MONSANTO

Food and Feed Safety and Nutritional Assessment of MON 87460 Drought Tolerant Corn (OECD Unique Identifier MON-8746Ø-4)

Conclusion Based on Data and Information Evaluated According to FDA's Policy on Foods from New Plant Varieties

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Certification

Monsanto Company is submitting this food and feed safety and nutritional assessment in compliance with the FDA's 1992 policy statement regarding foods derived from new plant varieties (57 FR 22984). At the agency's request, and where appropriate, this submission also complies with the recommendations contained in the proposed rule for Premarket Biotechnology Notice (PBN) Concerning Bioengineered Foods (66 FR 4706).

Specifically, as recommended in the proposed 21 CFR §192.25(a), the undersigned attests to the following:

1. It is the view of Monsanto Company (hereafter referred to as Monsanto) that: (i) MON 87460 is as safe and nutritious as other commercially available corn; and (ii) the intended uses of the food and feed derived from MON 87460 are in compliance with all applicable requirements of the Federal Food, Drug and Cosmetic Act.
2. Monsanto will make available to FDA, upon request, relevant data or other information not included in this submission, either during the course of FDA's evaluation of the submission, or for cause.
3. Monsanto will make relevant data or other information not included in this submission available to FDA either: (i) by allowing FDA to review and copy these data or information at Monsanto's offices in St. Louis, MO, during customary business hours; or (ii) by sending a copy of these data or information to FDA.
4. Monsanto makes no claim of confidentiality regarding either the existence of this submission, or any of the data or other information contained herein. However, Monsanto reserves the right to make a claim of confidentiality regarding any relevant data or other information not included in this submission, but requested by FDA, either in the course of its review of this submission, or for cause. Any such claim of confidentiality will be made at the time such data or information is provided, along with an explanation for the basis of the claim.
5. To the best of Monsanto's knowledge, this submission is representative and balanced, including information, unfavorable as well as favorable, pertinent to the evaluation of the safety, nutritional, or other regulatory issues that may be associated with MON 87460.

Signature: Date:

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Release of Information

Monsanto is submitting the information in this assessment for review by the FDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party except to the extent it is requested under the Freedom of Information Act (FOIA), 5 U.S.C., § 552; FDA complies with the provisions of FOIA and FDA's implementation regulations (21 CFR Part 20); and this information is responsive to the specific request. Except in accordance with the Freedom of Information Act, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

Abbreviations[1], Acronyms and Definitions

1× LB	Laemmli Buffer [62.5mM Tris-HCl, 5% (v/v) 2-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate, 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol, pH 6.8]
5× LB	Five times concentrated 1× LB
6-FAM	6-carboxyfluorescein
35S	Promoter and leader from the Cauliflower mosaic virus (CaMV) 35S RNA
AA	Amino Acid
AACC	American Association of Cereal Chemists
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7
AD8	Allergen, gliadin, and glutenin protein sequence database
ADF	Acid Detergent Fiber
AEC buffer	Buffer solution of 20 mM Tris-HCl, pH 7.0
ALLERGEN-SEARCH	Computer program for the search against known allergens
ALLPEPTIDES	Protein sequence database comprised of NRAA and SwissProt databases
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists Society
APHIS	Animal and Plant Health Inspection Service
APS	Analytical Protein Standard
ATP	Adenosine triphosphate
B	Border
Blp I	Restriction enzyme isolated from *Xanthomonas badrii*
bp	Base Pair
BSA	Bovine Serum Albumin
B. subtilis	*Bacillus subtilis*
bu/ac	Bushels per Acre
bw	Body Weight
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations

[1] Standard abbreviations, e.g., units of measure, are used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry*.

