the derived food/feed, (v) toxicological evaluation of the food/feed in rodents, and (vi) animal performance studies of the feed. The animal toxicological and performance studies address any unintended effects that may not be detected by the other four methods. The collective data confirm that food and feed derived from corn event MON 863 are safe for human and animal consumption. This paper describes the compositional analysis and comparison of MON 863 grain and forage with its conventional counterpart grown under similar conditions at eight different locations in the U.S. and Argentina. The remaining five aspects of the safety evaluation will be the subjects of future publications.

MATERIALS AND METHODS

Corn Samples for Compositional Analysis. Corn event MON 863 was produced using a modified *cry*3Bb1 coding sequence (GenBank Accession No. M89794), which encodes a protein with enhanced insecticidal activity against CRW (*Diabrotica* spp.). Corn event MON 863 also contains the neomycin phosphotransferase II (*npt*II) gene encoding the NPTII protein, which functions as a selectable marker. The seed source for this study was a hybrid containing event MON 863, and it was prepared as follows. Inbred corn line A634 was transformed to create event MON 863. The R0 plant of event MON 863 was crossed to inbred A1 and then backcrossed multiple times to bring the cry3Bb1 gene into a homozygous state. This inbred was then crossed with corn inbred 23CDC1 to create the hybrid of MON 863 used in this study. A hybrid formed from the cross of two related conventional inbreds A1 and 23CDC1 was used as the control.

Corn grain and forage samples were collected from field trials conducted in 1999 in the U.S. and Argentina. In the U.S. field trials, corn plants were grown at four replicated sites (Keokuk County, Iowa; Benton County, Iowa; York County, Nebraska; and Warren County, Illinois). Corn event MON 863 and its control were planted in a randomized complete block design with four blocks. In addition to the test and control corn hybrids, a total of 18 different commercial nontransgenic corn hybrids were grown in the field trials to serve as reference materials (see **Table 1**). The genetic purity of the CRW corn plants was maintained by bagging the tassels and ear shoots at anthesis and self-pollinating each plant by hand. The forage was collected at the late dough/early dent stage, and the grain was collected at normal kernel maturity. Forage and grain samples were harvested and shipped to Monsanto. The samples were ground to a fine powder in the presence of dry ice and maintained frozen until required for compositional analysis. The identity of forage and grain samples was based on sample handling records and Southern blot or polymerase chain reaction analyses of genomic DNA isolated from the forage and grain tissue.

Table 1. Reference Hybrids Included in Field Trials for Rootworm-Protected Corn Event MON 863

field trial location	vendor	hybrid
	1999 U.S. trials[a]	
Illinois	Novartis	N7590
Illinois	Golden Harvest	H2493
Illinois	Novartis	N7070
Illinois	Pioneer	P3394
Illinois	Holden's	228 × 283
Nebraska	Holden's	198 × 277
Nebraska	Holden's	HC33 × 185
Nebraska	Holden's	198 × 172
Nebraska	Holden's	277 × 218
Iowa[e]	Holden's	200 × 185
Iowa[e]	Holden's	228 × 184
Iowa[e]	Holden's	HC34 × 172
Iowa[e]	Holden's	HC34 × 277
Iowa[f]	Holden's	198 × 185
Iowa[f]	Holden's	197 × 273
Iowa[f]	Holden's	200 × 277
Iowa[f]	Holden's	198 × 284
Iowa[f]	Holden's	HC33 × 283
	1999 Argentina trials[b]	
Fontezuela 1[c]	DEKALB	DK757
Fontezuela 1[c]	DEKALB	Nid.Ax888
Fontezuela 1[c]	DEKALB	Titanium F1
Fontezuela 1[c]	DEKALB	Titanium I2
Fontezuela 2[d]	DEKALB	DK757
Fontezuela 2[d]	DEKALB	Nid.Ax888
Fontezuela 2[d]	DEKALB	Titanium F1
Fontezuela 2[d]	DEKALB	Titanium I2
Salto	DEKALB	DK757
Salto	DEKALB	Nid.Ax888
Salto	DEKALB	Titanium F1
Salto	DEKALB	Titanium I2
Rojas	DEKALB	DK757
Rojas	DEKALB	Nid.Ax888
Rojas	DEKALB	Titanium F1
Rojas	DEKALB	Titanium I2

[a] Each hybrid was replicated twice at each site. One replicate was randomly selected for analysis. [b] Each hybrid was replicated four times at each site. All replicates were analyzed for composition. [c] Field site was a separate location in Buenos Aires province, Argentina. [d] Field site was a separate location in Buenos Aires province, Argentina. [e] Field site was located in Benton County, Iowa. [f] Field site was located in Keokuk County, Iowa.

In the Argentinean field trials, grain and forage samples were collected from four replicated sites in the Buenos Aires province (two sites in Fontezuela and one site each in Salto and Rojas). In addition to the test and control corn hybrids, four different conventional commercial hybrid varieties were planted as reference materials (see **Table 1**). The genetic purity of the corn plants was maintained, and forage and grain samples were collected and identified as previously described for the U.S. field trials.

