JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Compositional Equivalency of Cry1F Corn Event TC6275 and Conventional Corn (*Zea mays* L.)

ROD A. HERMAN,*,† AMY M. PHILLIPS,† RANDY A. COLLINS,†
LAURA A. TAGLIANI,† FRED A. CLAUSSEN,‡ CHRISTOPHER D. GRAHAM,‡
BRENDA L. BICKERS,‡ TRAVIS A. HARRIS,‡ AND LEE M. PROCHASKA§

Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268, EPL Bio-Analytical Services, P.O. Box 109, 395 North Memorial Parkway, Harristown, Illinois 62537, and Pioneer Hi-Bred International, Inc., 7250 N.W. 62nd Avenue, Johnston, Iowa 50131

Maize (*Zea mays* L.) plants have been transformed to express a Cry1F insecticidal crystal protein originally isolated from *Bacillus thuringiensis* Berliner. This protein controls lepidopteran pests of maize, including the European corn borer, *Ostrinia nubilalis* (Hübner). As part of the safety assessment for crops containing transgenes, a compositional analysis of the food and feed is conducted. This analysis is designed to detect unintended changes in the nutrient and antinutrient content of the raw commodities produced by the crop due to the insertion of the genes into the genomic DNA of the plant (pleotropic effects). Samples of transgenic and nontransgenic maize forage and grain were collected from six field sites located in the U.S. and Canada. Forage samples were analyzed for proximates and minerals, and grain was further analyzed for fatty acids, amino acids, vitamins, secondary metabolites, and antinutrients. Results demonstrated that maize expressing the Cry1F protein was equivalent to nontransgenic maize with respect to these important components. Comparison of the variability within the nontransgenic and transgenic hybrid, as compared to composition values reported in the literature, suggest that factors other than transgenes may contribute more substantially to the composition of crops.

KEYWORDS: Cry1F; hybrid; maize; seed

INTRODUCTION

Maize (*Zea mays* L.) plants have been transformed to express a Cry1F insecticidal crystal protein originally isolated from *Bacillus thuringiensis* Berliner. This protein controls lepidopteran pests of maize, including the European corn borer, *Ostrinia nubilalis* (Hübner). Transformation event TC1507 expresses the Cry1F protein and has been commercialized under the trade name Herculex I. Currently under development is a second Cry1F transformation event, TC6275, which has an expression pattern that may be useful in some niche pest-control situations.

As part of the safety assessment for crops containing transgenes, a compositional analysis of the food and feed is conducted (*1*, *2*). This analysis is designed to detect unintended changes in the nutrient and antinutrient content of the raw commodities produced by the crop due to the insertion of the genes into the genomic DNA of the plant (pleotropic effects). Samples of transgenic and nontransgenic maize forage and grain were collected from six field sites located in the U.S. and Canada. Forage samples were analyzed for proximates and minerals, and grain was further analyzed for fatty acids, amino acids, vitamins, secondary metabolites, and antinutrients.

Transformation event TC6275 also contains a gene that renders the maize plants resistant to Liberty herbicide (glufosinate−ammonium). Because this herbicide may be used on this transformation event, some regulatory agencies also require a compositional analysis on crops treated with the herbicide. This is designed to determine if the use of the herbicide alters the composition of the crop. In this study, the composition of maize-optimized, Cry1F event TC6275 (with and without treatment with Liberty herbicide) was compared to a nontransgenic control and to composition values for conventionally bred maize as reported in the literature.

MATERIALS AND METHODS

Hybrid Cry1F maize seed was produced by crossing an inbred line containing event TC6275 (Cry1F) to a second nontransgenic inbred line. A genetically similar control hybrid was produced by crossing the same two inbred lines without the transgenes present (nontransgenic control). Seed was planted in conventionally tilled plots between May 15 and June 10, 2002, in a LaHoque loam soil at Rochelle, IL; in a Plano Silt loam soil at Wyoming, IL; in a Brookston/Crosby loam soil at Noblesville, IN; in a Hastings Silt loam soil in York, NE; in a Brant

* Corresponding author [telephone (317) 337-3551; fax (317) 337-3255; e-mail raherman@dow.com].
† Dow AgroSciences LLC.
‡ EPL Bio-Analytical Services.
§ Pioneer Hi-Bred International, Inc.

10.1021/jf049969n CCC: $27.50
Published on Web 04/10/2004

Silt loam soil at Thorndale, Ontario, Canada; and in a Ste-Sophie Sandy loam in Abbotsford, Quebec, Canada. Each field site contained three treatments: a nontransformed control hybrid, a Cry1F transgenic hybrid, and the same Cry1F transgenic hybrid treated with Liberty herbicide. Plots were arranged in a randomized complete block design with three replicate blocks. Plots were two rows wide (0.75 m row spacing) and approximately 7.7 m long. Each plot contained a one-row border on each side, and a minimum of 3 m separated blocks. Planting equipment and maintenance chemical use reflected local conventions. Liberty herbicide was applied to one set of Cry1F plots at the V4−V5 growth stage (June 10 to July 6) at 0.39−0.44 kg ai/ha and again at the V6−V7 growth stage (June 20 to July 18) at 0.47−0.50 kg ai/ha. Hand pollination ensured that sampled grain was self-pollinated.

Forage samples were collected at approximately the R4 growth stage. Samples consisted of three whole plants (aerial portion) per plot. Samples were chopped into sections of less than 5 cm in length and dried until a constant weight was achieved. Grain samples were collected at typical harvest (139−168 days after planting). Five self-pollinated ears were collected from each plot, dried to <15% moisture, and shelled. Samples were stored frozen (< −15 °C) prior to being analyzed.

Compositional Analyses. Compositional analyses were conducted to measure proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins (vitamin E, riboflavin, thiamin, β-carotene, and folic acid), antinutrients (phytic acid and trypsin inhibitor), and secondary plant metabolites (2-furaldehyde, raffinose, inositol, *p*-coumaric acid, and ferulic acid) levels in maize grain. Grain samples were ground prior to analysis to pass a 20-mesh screen. Proximates, ADF, NDF, crude fiber, calcium, and phosphorus levels were determined in maize forage. Forage samples were ground prior to analysis to pass a 1 mm screen. All compositional analyses were performed at EPL Bio-Analytical Services (Harristown, IL). Brief descriptions of the procedures used are given below.