CI	Confidence Interval
CL	Chile field production trial location Colina
CS	Coding Sequence
CSD	Cold Shock Domain
CSFII	Continuing Surveys of Food Intakes by Individuals
CSP	Cold Shock Protein
cspB	Coding sequence for CSPB from *B. subtilis*
CSPB	Cold Shock Protein B from *B. subtilis*
CT	Chile field production trial location Calera de Tango
CTAB	Cetyltrimethylammonium bromide
DAP	Days After Planting
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
DEEM-FCID	Dietary Exposure Evaluation Model Food Commodity Intake Database
DF	Dilution Factor
DLP	Dual Labeled Probe
dNTP	Deoxyribonucleotide triphosphate, a generic term referring to the four deoxyribonucleotides: dATP, dCTP, dGTP (deoxyguanosine triphosphate) and dTTP (deoxythymidine triphosphate)
ds	Double stranded
dT	Deoxythymidine
DW or dw	dry weight
DWCF	Dry Weight Conversion Factor
dwt	dry weight of tissue
E. coli	*Escherichia coli*
*Eco*O109 I	Restriction enzyme isolated from *E. coli*
EcoR V	Restriction enzyme isolated from *E. coli*
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EST	Expressed Sequence Tag
FA	Fatty Acid
FASTA	Algorithm used to find local high scoring alignments between a

	pair of protein or nucleotide sequences
FDA	Food and Drug Administration
FR	Federal Register
FW or fw	Fresh Weight
fwt	fresh weight of tissue
GRAS	Generally Recognized as Safe
H29	Histidine 29
HI	Harvest Index
*Hin*d III	Restriction enzyme isolated from *Haemophilus influenzae*
HPLC	High-performance liquid chromatography
HRP	Horseradish Peroxidase
I	Intron
IAE	U.S. field production trial location Benton County, Iowa
IAW	U.S. field production trial location Greene County, Iowa
ILSI-CCD	International Life Sciences Institute Crop Composition Database
IL	U.S. field production trial location Stark County, Illinois
IN	U.S. field production trial location Parke County, IN
I-*Ract1*	Intron from the rice actin gene
kb	kilobase
KS	U.S. field production trial location Pawnee County, Kansas
L	Leader
LUM	Chile field production trial location Lumbreras
L2V	Amino Acid Change in MON 87460–produced CSPB that substitutes lysine in position two to valine
Left Border	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA
LOD	Limit of Detection
LOQ	Limit of Quantitation
loxP	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase
MAFF	Ministry of Agriculture, Forestry and Fisheries (Japan)
MALDI-TOF	Matrix Assisted Laser Desorption Ionization - Time of Flight
MH+	Protonated mass ion
MHLW	Ministry of Health, Labor and Welfare (Japan)
MOE	Margin of Exposure

MON 863	A Monsanto corn product, producing the insecticidal *Bacillus thuringiensis* Cry3Bb1 protein and NPTII protein
MON 87460	A Monsanto corn product, and the subject of this application, which produces CSPB and NPTII proteins
mRNA	Messenger RNA
MS	Mass Spectrometry
MT/ha	Metric Tons Per Hectare
MWCO	Molecular weight cut-off
MW	Molecular Weight
n	Number of Observations
N/A	Not applicable
NA	Not available
NCGA	National Corn Grower's Association
NDF	Neutral Detergent Fiber
NE	U.S. field production trial location York County, Nebraska
NFDM	Non-fat Dry Milk
NOAEL	No Observable Effect Level
Not	Restriction enzyme isolated from *Nocardia otitidis*
nptII	Coding sequence of neomycin phosphotransferase II gene that confers resistance to neomycin and kanamycin
NPTII	Neomycin phosphotransferase II
OECD	Organization for Economic Co-operation and Development
OR	Origin of replication
ori-pBR322	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
ori V	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2
OSL	Overseason Leaf
OSR	Overseason Root
OSWP	Overseason Whole Plant
P	Promoter
PAR	Photosynthetically Active Radiation
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline containing Tween-20
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank

ppm	parts per million
PRESS	Predicted residual sums of squares
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene Difluoride
PVP	Polyvinyl Pyrrolidone
PV-ZMAP595	Plasmid vector used to develop MON 87460
QUI	Chile field production trial location Quillota
Ract1	The rice actin gene
Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
RNP	Ribonucleoprotein
RK2	Broad host range bacterial plasmid
rop	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
ROP	Repressor of primer protein
RP-HPLC	Reverse phase high-performance liquid chromatography
SAS®	Originally an acronym for Statistical Analysis System, now an integrated system of software products provided by the SAS Institute, Inc. headquartered in Cary, North Carolina, USA
SAX	Silica-based Anion Exchange
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
S.E.	Standard Error
SEQ	Sequential digestion assay (sequential treatment with SGF and SIF)
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SOP	Standard Operating Procedure
sp.	Species
ss	Single stranded
subsp.	Subspecies
SwissProt	A public protein database maintained by the Swiss Institute of Bioinformatics, Geneva, Switzerland, and the European Molecular Biology Laboratory
Taq	*Thermus aquaticus*, a thermophilic bacterium
T	Terminator (where used as a prefix to a gene sequence)

TCA	Trichloroacetic Acid
T/C/R	Test/control/Reference materials
T-DNA	Transfer DNA
TDF	Total Dietary Fiber
TE buffer	Tris-EDTA buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0, 1M NaCl)
TFA	Trifluoroacetic Acid
Ti	Tumor-inducing
TMB	3,3',5,5'-Tetramethylbenzidene
Tn5	Prokaryotic *E. coli* transposon from which the nptII coding sequence is derived.
T-nos	3' nontranslated transcript termination sequence of the nopaline synthase gene from *Agrobacterium tumefaciens*
TOXIN6	Toxin protein sequence database
Tris	Tris(hydroxymethyl)aminomethane
T-*tr7*	3' nontranslated sequence of the transcript 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation
TSSP	Tissue-Specific Site Pool
Tween-20	Polyoxyethylenesorbitan monolaurate
USDA	United States Department of Agriculture
U.S. EPA or EPA	United States Environmental Protection Agency
UV	Ultraviolet
v/v	volume to volume ratio
w/v	weight to volume ratio
w/w	weight to weight ratio
WCSP1	Wheat cold shock protein 1
Xba	Restriction enzyme isolated from *Xanthomonas badrii*

Narrative Summary

Food and Feed Safety and Nutritional Assessment of MON 87460

Monsanto has developed drought tolerant corn MON 87460 that reduces yield loss under water-limited conditions compared to conventional corn. Efficacy in MON 87460 is derived by expression of cold shock protein B (CSPB) from *Bacillus subtilis*.

Corn is a versatile crop that provides food, feed and fuel to the global economy. Growing economies in the developing world and the need to rely on alternative fuel sources in the developed world have led to a recent surge in corn demand. These demands are exceeding production, leaving global cereal stocks at a 25 year low. Diminished stocks magnify the impacts of supply disruptions. Climate change is expected to impact crop yields, potentially creating supply disruptions. The combination of these factors places a premium on yield stability in suboptimal environments.

Drought stress is the major cause of yield reduction in corn and its effects have far reaching global socio-economic implications. In North America alone, it is estimated that 40% of annual crop losses are caused by sub-optimal water availability. In both temperate and tropical regions the average annual corn yield loss attributable to moderate water deficits is approximately 15%. During periods of severe drought, these losses can be much higher and result in complete crop failure. Advances in breeding and agronomic practices have made significant contributions to increasing corn yield potential and improving drought tolerance. Biotechnology provides additional tools that can be used in combination with breeding and agronomic practices to enhance productivity.