Compositional Analyses. Compositional analyses were conducted to measure proximates (protein, fat, ash, carbohydrate, and moisture), acid detergent fiber (ADF) and neutral detergent fiber (NDF), amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamin E, folic acid, riboflavin, thiamin, phytic acid, and the trypsin inhibitor content of the grain. Proximates as well as ADF and NDF contents were measured

in the forage. The secondary metabolites, ferulic acid, *p*-coumaric acid, 2-furaldehyde, raffinose, and inositol, were measured in the grain. All compositional analyses were performed at Covance Laboratories, Inc. (Madison, WI). Brief descriptions of the methods utilized for the analyses are described below.

Proximate Analysis. Protein levels were estimated by determining the total nitrogen content using the Kjeldahl method (*22*, *23*). The protein was calculated from the total nitrogen using the formula $N \times 6.25$. The fat content of the grain was estimated by the Soxhlet extraction method (*24*). The fat content of the forage was determined by fat acid hydrolysis, followed by extraction with ether and hexane (*25*, *26*).

The ash content was determined by ignition in an electric furnace and quantitation of the ash by gravimetric analysis (*27*). The moisture content was determined by the loss of weight upon drying in a vacuum oven at 100 °C to a constant weight (*28*, *29*). Carbohydrate levels were estimated using the fresh weight-derived data and the following equation (*30*):

$$\%\ \text{carbohydrate} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{moisture})$$

Fiber Analysis. The ADF was estimated by treating the samples with an acidic boiling detergent solution to dissolve the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected and determined gravimetrically (*31*). The NDF was estimated by treating the samples with a neutral boiling detergent solution to dissolve the protein, enzymes, carbohydrate, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected and determined gravimetrically (*31*, *32*).

Minerals. To estimate the levels of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc, inductively coupled plasma emission spectrometry was used as described in the AOAC methods (*33*, *34*) and Dahlquist and Knoll (*35*). The sample was dried, precharred, and ashed overnight at approximately 500 °C. The ashed sample was treated with hydrochloric acid, taken to dryness, and placed in a solution of 5% (v/v) hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown samples, measured by the inductively coupled plasma, with the emission of a standard solution.

Amino Acid Composition. Three procedures described in the literature (*36*) were used to estimate the values for 18 amino acids in corn grain. The procedure for tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were then quantitated using an automated amino acid analyzer.

Fatty Acid Composition. The lipid in the grain samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride: methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (*37*).

Vitamin E. The vitamin E amount in the grain was determined following saponification to break down any fat and release the vitamin as described by Cort et al. (*38*). The saponified mixture was extracted with ethyl ether and then quantitated directly by high-performance liquid chromatography (HPLC) on a silica gel column.

Riboflavin. The amount of riboflavin was measured in grain samples following hydrolysis with dilute acid as described in the literature (*39*). The quantity of riboflavin in the sample hydrolysates was determined by comparing the growth of *Lactobacillus casei* measured turbidimetrically with the growth response in the presence of varying amounts of a riboflavin standard.

Thiamin. Thiamin was extracted by autoclaving the grain samples in the presence of weak acid followed by phosphatase digestion to release any bound thiamin (*40*−*42*). Thiamin was purified from the resulting solution by ion exchange chromatography and then converted to thiochrome with potassium ferricyanide. The thiochrome was extracted into isobutyl alcohol, and the levels were quantitated fluorometrically.

Folic Acid. Folic acid was analyzed using a published procedure (*43*, *44*) in which the grain was hydrolyzed by autoclaving in the presence of ascorbic acid. To release folic acid, the hydrolyzed material was digested by incubation for 18 h with an enzyme preparation from chicken pancreas. The quantity of folic acid in the sample was determined by comparing the growth of *L. casei* measured turbidimetrically with the growth response in the presence of varying amounts of a folic acid standard.

Phytic Acid. Phytic acid was quantitated in grain following extraction using ultrasonication as described by Lehrfeld (*45*, *46*). Purification and concentration of the extract were conducted using a silica-based anion exchange column followed by quantitation using a polymer HPLC column (PRP-1, 5 mm, 150 mm × 4.1 mm) fitted with a refractive index detector.

Trypsin Inhibitor. The trypsin inhibitor activity in grain was determined using AOCS method Ba 12-75 (*47*). The ground, defatted sample was suspended in dilute sodium hydroxide, and an appropriate dilution of the suspension was made. A series of aliquots with increasing levels of the diluted suspension was mixed with trypsin and the synthetic substrate, benzoyl-DL-arginine-*p*-nitroanilide. After 10 min, the action of trypsin was stopped by the addition of acetic acid, the mixture was centrifuged or filtered, and the absorbance of the supernatant or filtrate was measured at 410 nm. The trypsin inhibitor activity was calculated from the change in absorbance vs aliquot volume and expressed in trypsin inhibitor units (TIU)/mg fresh weight of sample.

Ferulic and p-Coumaric Acids. Ferulic and *p*-coumaric acids were assayed in grain using the method of Hagerman and Nicholson (*48*), in which the samples were extracted with methanol, and the extracts were hydrolyzed using 4 N sodium hydroxide, neutralized, and filtered. The levels of ferulic and *p*-coumaric acids were determined by reversed-phase HPLC with UV detection.