Proximate Analysis. An automated Kjeldahl method was used to determine total nitrogen content. Protein was calculated from nitrogen content with the formula 6.25*N* (*3*). Crude fat content was determined gravimetrically by an acid hydrolysis procedure (*4*). Ash content was determined by gravimetric measurement of the sample residue remaining after ignition in a muffle furnace (*5*). Moisture content of grain was determined by gravimetric measurement of weight loss after drying in a vacuum oven at 100 °C (*6*). Moisture content of forage was determined by gravimetric measurement of weight loss after drying in a forced air oven at 135 °C (*7*). Carbohydrate levels were estimated by use of the fresh weight-derived data and (*8*)

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

Fiber Analysis. An Ankom^{200} fiber analyzer was utilized in the analysis of ADF, NDF, and crude fiber. ADF was determined by digestion in an acid detergent solution (20 g of cetyl trimethylammonium bromide in 1 L of 1 N sulfuric acid) followed by a water rinse. The residue that remained was dried and quantified gravimetrically (*9*). NDF was determined by digestion in a neutral detergent solution (30 g of sodium lauryl sulfate, 18.6 g of ethylenediaminetetraacetic acid, 6.8 g of sodium tetraborate decahydrate, 4.6 g of sodium phosphate dibasic, and 10 mL of triethylene glycol in 1 L of distilled water). The residue that remained was dried and quantified gravimetrically (*10*). Analysis for the determination of crude fiber began with an acetone soak to remove the fat from the samples. The samples were then digested in 0.255 N sulfuric acid solution, followed by digestion in 0.313 N sodium hydroxide solution. The residue was then rinsed, dried, and weighed (*11*).

Fatty Acid Composition. The lipid fraction of grain was extracted with hexane by use of a Soxhlet apparatus. The lipids were saponified with 2% methanolic NaOH followed by methylation with 12% BF_3/methanol reagent. The fatty acid methyl esters were extracted with hexane and analyzed by gas chromatography with flame ionization detection (GC/FID). External standards were used to calibrate the GC/FID system (*12*−*14*).

Minerals. Levels of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) by an AOAC method (*15*). Grain and forage samples were dry-ashed in a muffle furnace at 650 °C. The resulting ash was dissolved in hot 50% nitric acid and diluted with deionized water. Emission intensity at the appropriate wavelength was determined for each element and compared to that of external standards to obtain quantitative results.

Amino Acid Composition. Amino acids in grain were determined by three methods (*16*−*18*). The procedure for tryptophan required base hydrolysis with lithium hydroxide. Analysis of tryptophan was performed by reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet detection. The sulfur-containing amino acids, cystine and methionine, required oxidation with performic acid prior to hydrolysis with hydrochloric acid. The remaining 15 amino acids were directly hydrolyzed with hydrochloric acid. The free amino acids liberated by acid hydrolysis were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and analyzed by HPLC with fluorescence detection.

2-Furaldehyde. Levels of 2-furaldehyde were determined by extraction with methanol and reverse-phase HPLC with ultraviolet detection (*19*). External standards were used to calibrate the HPLC system.

Inositol. Levels of inositol were determined by a derivatization procedure reported by Knapp (*20*). Samples were extracted with a mixture of ethanol and water. An aliquot of the extract was filtered, diluted with the ethanol−water solvent, and evaporated to dryness under a flow of nitrogen on a heating block. Standard solutions of inositol were evaporated along with samples for use as instrument calibration standards. Inositol in the dried residue was derivatized with butylboronic acid in pyridine. The derivatized extract was then analyzed by GC/FID.

Phytic Acid. Levels of phytic acid were determined by a modified version of an AOAC method (*21*). Phytic acid was extracted from the samples with dilute hydrochloric acid. The extracted phytate was isolated on an anion-exchange solid-phase extraction cartridge and eluted with dilute sodium chloride solution. The eluted phytate was then measured indirectly by determining the elemental phosphorus concentration by ICP-OES. The phytic acid concentration was calculated from the elemental phosphorus result by use of a molecular weight conversion factor.

Tocopherols. Tocopherols (α, β, γ, and δ) were extracted with hot hexane in a sealed glass vessel. The hexane extract was cooled, filtered, and analyzed by normal-phase HPLC with fluorescence detection (*22*). External standards were used to calibrate the HPLC system.

β-Carotene. β-Carotene content was determined by an AOAC method (*23*). Samples were extracted with an acetone/hexane solution. The extracts were assayed with a UV/visible spectrophotometer. The spectrophotometer was calibrated with external standards.

Trypsin Inhibitor. Trypsin inhibitor activity was determined by an approved method of the American Oil Chemists' Society (AOCS) (*24*). Samples were extracted with sodium hydroxide. Trypsin was added and reacted with the trypsin inhibitor in the extract. Unreacted trypsin in the extract was measured spectrophotometrically. Results were expressed in trypsin inhibitor units (TIU).

Raffinose. Raffinose was determined by an approved method of the American Association of Cereal Chemists (AACC) (*25*). Raffinose was extracted from the grain with a hot aqueous methanol solution. After cooling, the extract was filtered and analyzed by normal-phase HPLC with refractive index detection. External standards were used to calibrate the HPLC system.

Riboflavin and Thiamin. Thiamin (vitamin B1) and riboflavin (vitamin B2) were analyzed with modifications of an approved method of the AACC and a publication issued by Supelco (*26*, *27*). The B vitamins were extracted from grain with a 10% acetic acid solution. This extract was filtered and riboflavin was determined directly by HPLC with fluorescence detection. External standards were used to calibrate the HPLC system. Thiamine was determined by derivatizing an aliquot of the grain extract with potassium ferricyanide to produce thiochrome. Thiamine standard solutions were also derivatized for use