Knowing that stress response proteins allow organisms to adapt to and survive adverse environments, Monsanto scientists hypothesized that inserting a stress response protein into plants could impart a desirable phenotype. As part of a broad screening effort, the gene encoding CSPB from the soil bacterium *B. subtilis* was inserted into corn using *Agrobacterium*-mediated transformation. The plasmid used to create MON 87460 contains two expression cassettes. The first expression cassette produces CSPB and the second produces neomycin phosphotransferase II (NPTII), a selectable marker that confers tolerance to certain antibiotics such as neomycin and paromomycin. Transformed plants were tested for their ability to yield more grain than control plants under water-limited conditions. The CSPB protein expressed in MON 87460 exhibits key behaviors and properties that are similar to what is reported for bacterial cold shock proteins and cold shock domain-containing proteins in plants. It is important to note that MON 87460 represents the first of what are expected to be many yield-oriented traits produced through modern biotechnology using high throughput screening methods.

MON 87460 is expected to provide significant value to producers and consumers. Improved yields under water-limited conditions will help to ensure a stable grain supply, even in years with low rainfall. The greatest benefit from MON 87460 is expected to occur in regions that are prone to frequent drought stress such as the U.S. western dryland region, although other geographies would likely benefit from this event.

A multi-faceted approach was taken to characterize the genetic modification in MON 87460. The results confirm that MON 87460 contains a single insert with the

intended sequence, the insert is stably maintained over multiple generations, and the insertion will not result in unintended gene products with similarity to known allergens or toxins. The strategy used to characterize the genetic modification included: 1) Southern blot analyses to assay the entire corn genome for the presence of DNA derived from the transformation plasmid, PV-ZMAP595, to confirm that a single copy was inserted at a single site in the genome and that the insert is stably inherited; 2) DNA sequencing analyses to determine the exact sequence of the inserted DNA and allow a comparison to the transfer DNA (T-DNA) sequence of the transformation vector to confirm that only the expected sequences were integrated; and 3) a segregation analysis to confirm that the inserted traits are inherited according to Mendelian laws of genetics. Additionally, open reading frame bioinformatic analyses of the junction site between the insert and corn genomic DNA and of the *cspB* and *nptII* cassettes confirm that no relevant similarities exist between any putative polypeptides and known toxins or allergens. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA inserted at a single locus of the genome. The stability of the integrated DNA and absence of the backbone sequences in multiple generations of MON 87460 was also confirmed. These results are consistent with a single site of insertion that segregates in subsequent generations according to the Mendelian laws of genetics.

A multistep approach was also used to characterize the proteins expressed by MON 87460 as a result of the genetic modification. This detailed characterization confirms that the proteins are safe for human and animal consumption. The assessment involved: 1) characterizing the physicochemical and functional properties of each protein; 2) quantifying protein expression in plant tissues; 3) examining the similarity of each protein to known allergens, toxins and other biologically active proteins known to have adverse effects on mammals; 4) evaluating the digestibility of the proteins in simulated gastrointestinal fluids; 5) documenting the history of safe consumption of the proteins or their structural and functional homologues that lack documented adverse affects on human or animal health; and 6) investigating potential mammalian toxicity through a protein gavage assay. All data indicate that the CSPB and NPTII proteins are safe for human and animal consumption. Both proteins have histories of safe consumption and are expressed at low levels in MON 87460, particularly in grain. Both proteins lack similarity to known allergens, toxins and anti-nutritional proteins known to have adverse effects on humans and animals. Additionally, both proteins are readily digested in simulated gastric and intestinal fluids and neither protein exhibits any acute toxicity in a mammalian assay even when doses are several orders of magnitude greater than would be experienced under the most conservative exposure scenarios. Ultimately, the safety assessment supports the conclusion that dietary exposure to either the CSPB or NPTII proteins derived from MON 87460 poses no meaningful risks to human or animal health.