2-Furaldehyde. The levels of 2-furaldehyde were determined using the method of Albala-Hurtado et al. (*49*), in which the corn grain was extracted with 4% trichloroacetic acid, centrifuged, filtered, concentrated, and analyzed by reversed-phase HPLC with UV detection. The limit of quantitation (LOQ) for 2-furaldehyde was 0.5 ppm based on fresh weight.

Raffinose. The raffinose assay was based on two methods (*50*, *51*) in which the grain samples were extracted with deionized water and the extracts were treated with a solution of hydroxylamine hydrochloride in pyridine containing phenyl-α-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography with flame ionization detection.

Inositol. The amount of inositol was determined in grain following hydrolysis of the samples with dilute hydrochloric acid at elevated temperatures (*52*, *53*). The quantity of inositol in each sample was determined by comparing the growth of *Saccharomyces carlsbergenesis* measured turbidimetrically with the growth response in the presence of varying amounts of an inositol standard.

Statistical Analysis of Composition Data. The following 16 analyses with >50% of the observations at or below the LOQ of the assay were excluded from statistical analysis: sodium, 2-furaldehyde, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. For 22:0 behenic acid, two observations in the U.S. study and seven observations in the Argentinean study were below the LOQ. In addition, there were two observations for trypsin inhibitor and total fat in the U.S. and Argentinean data sets, respectively, that were below the LOQ. To include a complete data set for 22:0 behenic acid, trypsin inhibitors, and total fat in the statistical analysis, values equal to half the LOQ were assigned for the missing data points. Two outliers identified by the studentized PRESS residuals procedure (vitamin E and *p*-coumaric acid) from two of the commercial reference plots in Argentina were excluded from the statistical analysis (*54*). Except for moisture, all component values were converted from a fresh

weight to a dry weight basis and into their respective units described in **Tables 2−6**. There were a total of 51 components evaluated (seven in forage and 44 in grain) in the U.S. study and 58 components evaluated (seven in forage and 51 in grain) in the samples from Argentina.

Statistical analyses of the composition data were conducted using a mixed model analysis of variance for a combination of all sites for both the U.S. and the Argentinean studies. The combined trial analysis 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 = line effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by line interaction, and e_{ijk} = residual error. In these analyses, corn event MON 863 was compared to the nontransgenic control. For each comparison, the p value for a test of the corn event MON 863 mean equal to the control mean, the observed difference of the corn event MON 863 from the control, and the lower and upper 95% confidence intervals for the mean difference of the corn event MON 863 from the control were calculated.

Compositional data from the conventional commercial reference varieties were not included in the statistical analysis for either study. However, a range of the reference values was determined for each component across all sites. Additionally, the commercial reference data were used to develop population tolerance intervals. A tolerance interval is an interval with a specified degree of confidence, $100(1 - a)\%$, which contains at least a specified proportion, p, of an entire sampled population for the parameter measured (*55*). For each compositional analysis component, tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the values expressed in the population of commercial lines. Because negative quantities are not possible, calculated lower tolerance bounds that were negative were set to zero. Because there were only four different commercial varieties in the Argentinean field trial (see **Table 1**), samples from six commercial varieties grown in the European Union in 1999, handled in the same manner, and analyzed in the same laboratory using the same methods were included to construct the 99% tolerance interval for the Argentinean study. The field sites for these additional commercial varieties were in Sancho Abarca, Spain; Bagnarola, Italy; and Mulazzano, Italy. The commerical varieties with the supplier noted in parentheses were DK626 (DEKALB), Donana (Semillas Fito), Santos (DEKALB), Aramis (DEKALB), Eleonora (Pioneer), and Cecilia (Pioneer). SAS software (*56*) was used to generate all summary statistics and perform all analyses, which were conducted by Certus International, Inc. (Chesterfield, MO).

RESULTS AND DISCUSSION

The safety assessment of genetically modified crops has relied on a comparative approach that focuses on similarities and differences between the food and the feed derived from a genetically enhanced crop and its conventional counterpart (*57−60*). In this study, the nutritional composition of corn event MON 863 was compared to a nontransgenic control with a similar genetic background, which was grown in the same field trials in the United States and Argentina. In addition, the compositional profile of corn event MON 863 was compared to traditional corn varieties grown in the U.S., Argentina, and the European Union using a 99% tolerance interval to describe the compositional variability in the population of conventional corn hybrids in the marketplace. Finally, the composition values for corn event MON 863 were compared with values obtained from the published literature or historical conventional control values determined in previous studies.

Proximate, Fiber, and Mineral Composition. Compositional analysis results for corn grain and corn forage are presented in **Tables 2** and **3**, respectively. These results demonstrate that the levels of proximate components (fat, protein, ash, and carbohydrate), fiber (ADF and NDF), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, and zinc) in the grain as well as proximates and fiber of forage from corn event MON 863 were comparable to those in the grain and forage of the nontransgenic control. In addition, these values were either within published literature ranges, within the tolerance interval determined for commercial varieties evaluated in this study, and/or within the range of historical conventional control values determined in previous studies. No statistically significant differences were observed for these analytes with the exception of copper in the Argentinean field trials (see **Table 2**). The content of copper in the grain of corn event MON 863 was not significantly different statistically from the nontransgenic control in the U.S. field trials, and the range of values at both locations was shown to fall within the 99% tolerance interval for the commercial varieties. These results demonstrate that the levels of copper for corn event MON 863 were within the same population as nontransgenic, commercially available corn varieties.