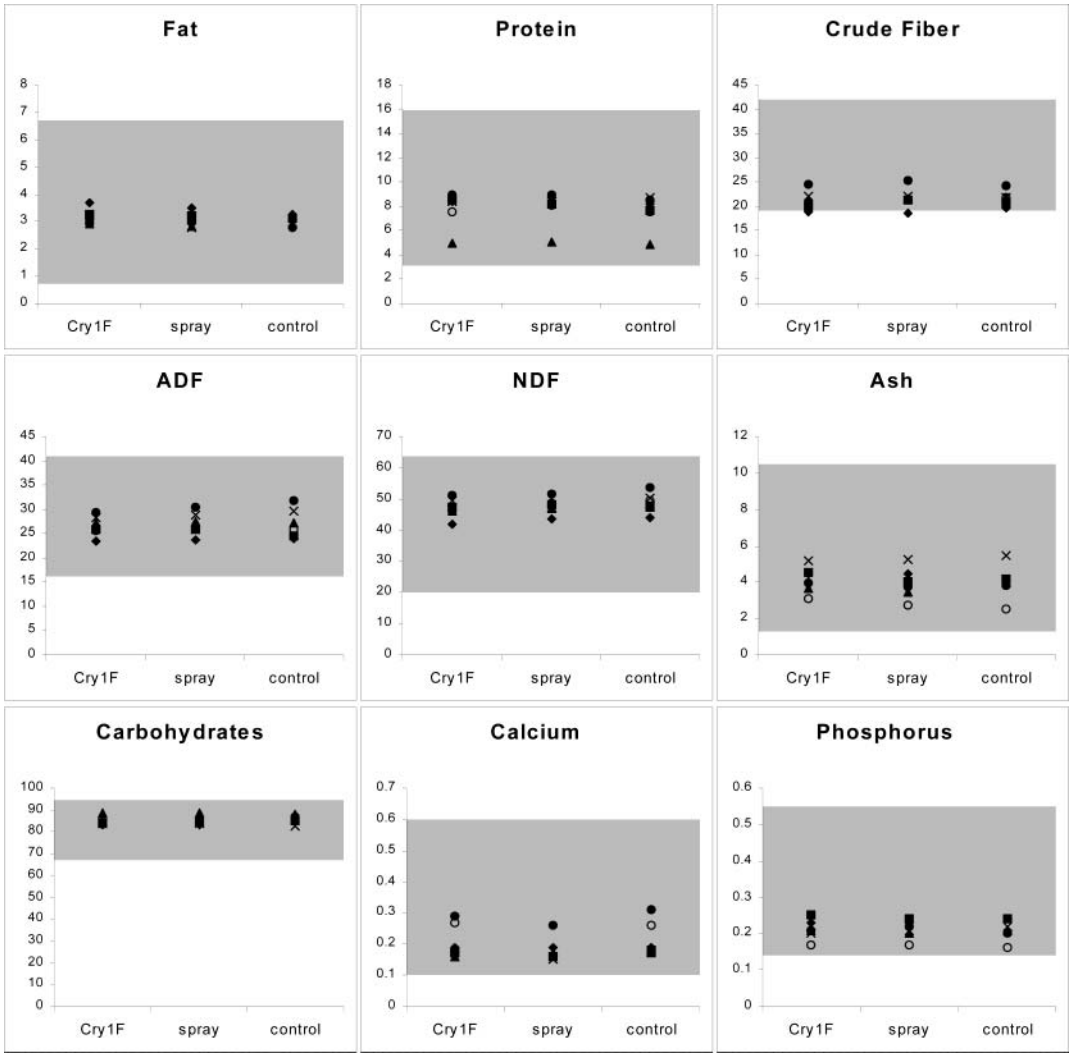

Figure 1. Proximates and minerals (percent dry weight) in Cry1F forage (Cry1F), glufosinate-treated Cry1F forage (spray), and nontransgenic forage (control). Means at each location shown: (◆) Rochelle, IL; (■) Wyoming, IL; (▲) Noblesville, IN; (×) York, NE; (○) Ontario, Canada; (●) Quebec, Canada. Literature ranges are shaded. ADF, acid detergent fiber; NDF, neutral detergent fiber.

as instrument calibration standards. Thiochrome was then determined by HPLC with fluorescence detection.

Ferulic and p-Coumaric Acids. Phenolic acids were determined by published methods (*28*−*31*). Grain was hydrolyzed with sodium hydroxide. The hydrolysate was acidified and extracted with an ethyl ether/ethyl acetate mixture. After evaporation of the extraction solvent, the residue was redissolved in acetonitrile and analyzed by reverse-phase HPLC with UV detection. External standards containing both phenolic acids were used to calibrate the HPLC system.

Folic Acid. Folic acid was determined by an approved method of the AACC. (*32*) Ground maize grain was subjected to hydrolysis and digestion by protease and amylase enzymes to release the folates from the grain. A conjugase enzyme was used to convert the naturally occurring folypolyglutamates to folydiglutamates. An aliquot of the extracted folates was mixed with a folate-free microbiological growth medium. The mixture was inoculated and incubated with *Lactobacillus casei*, subspecies rhamnosis. The total folate content was determined by measuring the turbidity of the *L. casei* subspecies rhamnosis growth response in the sample and comparing it to the turbidity of the growth response in the folic acid standards.

Statistical Analysis. Analysis of variance was conducted across the six field locations wtih a mixed model. Locations and replicates within a location were each treated as random variables. The Cry1F maize treatment and the Liberty-sprayed Cry1F treatment were each compared to the nontransgenic control by a *t*-test with *p* values adjusted via a Dunnett procedure (*33*). The denominator degrees of freedom were calculated by a general Satterthwaite approximation. Significant differences are declared at the 95% confidence level.

Figures were constructed where the mean value (at each location) for each analyte and matrix was plotted along with published ranges for the analyte in conventional maize (*34*−*37*) (**Figures 1**−**7**).