A detailed compositional and nutritional comparison of MON 87460, the control and commercially available corn varieties confirmed that MON 87460 is as safe and nutritious as conventional corn. The compositional comparisons were made using grain and forage produced under three sets of production conditions over two field seasons. In the first season corn was grown at six sites in the U.S. under conditions typical of local corn production. In the second season, corn was grown in Chile at three sites under both well-watered and water-limited conditions. This range of production conditions across seasons represents typical environments in which MON 87460 will be cultivated. Results

from the U.S. showed that, for 407 (93.7%) of the 434 comparisons made between the MON 87460 test and the control, there were no significant differences ($p>0.05$). The 27 detected differences were not consistent across sites, were small in magnitude and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference varieties. Results from well-watered plots in the second season study showed that, for 230 (94.3%) of the 244 comparisons made between MON 87460 and the control, there were no significant differences ($p>0.05$). The 14 detected differences were not consistent across sites, were small in magnitude and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference varieties. Results from the water-limited plots in the second season study showed that for 233 (95.5%) of the 244 comparisons made between MON 87460 and the control, there were no significant differences ($p>0.05$). The nine detected differences were not consistent across sites, were small in magnitude and the mean component values of the test and control substances were within the 99% tolerance interval established from the commercial reference varieties. Samples from the second season study were also analyzed for 11 secondary metabolites that are potentially associated with drought stress. Results from this additional analysis further confirm that MON 87460 is compositionally equivalent to conventional corn. All compositional analyses, therefore, support the conclusion that MON 87460 is equivalent to conventional corn when grown under a range of typical environmental conditions.

The data and information presented in this summary demonstrate that the foods and feeds derived from MON 87460 are as safe and nutritious as the comparable foods and feeds derived from conventional corn. This conclusion is based on several lines of evidence. The first is the detailed molecular characterization of the inserted DNA. Results confirm the insertion of a single functional copy of the *cspB* and *nptII* expression cassettes at a single locus within the genome. The second is a detailed characterization of the CSPB and NPTII proteins produced in MON 87460. Both proteins have extensive histories of safe use and their source organisms are ubiquitous in the environment. Data demonstrate that the two proteins are unlikely to cause allergenic or toxic effects and both human and animal exposures will be low. Safety assessments for humans and animals confirm that there are no meaningful risks from dietary exposure to CSPB or NPTII. Finally, compositional and nutritional assessments of MON 87460 grain and forage produced under a range of typical environmental conditions demonstrate that MON 87460 is compositionally equivalent to conventional corn. All data strongly support the conclusion that food and feed derived from MON 87460 will be as safe and nutritious as food and feed derived from conventional corn and that the sale and consumption of MON 87460 would be in compliance with FDA's 1992 "Statement of Policy: Foods Derived from New Plant Varieties" as well as the Federal Food, Drug and Cosmetic Act.

PART II: SYNOPSIS OF CONSULTATION SUMMARY

SECTION 1. Name and Address of the Submitter

The submitter of this safety and nutritional assessment summary for corn MON 87460 is:

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167

Communications with regard to this submission should be directed to [redacted], Regulatory Affairs Manager, at the Monsanto address. He can also be contacted by telephone [redacted].

SECTION 2. The Subject of this Summary and the Plant Species from which it was Derived

The subject of this summary is MON 87460, a corn product derived from a Monsanto proprietary corn inbred.

SECTION 3. Distinctive Designations given to the Subject of this Summary

The event that is the subject of this summary has been designated MON 87460. In accordance with OECD's "Guidance for the Designation of a Unique Identifier for Transgenic Plants," MON 87460 has been assigned the unique identifier MON-8746Ø-4.

SECTION 4. Identity and Sources of the Genetic Material Introduced into MON 87460

MON 87460 was developed through *Agrobacterium*-mediated transformation of corn to using the binary plasmid vector, PV-ZMAP595. This plasmid contains two expression cassettes. The first expression cassette produces *Bacillus subtilis* cold shock protein B (CSPB) and the second expression cassette produces neomycin phosphotransferase II (NPTII) from *Escherichia coli* strain K12, a selectable marker that confers tolerance to certain antibiotics such as neomycin and paromomycin. A detailed description of the transformation and selection process is provided in Part IV.