Amino Acid Composition. The levels of the 18 amino acids measured in the grain of corn event MON 863 were comparable to those in the grain of the nontransgenic control (**Table 4**). These values were either within published literature ranges, within the 99% tolerance interval for commercial varieties evaluated in 1999 field trials, and/or within the range of historical conventional control values determined from previous studies.

For a majority of the amino acids, there were no differences between the corn event MON 863 and the control in the U.S. and Argentinean trials. However, small statistically significant differences between the MON 863 and the control expressed as a percentage of the control (2.3−5.3%) were observed for arginine, cysteine/cystine, leucine, and proline (see **Table 4**). None of these differences occurred in both the U.S. and the Argentinean trials, and in all cases, the range of values found for corn event MON 863 fell within the 99% tolerance interval for conventional commercial varieties grown in the same year. These results demonstrate that the levels of these amino acids were within the same population as nontransgenic, commercially available corn varieties.

Fatty Acid Composition. The levels of fatty acids in the grain of corn event MON 863 were comparable to those observed in the grain of the nontransgenic control (**Table 5**). All values were either within published literature ranges, within the 99% tolerance interval determined for commercial varieties evaluated in 1999 field trials, and/or within the range of historical conventional control values determined from previous studies. Statistically significant differences between the corn event MON 863 and the nontransgenic control were observed in the levels of 20:1 eicosenoic acid, 18:2 linoleic acid, 16:0 palmitic acid, and 18:0 stearic acid for the Argentinean field trials. However, the magnitude of the differences expressed as a percentage of the control value was small (2.3−8.4%), and in no case was a fatty acid level found to be statistically different in corn event MON 863 when compared to the control at more than one site. Furthermore, the range of values found for these fatty acids was in all cases within the 99% tolerance interval for the commercial varieties grown in the 1999 field trials demonstrating that corn event MON 863 was within the same population as conventional, commercially available corn varieties.

Phytic Acid, Trypsin Inhibitor, Vitamin E, Riboflavin, Thiamin, and Folic Acid Composition. Various other compounds present in corn were evaluated because of their nutritive

Table 2. Fiber, Mineral, and Proximate Composition of Grain from Rootworm-Protected Corn Event MON 863

	1999 U.S. trials[a]			1999 Argentina trials[b]				
component[c]	MON 863 mean (range)[h]	control[d] mean (range)[h]	comm. hybrids[e] tolerance interval[f]	MON 863 mean (range)[h]	control[d] mean (range)[h]	comm. hybrids[e] tolerance interval[f]	literature (range)	historical[g] (range)[h]
protein	11.60 (10.43–12.82)	12.19 (10.45–13.80)	5.47, 16.57	10.39 (9.54–11.36)	10.40 (9.30–10.92)	3.37, 16.57	(6.0–12.0)[i] (9.7–16.1)[i]	(9.0–13.6)
total fat	3.77 (3.00–4.56)	3.64 (3.05–4.29)	1.68, 4.64	3.59 (3.00–4.42)	3.60 (2.83–3.94)	1.26, 6.25	(3.1–5.7)[i] (2.9–6.1)[i]	(2.4–4.2)
ash	1.35 (0.84–1.71)	1.41 (0.89–1.89)	0.26, 2.06	1.55 (1.34–1.81)	1.51 (1.32–1.80)	0.97, 1.76	(1.1–3.9)[i]	(1.2–1.8)
ADF[k]	4.45 (3.49–5.23)	4.50 (3.62–5.89)	1.98, 6.62	3.47 (2.65–4.84)	3.25 (2.58–4.44)	1.35, 5.75	(3.3–4.3)[i]	(3.1–5.3)
NDF[k]	11.64 (9.21–13.47)	12.02 (10.31–15.82)	6.51, 16.28	12.67 (9.70–19.86)	11.60 (8.49–18.12)	4.35, 17.20	(8.3–11.9)[i]	(9.6–15.3)
carbohydrates	83.30 (81.83–85.00)	82.76 (80.70–84.80)	78.97, 90.36	84.58 (83.28–87.10)	84.49 (83.84–85.92)	77.60, 92.24	not reported in this form	(81.7–86.3)
moisture	10.03 (8.54–11.20)	10.23 (8.60–11.40)	5.09, 18.62	12.52 (11.10–15.10)	12.73 (11.60–15.30)	0, 20.94	(7–23)[i]	(9.4–15.8)
calcium	0.0052 (0.0041–0.0064)	0.0053 (0.0043–0.0089)	0.0022, 0.0073	0.0041 (0.0028–0.0051)	0.0044 (0.0033–0.0055)	0.0016, 0.0090	(0.01–0.1)[i]	(0.003–0.006)
copper	2.26 (1.72–3.18)	2.19 (1.60–2.88)	0.25, 2.70	2.29[l] (1.88–2.63)	2.82 (2.32–3.22)	0, 3.91	(0.9–10)[i]	na[m]
iron	23.55 (21.13–26.36)	24.18 (20.57–28.16)	12.52, 35.06	24.91 (21.97–31.67)	25.33 (22.84–27.19)	2.49, 37.25	(1–100)[i]	na
magnesium	0.13 (0.12–0.14)	0.14 (0.12–0.16)	0.082, 0.17	0.13 (0.12–0.16)	0.13 (0.12–0.14)	0.074, 0.17	(0.09–1.0)[i]	na
manganese	5.81 (3.75–7.40)	6.15 (4.01–8.28)	0, 12.84	7.74 (5.95–9.72)	7.58 (6.04–9.05)	0.90, 11.97	(0.7–54)[i]	na
phosphorus	0.40 (0.37–0.45)	0.42 (0.39–0.46)	0.21, 0.47	0.35 (0.30–0.41)	0.36 (0.31–0.39)	0.25, 0.39	(0.26–0.75)[i]	(0.288–0.363)
potassium	0.43 (0.40–0.48)	0.44 (0.39–0.48)	0.28, 0.48	0.43 (0.38–0.49)	0.43 (0.41–0.46)	0.23, 0.52	(0.32–0.72)[i]	na
zinc	22.15 (17.95–25.25)	23.68 (18.77–28.14)	6.31, 37.95	27.15 (23.50–30.31)	28.13 (24.38–31.63)	6.10, 40.05	(12–30)[i]	na