RESULTS AND DISCUSSION

Forage samples were analyzed for seven proximates and two minerals (**Figure 1**). The Cry1F forage that was treated with Liberty had significantly ($\alpha = 0.05$) less calcium (0.200% dry weight) than the nontransgenic control (0.216% dry weight), but the Cry1F forage that was not treated with Liberty was not significantly different than the nontransgenic control (0.206% dry weight). Although significantly different, the calcium level in the Liberty-treated Cry1F forage differed from the nontransgenic control by less than 7.5%. The Cry1F forage that was not treated with Liberty had significantly less neutral detergent fiber (NDF) (46.97% dry weight) compared to the nontransgenic control (48.75% dry weight), but the Liberty-treated Cry1F forage (47.64% dry weight) was not significantly different from the nontransgenic control. Although significantly different, the NDF content of the Cry1F forage that was not treated with Liberty differed from the nontransgenic control by less than 4%. Mean values for both calcium and NDF were within the

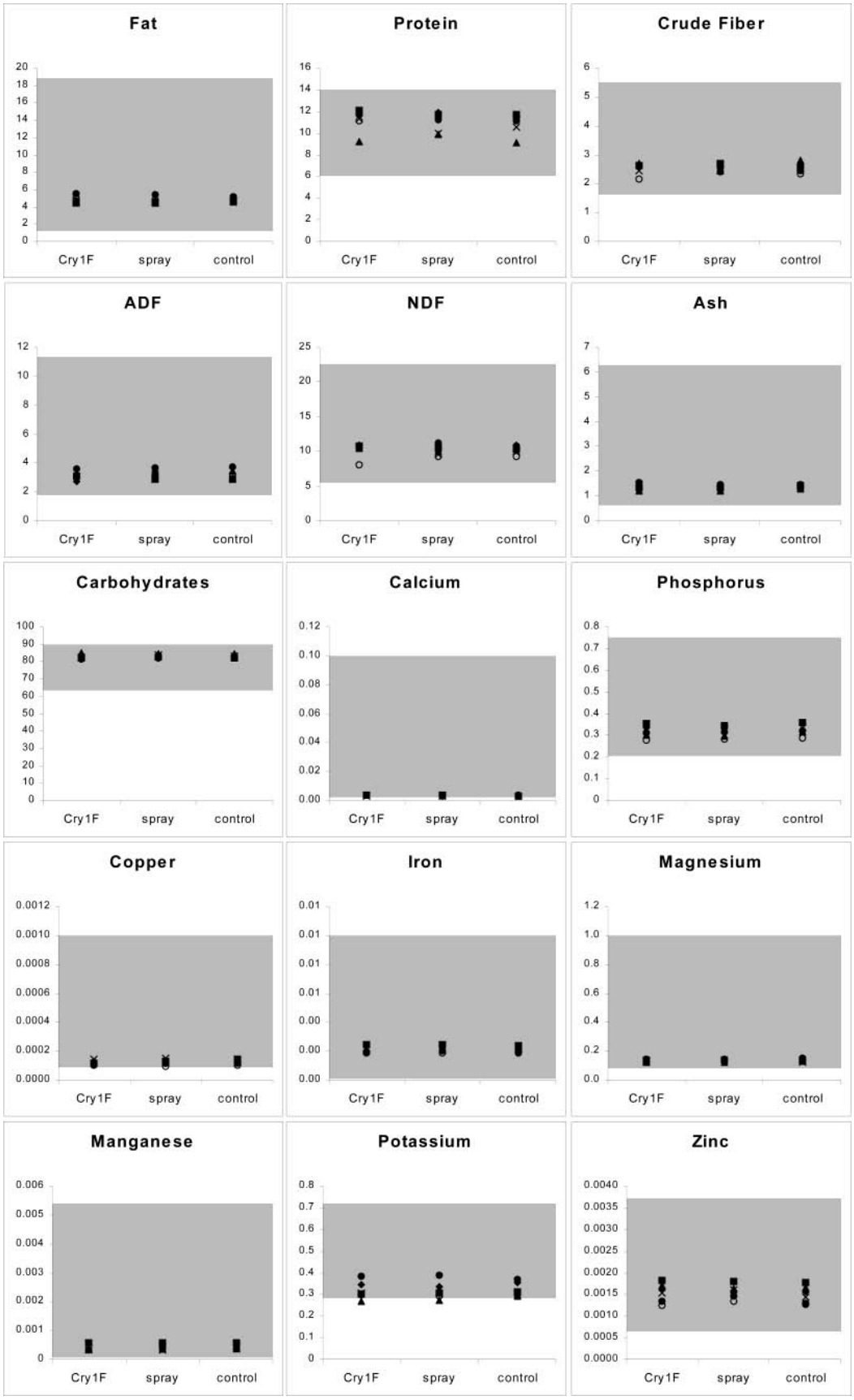

Figure 2. Proximates and minerals (percent dry weight) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**. Sodium was also measured in grain, but levels were below detection in all samples (<0.0005% dry weight).

range of values reported in the literature for all treatments (**Figure 1**). In no case were the Cry1F forage and Liberty-treated Cry1F forage values both significantly different from the nontransgenic control forage. Where significant differences

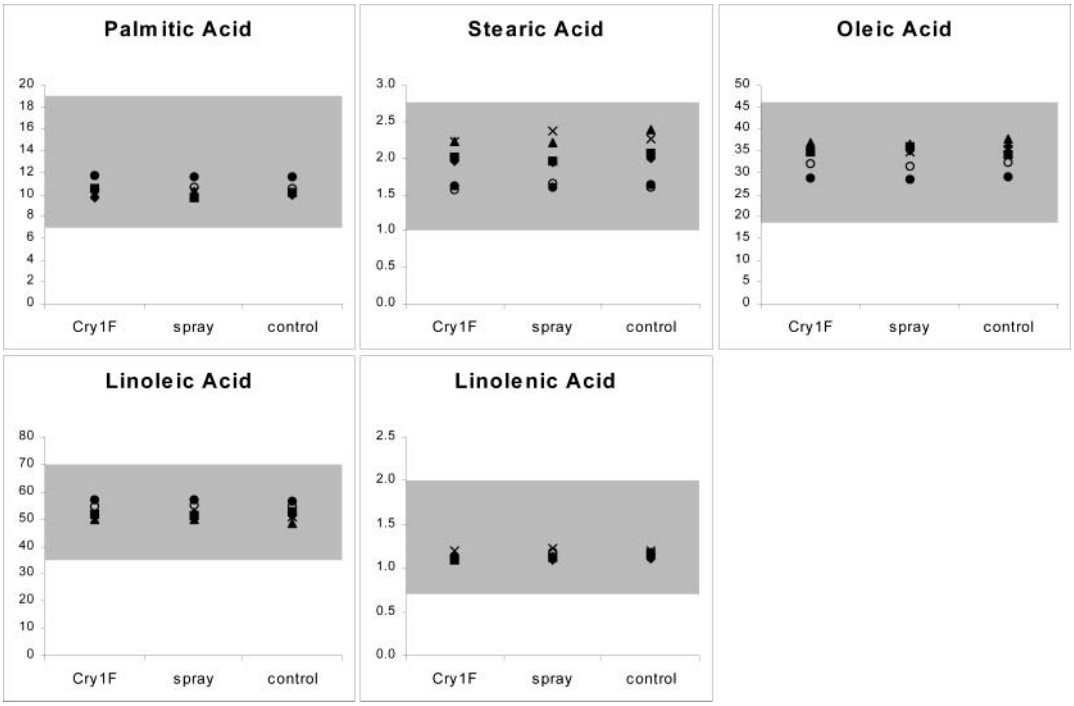

Figure 3. Fatty acids (percent of total fatty acids) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**.