The expression cassette for the coding sequence of the CSPB protein consists of the promoter (P-*Ract1*) (McElroy et al., 1990) and the leader and intron from the rice actin gene (L-*Ract1*, I-*Ract1*) (McElroy et al., 1991), the *cspB* coding sequence from *B. subtilis* (Willimsky et al., 1992), and the 3' nontranslated sequence of the *transcript 7* gene from *Agrobacterium tumefaciens* (T-*tr7*; Dhaese et al., 1983), which terminates transcription and provides the signal for mRNA polyadenylation.

The *nptII* gene expression cassette that produces the NPTII protein consists of the *loxP* sequence from Bacteriophage P1 for the recombination site recognized by Cre recombinase (Russell et al., 1992), the 35S promoter from the cauliflower mosaic virus (P-*35S;* Odell et al., 1985), the coding sequence for NPTII from *E. coli* (*nptII*; Fraley et al., 1983), the 3' nontranslated region of the nopaline synthase (T-*nos*) coding region

from *Agrobacterium tumefaciens* T-DNA which terminates transcription and directs polyadenylation (Bevan et al., 1983), and a second *loxP* site.

The molecular analyses described in Part IV demonstrate that MON 87460 contains a single copy of introduced T-DNA (~5.2 kb) inserted at a single locus. This insert contains one intact copy each of the *cspB* and *nptII* gene expression cassettes. There are no detectable plasmid backbone sequences, except for sequences common with the T-DNA, and no additional elements, linked or unlinked to intact cassettes, from transformation vector PV-ZMAP595. Comprehensive molecular analysis supports the conclusion that the insert in MON 87460 encodes only the two expected full-length proteins, CSPB and NPTII.

SECTION 5. The Intended Technical Effect of MON 87460

Plants have a variety of responses that allow them to adapt to and reproduce under stress conditions. In crop plants, the most desirable stress responses are those that protect end of season yields. Crop productivity is the summation of a series of events that take place throughout the growing season, with certain periods being more sensitive to stress than others. Protecting crops during their most critical growth stages is the key to yield improvement. In corn, the most dramatic adverse impacts on yield occur as a result of drought stress during flowering.

Knowing that stress response proteins allow organisms to survive in adverse environments, Monsanto scientists hypothesized that inserting a stress response protein into plants could impart a desirable phenotype. As part of a broad screening effort, the gene encoding CSPB from the soil bacterium, *B. subtilis,* was inserted into corn and the resulting plants were tested for their ability to yield more grain than non-transformed plants under water-limited conditions.

The insertion of the *cspB* gene in MON 87460 confers tolerance to water-limited conditions that would otherwise negatively impact yield. The *nptII* gene was inserted to facilitate selection of plants containing *cspB* during early product development. MON 87460 was chosen for development based on its yield advantage under water-limited conditions compared to the control and absence of negative pleiotropic effects on plant performance.

SECTION 6. The Applications and Uses of MON 87460

Limited water availability is the single most important factor that reduces global crop yields. In North America alone, it is estimated that 40% of annual crop losses are due to sub-optimal water availability (Boyer, 1982). In both temperate and tropical regions the average annual corn yield loss attributable to moderate water deficits is approximately 15% (Barker et al., 2005). During periods of severe drought, these losses can be much higher and result in complete crop failure. Within the U.S., the western dryland, a region representing approximately 15% of total U.S. corn acres and spanning states from South Dakota to Texas, is most susceptible to corn yield losses caused by limited rainfall. Annual precipitation in this region averages between 12 to 20 inches (30 to 50 cm) (http://prism.oregonstate.edu). Other geographies with similar conditions such as parts of Africa may also benefit from this event.

While much progress has been made to improve corn yield in water-limited environments through breeding and cultural practices, there remains potential for additional improvement. Increased corn yield per acre will help reduce the total number of acres needed to meet the needs for food, feed and biofuel uses. Positive impacts on yield and increased yield stability will provide value to producers, consumers, and the environment.

SECTION 7. Applications for which MON 87460 is Not Suitable

Monsanto Company is not aware of food or feed uses of conventional corn that are not applicable to MON 87460.