[a] Data from four replicated U.S. sites. [b] Data from four replicated sites in Argentina. [c] Percent dry weight of sample except for moisture as percent fresh weight and copper, iron, manganese, and zinc as mg/kg dry weight. [d] Nontransgenic control. [e] Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999. [f] Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero. [g] Range for nontransgenic control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual value across sites for each line. [i] Ref *68*. [j] Ref *69*. [k] ADF = acid detergent fiber; NDF = neutral detergent fiber. [l] Statistically different from the control at the 5% level ($p < 0.05$). [m] na = not available.

Table 3. Fiber and Proximate Composition of Forage from Rootworm-Protected Corn Event MON 863

	1999 U.S. trials[a]			1999 Argentina trials[b]				
component[c]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	literature[g] (range)	historical[h] (range)[i]
protein	8.62 (6.91–10.40)	8.33 (5.99–10.55)	4.94, 11.97	8.92 (7.59–10.04)	9.52 (8.35–10.60)	0.22, 15.79	(5.11–10.27)	(4.8–8.4)
total fat	2.40 (0.92–3.16)	2.35 (1.30–3.33)	1.03, 3.24	1.59 (0.81–2.65)	1.56 (0.71–2.37)	0, 4.49	(0.35–3.62)	(1.4–2.1)
ash	4.73 (3.62–5.65)	5.00 (3.81–6.27)	3.04, 5.58	6.51 (4.24–8.08)	6.32 (4.88–8.23)	2.33, 7.70	(2.00–6.60)	(2.9–5.1)
ADF[j]	28.67 (21.74–43.30)	28.41 (23.39–32.08)	9.33, 45.44	26.79 (22.55–31.27)	27.22 (22.83–30.32)	15.09, 34.96	(18.32–40.99)	(21.4–29.2)
NDF[j]	43.25 (37.97–49.67)	42.94 (37.32–51.85)	22.71, 56.02	42.87 (35.21–48.21)	43.20 (39.15–47.21)	24.59, 55.98	(26.37–54.45)	(39.9–46.6)
carbohydrates	84.24 (82.29–86.32)	84.32 (80.78–87.21)	81.22, 88.97	82.98 (80.74–85.10)	82.61 (81.09–84.68)	78.37, 91.73	(83.16–91.55)	(84.6–89.1)
moisture	71.09 (69.30–73.10)	71.68 (69.80–74.50)	62.70, 77.69	73.32 (70.10–75.10)	74.13 (70.20–77.70)	56.69, 87.10	(55.30–75.30)	(68.7–73.5)

[a] Data from four replicated U.S. sites. [b] Data from four replicated sites in Argentina. [c] Percent dry weight of sample except for moisture. [d] Nontransgenic control. [e] Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999. [f] Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero. [g] Ref *60*. [h] Range for control hybrids planted in Monsanto Company field trials conducted in 1994 and 1995. [i] Range denotes the lowest and highest individual value across sites for each line. [j] ADF = acid detergent fiber; NDF = neutral detergent fiber.

value. Phytic acid, the hexakis-o-phosphate of *myo*-inositol, is widely distributed in plants (*61*). Seeds accumulate up to 90% of stored organic phosphate as phytic acid, and it has been shown to limit the uptake of minerals such as calcium in higher animals. The trypsin inhibitors in several types of corn have been compared and found to be similar in physicochemical and immunological properties (*62*). The trypsin inhibitors of soybeans have been well-studied and have been shown to affect the nutritive value of raw soybeans (*63*); however, the levels of these materials in soybeans are significantly higher than those measured in corn. Corn is also considered to be a good source of vitamin E and thiamin (*64*).