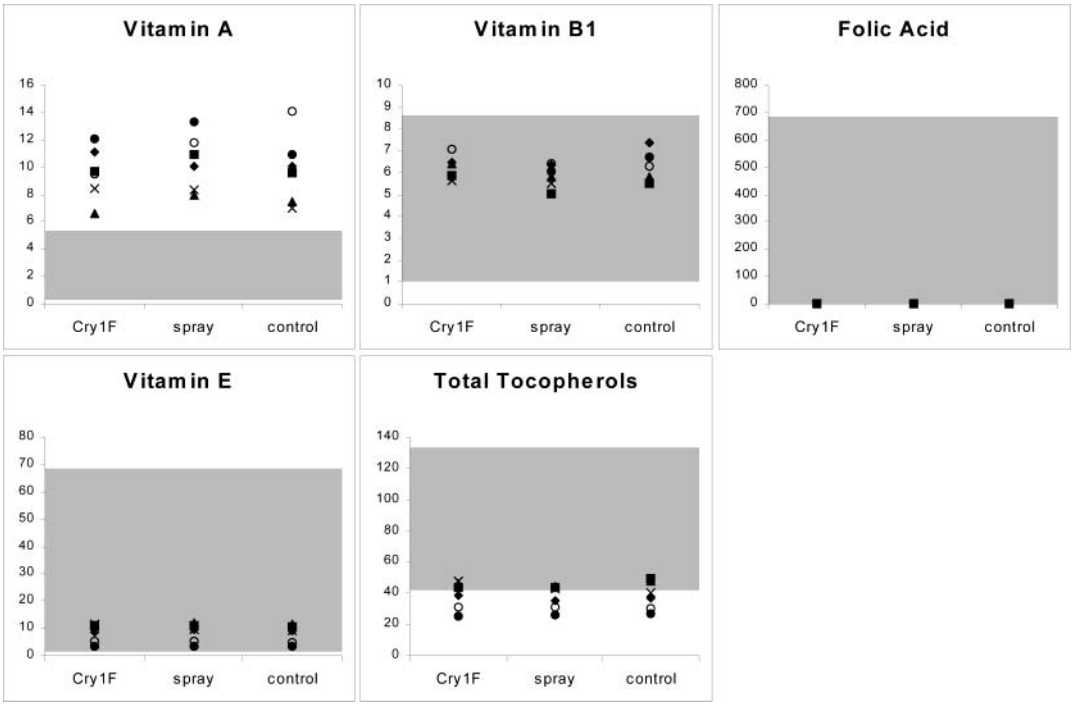

Figure 4. Vitamins (milligrams per kilogram of dry weight) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**. Grain samples were also analyzed for vitamin B2, but this analyte was not detected at any location (<1 mg/kg of dry weight).

occurred for the forage analyses, less than a 7.5% deviation from the nontransgenic control was seen. It is apparent from **Figure 1** that factors other than the transgenes are largely responsible for the levels of proximates and minerals in forage. On the basis of this analysis, the two Cry1F treatments and the nontransgenic control appear to produce compositionally equivalent forage.

Grain samples were analyzed for seven proximates and nine minerals (**Figure 2**). No significant treatment effects were seen for the proximates ($\alpha = 0.05$). The Liberty-treated Cry1F grain had significantly less phosphorus (0.319% dry weight) than the nontransgenic control (0.330% dry weight), but the non-Liberty-treated Cry1F grain (0.322% dry weight) was not significantly different from the nontransgenic control. The Liberty-treated Cry1F grain differed from the nontransgenic control by less than 4%. Phosphorus levels for all treatments were within the ranges reported in the literature. Calcium values were lower than those reported in the literature for all treatments including the