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PART III: STATUS OF SUBMISSIONS TO OTHER REGULATORY AGENCIES

SECTION 1. Status of Submission to USDA-APHIS

Monsanto will be requesting a Determination of Nonregulated Status for MON 87460, including all progenies derived from crosses between MON 87460 and other corn, from the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) in 2009. Under regulations administered by USDA-APHIS (7 CFR 340), MON 87460 is currently considered a "regulated article." Monsanto will continue to conduct all field tests for MON 87460 in strict compliance with USDA field regulations until a Determination of Nonregulated Status is obtained for MON 87460.

SECTION 2. Status of Submission to EPA

In October 2007, the U.S. Environmental Protection Agency (EPA) informed Monsanto that corn engineered to resist drought is not a pesticide and the agency will not regulate this product. EPA requested that Monsanto provide copies of correspondence with FDA and USDA to them as a courtesy.

SECTION 3. Status of Submissions to Foreign Governments

Regulatory submissions for import and/or production approvals will be made to countries that import significant U.S. corn grain or derived food and feed products and have regulatory approval processes in place. These will include submissions to a number of foreign government regulatory agencies including, but not limited to the Canadian Food Inspection Agency (CFIA) and Health Canada, Mexico's Intersectoral Commission for Biosafety of Genetically Modified Organisms (CIBIOGEM), Japan's Ministries of Agriculture, Forestry and Fisheries (MAFF) and Health, Labor and Welfare (MHLW), and the European Commission of the European Union. As appropriate, notifications of import will be made to importing countries that do not have a formal approval process.

PART IV: DEVELOPMENT OF MON 87460

SECTION 1. Corn as a Crop

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere. Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total production. In the U.S., corn is a highly productive crop, yielding an average of 151.1 bushels per acre in the U.S. during 2007 (NCGA, 2008). Its comparatively high yield makes it one of the most economical sources of metabolizable energy for feeds, and of starch and sugar for food and industrial products. In the U.S., demand for corn is driven by the demand for feed and fuel. In 2007 animal feed accounted for over 45% of corn consumption followed by nearly 25% for ethanol, and approximately 10% for food and industrial uses with the remainder being exported (NCGA, 2008).

Corn is used globally for food, feed, and fuel, and in recent years the demand for corn has increased, leading to higher prices for raw grain and its derivatives (NCGA, 2008). OECD-FAO's joint 2008-2017 Agricultural Outlook forecasted that corn prices will remain 40-60% higher in the next decade than they have been for the last decade. The report also concluded that increased yields on existing agricultural land will be more important to improving commodity supplies than bringing new land into cultivation. In developing countries, economic growth, changing diets, and growing populations are driving added demand. In developed countries, fuel uses are the largest source of new demand. These factors along with diminished stocks and climate change will lead to variability in agricultural product supply and possibly result in price spikes (OECD/FAO, 2008).

1.1. Scientific name and taxonomic classification of corn

Several hypotheses exist on the origin of corn but the preponderance of evidence supports the hypothesis that corn descended from teosinte (Galinat, 1988). The teosinte genome is similar to corn; teosinte easily crosses with corn, and teosinte has several morphological traits similar to corn. Teosinte has a more weedy appearance and more tillers than modern corn varieties. The one major distinguishing difference between corn and teosinte is the female inflorescence, or ear. Modern corn varieties have one to three lateral branches that terminate in an ear with 8 to 24 rows of kernels that is enclosed in modified leaves or husks. Teosinte also has lateral branches, but they terminate in two-rowed spikes of perhaps 12 fruit cases, with each fruit case having one seed enclosed by an indurated glume (Goodman and Brown, 1988).

Corn (*Zea Mays* L.) is a member of the tribe Maydae, which is included in the subfamily Panicoideae of the grass family Gramineae. Table IV-1 summarizes the taxonomic classification of corn and its close relatives.

The genera included in the tribe Maydae include *Zea* and *Tripsacum* in the Western Hemisphere, and *Coix*, *Polytoca*, *Chionachne*, *Schlerachne*, and *Trilobachne* in Asia. Although some researchers have implicated the Asian genera in the origin of corn, the

evidence for them is not as extensive and convincing as for the genera located in the Western Hemisphere.