Table 4. Amino Acid Composition of Grain from Rootworm-Protected Corn Event MON 863

	1999 U.S. trials[b]			1999 Argentina trials[c]				
amino acid[a]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	literature[g] (range)[i]	historical[h] (range)[i]
alanine	7.74 (7.65–7.85)	7.79 (7.46–7.98)	6.94, 8.46	7.74 (7.47–7.98)	7.84 (7.46–8.06)	7.09, 8.31	(6.4–9.9)	(7.2–8.8)
arginine	4.43[j] (4.21–4.68)	4.33 (4.09–4.63)	3.38, 5.22	4.24 (3.14–4.87)	4.24 (3.49–5.33)	3.00, 5.75	(2.9–5.9)	(3.5–5.0)
aspartic acid	6.51 (6.38–6.72)	6.45 (6.30–6.67)	5.54, 7.65	6.71 (6.25–7.22)	6.60 (6.30–6.99)	5.60, 7.68	(5.8–7.2)	(6.3–7.5)
cysteine/ cystine	2.20[j] (1.98–2.40)	2.09 (1.99–2.29)	1.59, 2.65	2.22 (2.11–2.33)	2.20 (1.98–2.30)	1.31, 3.02	(1.2–1.6)	(1.8–2.7)
glutamic acid	19.39 (18.99–19.91)	19.56 (18.97–20.26)	17.55, 21.25	18.97 (18.36–19.35)	19.21 (18.61–19.77)	15.91, 22.38	(12.4–19.6)	(18.6–22.8)
glycine	3.60 (3.45–3.74)	3.53 (3.32–3.72)	2.81, 4.46	3.78 (3.59–4.01)	3.71 (3.58–3.89)	2.29, 5.26	(2.6–4.7)	(3.2–4.2)
histidine	2.84 (2.70–2.95)	2.83 (2.72–2.94)	2.37, 3.35	3.02 (2.85–3.19)	2.99 (2.79–3.21)	2.22, 3.71	(2.0–2.8)	(2.8–3.4)
isoleucine	3.67 (3.45–3.89)	3.74 (3.61–3.87)	3.20, 4.17	3.73 (3.54–3.91)	3.71 (3.55–3.88)	3.18, 4.13	(2.6–4.0)	(3.2–4.3)
leucine	13.36[j] (12.88–13.65)	13.65 (13.27–14.17)	11.30, 15.63	12.90 (12.14–13.35)	12.99 (12.59–13.44)	9.76, 16.17	(7.8–15.2)	(12.0–15.8)
lysine	2.92 (2.65–3.26)	2.88 (2.67–3.08)	1.87, 3.89	3.01 (2.69–3.40)	2.93 (2.68–3.21)	1.79, 4.28	(2.0–3.8)	(2.6–3.5)
methionine	2.28 (1.89–2.49)	2.24 (1.96–2.58)	1.34, 2.74	2.01 (1.77–2.17)	2.08 (1.89–2.38)	1.03, 3.01	(1.0–2.1)	(1.3–2.6)
phenylalanine	4.99 (4.93–5.06)	5.04 (4.95–5.23)	4.53, 5.66	5.03 (4.88–5.18)	5.02 (4.92–5.15)	4.25, 5.75	(2.9–5.7)	(4.9–6.1)
proline	8.73 (8.30–9.21)	8.78 (8.60–9.05)	8.04, 10.35	9.35[j] (8.86–9.82)	9.68 (9.17–10.56)	8.47, 10.48	(6.6–10.3)	(8.7–10.1)
serine	4.70 (3.93–5.09)	4.67 (4.20–4.94)	3.76, 5.69	4.93 (4.62–5.26)	4.92 (4.56–5.29)	4.11, 5.52	(4.2–5.5)	(4.9–6.0)
threonine	3.41 (3.16–3.60)	3.36 (3.16–3.49)	2.93, 3.83	3.32 (2.76–3.60)	3.31 (2.87–3.61)	2.87, 3.99	(2.9–3.9)	(3.3–4.2)
tryptophan	0.66 (0.60–0.83)	0.65 (0.60–0.68)	0.37, 0.90	0.56 (0.51–0.61)	0.58 (0.51–0.66)	0.23, 0.94	(0.5–1.2)	(0.4–1.0)
tyrosine	3.63 (3.33–3.77)	3.48 (2.71–3.82)	2.15, 4.65	3.45 (2.81–3.66)	3.00 (1.93–3.66)	2.38, 4.19	(2.9–4.7)	(3.7–4.3)
valine	4.94 (4.71–5.13)	4.94 (4.64–5.12)	4.15, 5.63	5.03 (4.82–5.19)	4.98 (4.77–5.16)	4.49, 5.47	(2.1–5.2)	(4.2–5.3)

[a] Values expressed as percent of total amino acids. [b] Data from four replicated U.S. sites. [c] Data from four replicated sites in Argentina. [d] Nontransgenic control. [e] Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999. [f] Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero. [g] Ref *70*. Values are percent of total protein. [h] Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995; values are percent of total protein. [i] Range denotes the lowest and highest individual value across sites. [j] Value statistically and significantly different than the control at the 5% level ($p < 0.05$).