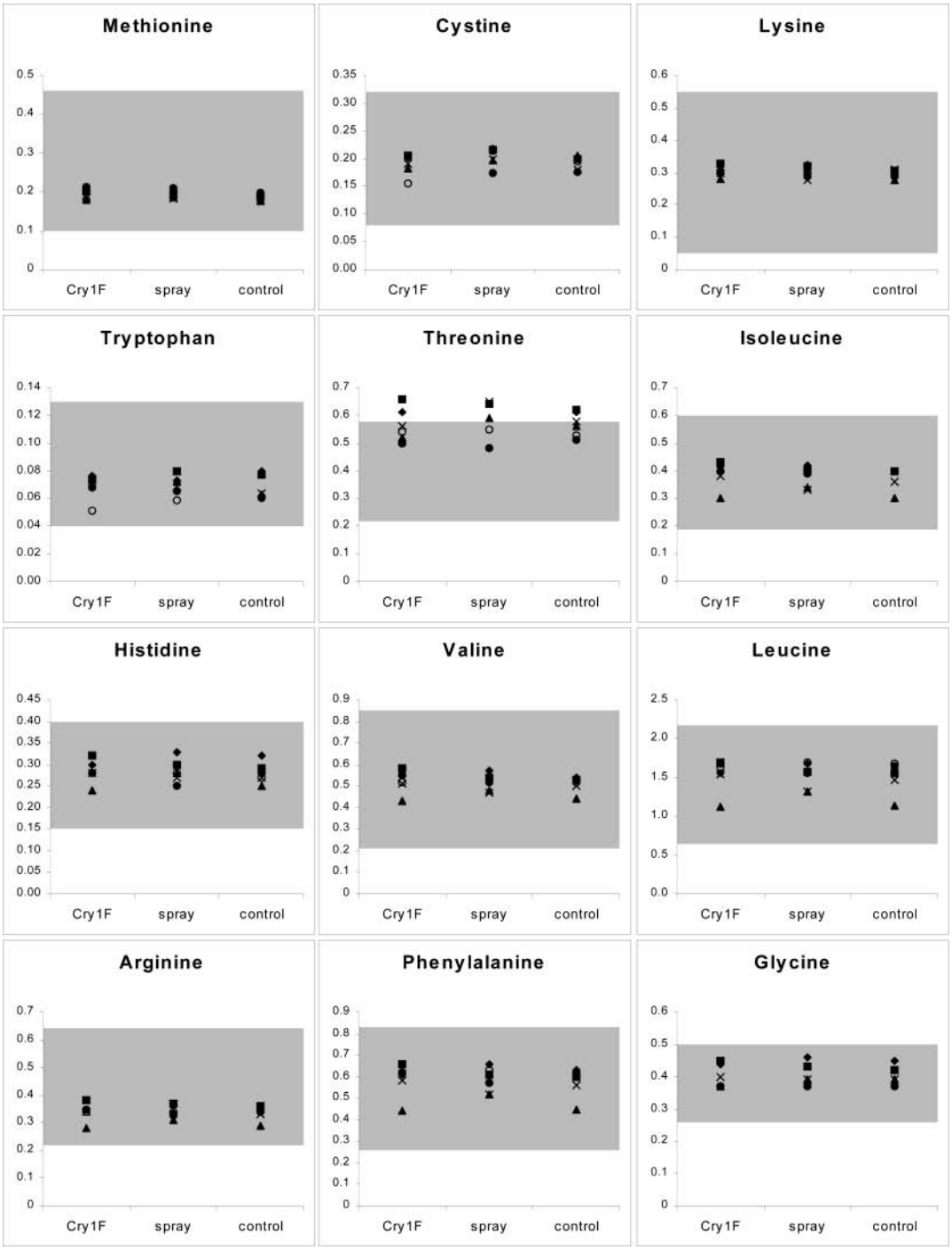

Figure 5. Essential amino acids (percent dry weight) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**.

nontransgenic control, indicating that this was not an effect of the transgenes. Sodium was also measured in grain, but levels were below detection (<0.0005% dry weight), so results are not illustrated in **Figure 2**. This is consistent with literature values for sodium in maize grain (0−0.15%) (*36*). It is clear from Figure 2 that factors other than the transgenes are largely responsible for the levels of proximates and minerals in grain. On the basis of the proximate and mineral analyses, the two Cry1F treatments and the nontransgenic control appear to produce compositionally equivalent grain.

Grain samples were further analyzed for five fatty acids (**Figure 3**), six vitamins (**Figure 4**), 12 essential amino acids (**Figure 5**), six nonessential amino acids (**Figure 6**), and five secondary metabolites and two antinutrients (**Figure 7**). No significant treatment effects were detected for any of these analytes ($\alpha = 0.05$). The vitamin A content (measured as β-carotene) for the grain samples was above the values reported in the literature for conventional maize for all treatments, including the nontransgenic control (**Figure 4**), indicating that this was not caused by the transgenes. The total tocopherols in the grain samples from all treatments, including the nontransgenic control, were generally lower than those reported in the literature, also indicating that this deviation was not caused by the transgenes. All other analyses indicated similarity to conventionally bred maize. Grain samples were also analyzed for vitamin B2, but this analyte was not detected in any samples

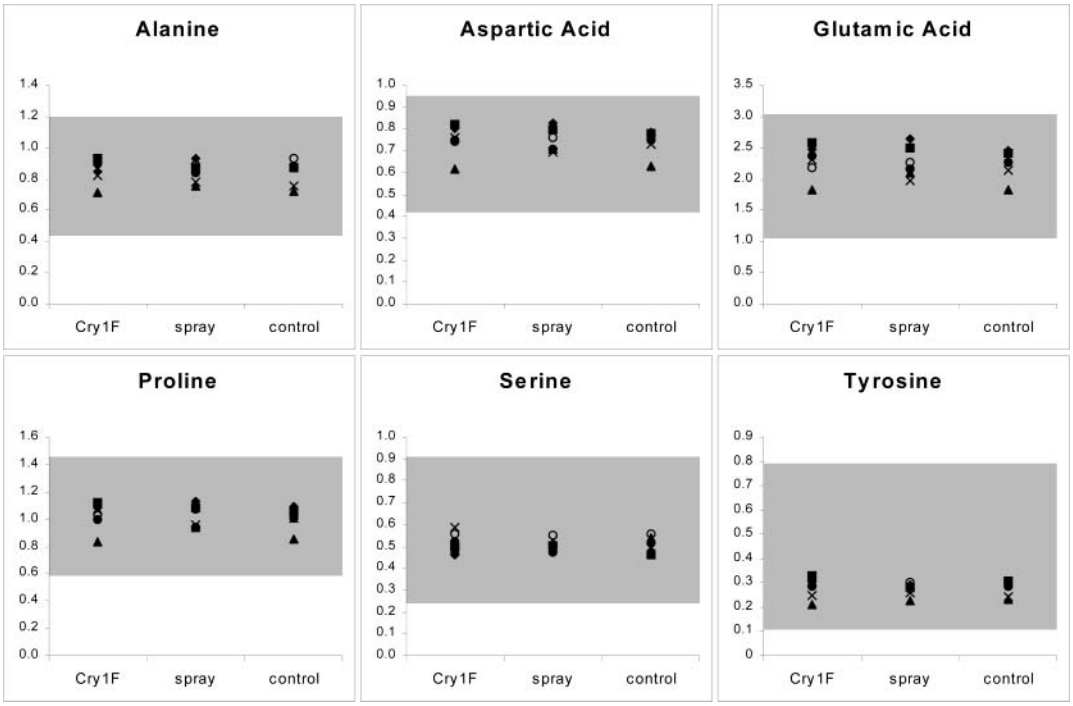

Figure 6. Nonessential amino acids (percent dry weight) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**.