The results show that the content of phytic acid, trypsin inhibitor, vitamin E, thiamin (vitamin B_1), riboflavin (vitamin B_2), and folic acid in the grain of corn event MON 863 was comparable to that observed in the grain of the nontransgenic control (**Table 6**). These values were either within published literature ranges, within the 99% tolerance interval for the commercial varieties in the 1999 field trials, and/or within the range of historical conventional control values determined from previous studies. Statistically significant differences in the levels of phytic acid between the corn event MON 863 and the nontransgenic control were observed in the data from both the U.S. and the Argentinean field trials (see **Table 6**). However, in the U.S. trials, phytic acid levels were below the control values, and in the Argentinean trials, phytic acid levels were slightly elevated as compared with the control. Furthermore, the levels of phytic acid measured in corn event MON 863 were within the 99% tolerance interval for the commercial varieties demonstrating that the MON 863 event and conventional commercial varieties are considered to be part of the same population. Therefore, these differences are not considered to be biologically relevant. The amount of vitamin E in corn event MON 863 was statistically different from the control in the U.S., but not Argentinean, trials. In addition, the values for MON 863 were shown to fall within the 99% tolerance interval for the conventional commercial varieties indicating that these small differences are not biologically relevant.

Secondary Metabolite Composition. The secondary metabolites 2-furaldehyde, ferulic acid, *p*-coumaric acid, raffinose, and inositol have all been shown to be present in corn grain or processed corn components. The pentosans in corncobs, oat hulls, and other crop residues are a major source of 2-furaldehyde (furfural) (*65*). Ferulic and *p*-coumaric acids in plants are derived from the aromatic amino acids, phenylalanine and tyrosine (*66*), and serve as precursors for a large group of phenylpropanoid compounds including flavonoids and coumarins. Raffinose is a nondigestible oligosaccharide that is considered to be an antinutrient due to gas production and the resulting flatulence caused by its consumption (*67*).

The levels of 2-furaldehyde were below the LOQ (<0.5 ppm fresh weight) for all corn grain samples analyzed from the 1999 Argentinean field trials. As shown in **Table 6**, the levels of ferulic acid, *p*-coumaric acid, raffinose, and inositol in the grain

Table 5. Fatty Acid Composition of Grain from Rootworm-Protected Corn Event MON 863

	1999 U.S. trials[b]			1999 Argentina trials[c]				
fatty acid[a]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	MON 863 mean (range)[i]	control[d] mean (range)[i]	comm. hybrids[e] tolerance interval[f]	literature[g] (range)	historical[h] (range)[i]
arachidic (20:0)	0.41 (0.39–0.44)	0.40 (0.39–0.42)	0.30, 0.51	0.34 (0.32–0.37)	0.35 (0.32–0.39)	0.16, 0.60	(0.1–2)	(0.3–0.5)
behenic (22:0)	0.18 (0.17–0.21)	0.18 (0.15–0.21)	0.055, 0.30	0.15 (0.073–0.18)	0.15 (0.086–0.17)	0.054, 0.28	(not reported)	(0.1–0.3)
eicosenoic (20:1)	0.30 (0.28–0.35)	0.30 (0.28–0.35)	0.18, 0.42	0.24[j] (0.22–0.27)	0.25 (0.24–0.27)	0.19, 0.39	(not reported)	(0.2–0.3)
linoleic (18:2)	62.23 (60.02–63.21)	62.47 (61.55–63.60)	50.21, 70.86	63.99[j] (62.14–65.09)	62.58 (61.41–63.63)	49.72, 69.67	(35–70)	(55.9–66.1)
linolenic (18:3)	1.20 (1.13–1.29)	1.24 (1.09–1.45)	0.75, 1.51	1.17 (1.12–1.20)	1.19 (1.15–1.23)	0.76, 1.58	(0.8–2)	(0.8–1.1)
oleic (18:1)	22.00 (20.97–23.55)	21.87 (21.00–22.53)	13.28, 36.31	21.53 (20.68–22.45)	22.03 (21.20–22.92)	18.41, 31.88	(20–46)	(20.6–27.5)
palmitic (16:0)	12.01 (11.61–12.56)	11.88 (11.66–12.20)	7.74, 13.87	10.70[j] (9.86–11.47)	11.68 (11.35–12.06)	5.63, 17.42	(7–19)	(9.9–12.0)
stearic (18:0)	1.66 (1.40–1.86)	1.66 (1.33–1.81)	1.04, 2.68	1.88[j] (1.67–2.34)	1.76 (1.64–1.91)	0.80, 2.44	(1–3)	(1.4–2.2)

[a] Value of fatty acids expressed as % of total fatty acid. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). [b] Data from four replicated U.S. sites. [c] Data from four replicated sites in Argentina. [d] Nontransgenic control. [e] Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999. [f] Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero. [g] Ref *70*. Values expressed as % of total fat except for palmitic acid (16:0), which is expressed as % of triglyceride fatty acids. [h] Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995; values are expressed as % of total fatty acids. [i] Range denotes the lowest and highest individual value across sites. [j] Statistically and significantly different from the control at the 5% level ($p < 0.05$).