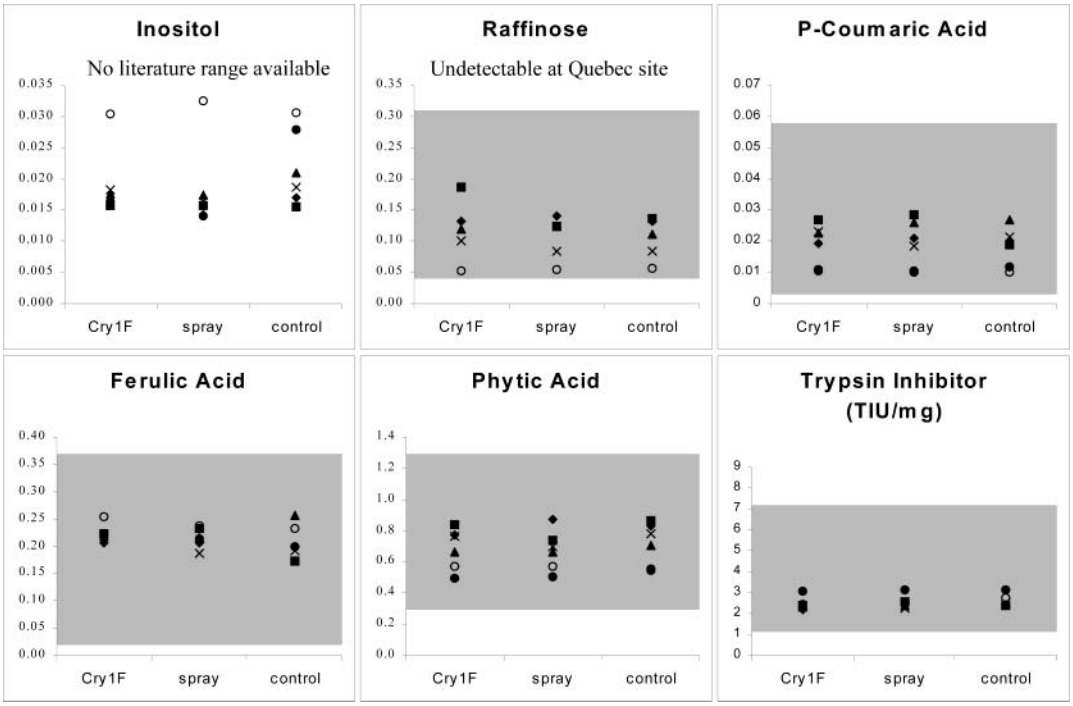

Figure 7. Secondary metabolites and antinutrients (percent dry weight except as indicated) in Cry1F grain (Cry1F), glufosinate-treated Cry1F grain (spray), and nontransgenic grain (control). Symbols and notation are as described for **Figure 1**. TIU, trypsin inhibitor units. Grain samples were also analyzed for furfural, but levels were below detection in all samples (<0.0001% dry weight).

(<1 mg/kg of dry weight) and thus results are not illustrated in **Figure 4**. Furfural levels were also below detection in all samples (<0.0001% dry weight) and thus results are not depicted in **Figure 7**. Literature values for the furfural content in maize grain are not available. It is clear from **Figures 3**−**7** that factors other than the transgenes are largely responsible for the levels of fatty acids, vitamins, amino acids, secondary metabolites, and antinutrients in grain. On the basis of these analyses, the two Cry1F treatments and the nontransgenic control appear to produce compositionally equivalent grain.

A total of 61 compositional analyses were completed across forage and grain samples for each treatment. Forage and grain samples were collected from six sites in North America and from each of three replicates at each site. Fifty-eight of these analytes were amenable to a statistical analysis, with three analytes being undetectable in all samples. Of the 58 analyses,

three treatment effects were detected at the 95% confidence level, which is consistent with random chance (5%). Also, for no analyte did both Cry1F treatments (with and without Liberty treatment) differ significantly from the nontransgenic control, and for these significant differences, deviations from the nontransgenic control were all less than 7.5%, and were all within the published ranges for conventionally bred corn.

The horizontal alignment of the analyte values in **Figures 1−7** clearly illustrates the similar composition of the Cry1F, Liberty-treated Cry1F, and nontransgenic control. The consistency of the analyte values for the treatments evaluated here, relative to many of the ranges of values reported in the literature, indicate that factors other than the transgenes and Liberty treatments are largely responsible for the variability in the composition of maize forage and grain.

Equivalency between the composition of crops expressing insecticidal crystal proteins and/or herbicide-inactivating enzymes and the composition of conventionally bred crops is expected. The insertion of a small number of well-characterized genes into a genome of nearly 50 000 genes (*38*) would not be predicted to have a substantial unexpected effect on the composition of a crop. This is especially true because only transgenic events that are agronomically satisfactory and that show nutritional equivalence to conventional maize in animal performance studies are selected for commercial development. These latter studies eliminate transformation events that might affect the nutrition of the crop or its agronomic performance based on whole-organism evaluations.

Traditional breeding techniques rely on genetic recombination to create new variation, and many genes of unknown function are often selected to achieve a desired phenotype. The range in composition values seen for conventionally bred maize highlights the degree of variation that currently exists in commercial hybrids. The data presented in **Figures 1−7** indicate that background genetics and/or growing conditions may largely contribute to differences in crop composition. Likewise, the use of many different types of herbicides in conventional crops makes it unlikely that a new herbicide used to control weeds in a transgenic crop will cause compositional effects outside of the normal range. The likelihood that the variation would be extreme enough to be detrimental is even more remote.

Compositional equivalency studies are designed to determine if the insertion of transgenes affects the food and feed quality of a crop. As we continue to generate compositional equivalency data in the evaluation of transgenic events, consideration of the value of the studies will be important. The cost of a compositional study typically approaches $200 000 for each growing season within each country, and studies in North America, Europe, and Argentina are currently required for registering products globally (with 2 years of data required for some countries). In the future, alternative approaches to safety and equivalency evaluations, balanced with potential risk, should be considered to determine if the scope of current compositional equivalency studies provides a benefit, beyond that provided by agronomic and animal performance studies, that is commensurate with their cost.

ACKNOWLEDGMENT

We thank Bob Pauli, Aaron DeVries, Patrick Garcia, and Annette Leslie for technical assistance during the field portion of this study. We also thank Penny Hunst, Katherine Armstrong, Don Stuart, Brad Shurdut, Ron Meeusen, and John Cuffe for reviewing the draft manuscript.