Table 6. Phytic Acid, Trypsin Inhibitor, Vitamin E, Thiamin, Riboflavin, Folic Acid, and Secondary Metabolite Content of Grain from Rootworm-Protected Corn Event MON 863

	1999 U.S. trials[a]			1999 Argentina trials[b]				
component	MON 863 mean (range)[h]	control[d] mean (range)[h]	comm. hybrids[d] tolerance interval[e]	MON 863 mean (range)[h]	control[c] mean (range)[h]	comm. hybrids[d] tolerance interval[e]	literature[f] (range)	historical[g] (range)[h]
phytic acid (% dw)	1.11[i] (0.92–1.28)	1.23 (1.01–1.37)	0.39, 1.33	0.76[i] (0.61–1.05)	0.60 (0.42–0.76)	0.36, 0.97	to 0.9%	na[j]
trypsin inhib. (TIU/mg dw)	2.30 (0.56–3.10)	2.48 (1.91–3.45)	0, 4.25	3.82 (2.89–4.76)	3.83 (2.19–5.05)	0, 6.98	na	na
folic acid (μg/g dw)	na	na	na	0.71 (0.48–1.02)	0.68 (0.59–0.75)	na	na	na
thiamin (mg/100 g dw)	na	na	na	0.28 (0.21–0.41)	0.27 (0.23–0.33)	na	(0.3–0.86)	na
riboflavin (μg/g dw)	na	na	na	1.35 (0.93–1.76)	1.27 (0.91–1.74)	na	(0.25–5.6)	na
vitamin E (mg/g dw)	0.011[i] (0.0062–0.014)	0.013 (0.0088–0.016)	0, 0.019	0.0089 (0.0070–0.014)	0.0080 (0.0060–0.011)	0, 0.028	(0.017–0.047)	(0.008–0.015)[k]
ferulic acid (% dw)	na	na	na	0.24 (0.20–0.40)	0.23 (0.19–0.27)	0.17, 0.28	na	(0.17–0.27)k
inositol (μg/g dw)	na	na	na	1564.01 (1355.93–1820.25)	1494.18 (1244.34–1704.55)	na	na	na
p-coumaric acid (%dw)	na	na	na	0.023 (0.016–0.047)	0.020 (0.016–0.026)	0.0022, 0.037	na	(0.011–0.030)[k]
raffinose (% dw)	na	na	na	0.12 (0.10–0.15)	0.11 (0.091–0.13)	0, 0.35	(0.028–0.074)[l]	(0.053–0.16)[k]

[a] Data from four replicated U.S. sites. [b] Data from four replicated sites in Argentina. [c] Nontransgenic control. [d] Commercial hybrids planted at each trial site. The commercial hybrids for Argentina also included six hybrids grown in the E.U. during 1999. [e] Tolerance interval is specified to contain 99% of the commercial line population; negative limits are set to zero. [f] Ref *64*. [g] Range for control hybrids planted in Monsanto Company field trials conducted between 1993 and 1995. [h] Range denotes the lowest and highest individual value across sites for each hybrid. [i] Statistically and significantly different from the control at the 5% level ($p < 0.05$). [j] na = not available. [k] Range for 13 commercial hybrids planted in Monsanto Company field trials or purchased from growers in 1998. [l] The range of sample values listed from ref *71*.

of corn event MON 863 were comparable to the levels found in the grain from the nontransgenic control. No statistically significant differences were observed in the comparisons conducted for the Argentinean field trials. These secondary metabolites were not analyzed in the grain samples from the U.S. trials.

In conclusion, the results of compositional analyses derived from eight field sites in the U.S. and Argentina demonstrate that the grain and forage of corn event MON 863 are comparable in composition with those of the nontransgenic control and conventional corn varieties. The use of growing seasons in diverse geographical areas and incorporation of reference corn hybrids into field trials suggest that the few statistically significant differences observed are most likely due to random chance and unlikely to be of biological relevance. The composition of corn event MON 863 was shown to fall within the 99%

tolerance interval for components in nontransgenic commercial corn varieties grown as part of 1999 field trials in the U.S., Argentina, and Europe and also within the ranges of values reported for nontransgenic corn in the literature as well as in historical data. These latter comparisons are important and relevant because it is well-recognized that the composition of any crop, including corn, varies as a result of many factors, including variety, growing conditions, and methods of analysis. The values for all components in corn event MON 863 fell within the range of natural variability found in nontransgenic corn varieties.

The analysis of the data reported herein confirms that the tolerance interval is a useful statistical tool that can account for extant natural variability in biochemical composition as demonstrated in a previous study (*55*). From the perspective of safety assessment, the biochemical sampling described in this paper provides a robust measure of any potential unexpected effects that may be due to the insertion of the *cry*3Bb1 gene into the corn genome. It has been shown by targeted nutritional analysis that the genetic enhancement of conventional corn to produce corn event MON 863 did not produce significant changes in any of the biologically and nutritionally important components analyzed. On the basis of the principle of substantial equivalence as articulated by the World Health Organization, the Organization for Economic Cooperation and Development, and the United Nations Food and Agriculture Organization, these data support the conclusion that CRW corn event MON 863 is as safe and nutritious as conventional varieties of corn on the market today.

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