LITERATURE CITED

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

(2) Sidhu, R. S.; Hammond, B. G.; Fuchs, R. L.; Mutz, J.; Holden, L. R.; Goerge, B.; Olson, T. Glyphosate-tolerant corn: the composition and feeding value of grain from glyphosate-tolerant corn is equivalent to that of conventional corn (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2305−2312.

(3) Foss-Tecator. In *Kjeltec 2300 Site Preparation, Installation and Operating Guide*; Foss-Tecator AB: Hoganas, Sweden, 1999.

(4) AOAC. Fat in flour. Method 922.06. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 32, p 5.

(5) AOAC. Ash of flour. Method 923.03. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 32, p 2.

(6) AOAC. Solids (total) and moisture in flour. Method 925.09. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 32, p 1.

(7) AOAC. Loss on drying (moisture) for feeds. Method 930.15. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.: Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 4, p 2.

(8) USDA. Energy value of foods. In *Agriculture Handbook No. 74*; United States Department of Agriculture: Washington, DC, 1973; pp 2−11.

(9) Ankom Technology. Method for determining acid detergent fiber. In *Operator's Manual Ankom$^{200/220}$ Fiber Analyzer*; Ankom Technology: Fairport, NY, 1999; pp 9−10.

(10) Ankom Technology. Method for determining neutral detergent fiber. In *Operator's Manual Ankom$^{200/220}$ Fiber Analyzer*; Ankom Technology: Fairport, NY, 1999; pp 11−12.

(11) Ankom Technology. Method for determining crude fiber. In *Operator's Manual Ankom$^{200/220}$ Fiber Analyzer*; Ankom Technology: Fairport, NY, 1999; pp 15−16.

(12) AOAC. Fat acidity-grains. Method 939.05. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 32, p 34.

(13) AOCS. Determination of fatty acids in edible oils and fats by capillary GLC. Method Ce 1e-91. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; Firestone, D., Ed.; American Oil Chemist's Society: Champaign, IL, 1997.

(14) AOCS. Preparation of methyl esters of fatty acids. Method Ce 2−66. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; Firestone, D., Ed.; American Oil Chemist's Society: Champaign, IL, 1997.

(15) AOAC. Determination of lead, cadmium, copper, iron and zinc in foods. Method 999.11. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt, 9, p 19.

(16) Liu, H. J. Determination of amino acids by precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. A* **1994**, *670*, 59−66.

(17) Waters Method, Analysis of Amino Acids in Feeds and Foods Using a Modification of the ACCQ Tag Method for Amino Acid Analysis.

(18) Togers, Stephen R.; Pesti, Gene M. Determination of Tryptophan from Feedstuffs using Reverse Phase High-Performance Liquid Chromatography. *J. Micronutr. Anal.* **1990**, *7*, 27−35.

(19) Bredie, W. L. P.; Mottram, D. S.; Guy, R. C. E. Aroma volatiles generated during extrusion cooking of maize flour. *J. Agric. Food Chem.* **1998**, *46*, 1479−1487.
(20) Knapp, D. R. *Handbook of Analytical Derivatization Reactions*; Wiley: New York, 1979: p 562.
(21) AOAC. Phytate in foods. Method 986.11. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 32, pp 57−58.
(22) Anonymous. High performance liquid chromatography of tocols in corn grain. *J. Am. Oil Chem. Soc*. **1984**, *61*(7).
(23) AOAC. Carotene in fresh plant materials and silages. Method 941.15. In *Official Methods of Analysis of AOAC International*, 17th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists International: Gaithersburg, MD, 2000; Chapt. 45, pp 45−46.
(24) AOCS. Trypsin inhibitor activity. Method Ba 12-75. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 1997.
(25) AACC. Determination of simple sugars in cereal products—HPLC method. Method 80−04. In *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; American Association of Cereal Chemists: Minneapolis, MN, 2000.
(26) Supelco. Applications, vitamins, water soluble (HPLC). In *Supelco Chromatography Products Guide*; Sigma−Aldrich Company: St. Louis, MO; p 543.
(27) AACC. Thiamine−thiochrome method. Method 86-80. In *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; American Association of Cereal Chemists: Minneapolis, MN, 2000.
(28) Figueroa-Espinoza, M.-C.; Morel, M.-H.; Rouau, X. Effect of lysine, tyrosine, cysteine, and glutathione on the oxidative cross-linking of feruloylated arabinoxylans by a fungal laccase. *J. Agric. Food Chem.* **1998**, *46*, 2583−2589.
(29) Classen, D.; Arnason, J. T.; Serratos, J. A.; Lambert, J. D. H.; Nozzolillo, C.; Philogene, B. J. R. Correlation of phenolic acid content of maize resistant to *Sitophilus zeamais*, the maize weevil, in CMMYT'S collections. *J. Chem. Ecol.* **1990**, *16*, 301−315.
(30) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, *30*, 330−334.
(31) Sosulski, F.; Krygier, K.; Hogge, L. Free, esterified and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereals and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 337−340.
(32) AACC. Total folate in cereal products—microbiological assay using trienzyme extraction. Method 86−47. In *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; American Association of Cereal Chemists: Minneapolis, MN, 2000.
(33) SAS Institute. *SAS/STAT User's Guide*, Version 8; SAS Institute: Cary, NC, 1999.
(34) OECD. *Consensus Document on Compositional Considerations for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-nutrients, and Secondary Plant Metabolites*; Organization of Economic Co-operation and Development: 2002.
(35) Watson, S. A. Corn: Amazing maize. General properties. In *CRC Handbook of Processing and Utilization in Agriculture, Vol. II: Part 1 Plant Products*; Wolff, I. A., Ed.; CRC Press: Boca Raton, FL, 1982; pp 3−29.
(36) Watson, S. A. Structure and composition. In *Corn: Chemistry and Technology*; Watson, S. A., Randsted, P. E., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987; pp 53−82.
(37) ILSI (International Life Sciences Institute). ILSI Crop Composition Database, Version 1.0, www.cropcomposition.org, 2003.
(38) Bennetzen, J. L.; Chandler, V. L.; Schnable, P. National Science Foundation-sponsored workshop report. maize genome sequence project. *Plant Physiol.* **2001**, *127*, 1572−1578.

Received for review January 6, 2004. Revised manuscript received March 3, 2004. Accepted March 4, 2004.

JF049969N