Table IV-1. Taxonomic Classification of Corn and its Close Relatives

Family - Gramineae
- Subfamily - Panicoideae
  - Tribe - Maydae
    - Western Hemisphere:
    - I. Genus - *Zea*
      - A. Subgenus - *Luxuriantes*
        1. *Zea luxurians* (2n = 20)
        2. *Zea perennis* (2n = 40)
        3. *Zea diploperennis* (2n = 20)
      - B. Subgenus - *Zea*
        1. *Zea mays* (2n = 20)
           Subspecies
           1. *Z. mays parviglumis* (2n = 20)
           2. *Z. mays huehuetenangensis* (2n = 20)
           3. *Z. mays mexicana* (Schrad.) (2n = 20)

1.2. Growth and reproductive characteristics of corn

Corn development is measured in V (vegetative) stages and R (reproductive) stages. The V number corresponds to the number of leaves with a visible collar or ligule. Leaves initiate at the growing point, or meristem, which in corn and other grasses is at the base of the plant. At V5, ear shoot formation is complete and all leaves have initiated. Until V6, the growing point of the plant is below ground. Beginning at V10, corn plants accumulate a reserve of nutrients and dry weight to support reproductive development and grain production. V stages proceed through VT (tassel emergence or anthesis) and the R stages begin at silking (R1). R2 and R3 represent early to mid grainfill while R4 and R5 represent mid to late grainfill. R6 is physiological maturity (Hanway, 1982). Figure IV-1 presents the growth stages of corn.

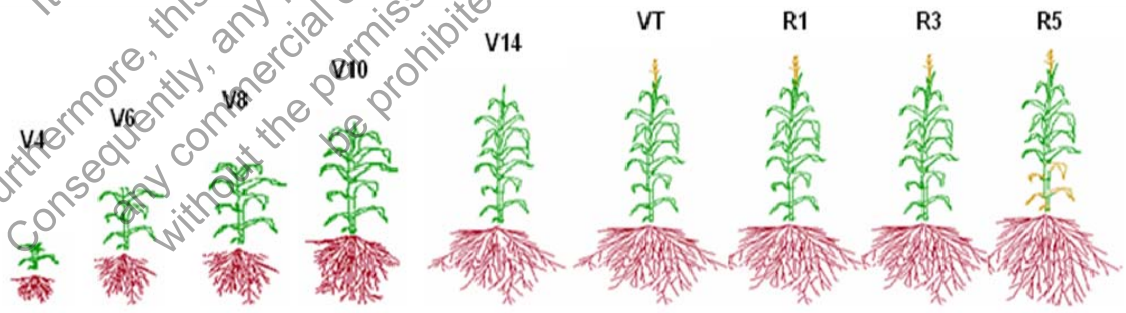


Figure IV-1. Growth Stages of Corn from Early Vegetative (V4) through Late Grainfill (R5)

Corn yield is driven by both kernel number and kernel weight. Kernel number is a function of how many ovules are fertilized and supported through maturity and is the primary determinant of overall yield (Westgate et al., 2004). Kernel weight is a function

of the amount of dry matter available to support kernel development and the amount of water available during grainfill. Harvest index (HI) is the ratio of grain biomass to shoot biomass and is used to quantify the relationship between sink tissue and source tissue. In modern hybrids HI is approximately 0.5 (Westgate et al., 2004). The amount of shoot biomass available at the end of the season to support kernel development is a function of the amount of photosynthetically active radiation (PAR) the plant is able to intercept. This in turn is a function of the amount of leaf area exposed to sunlight, which is determined by the rate of leaf expansion. Sufficiently severe, adverse conditions during any growth stage can limit plant growth and therefore limit yield (Westgate et al., 2004).

1.3. History of corn development

From its likely origin as a wild grass, corn has undergone continuous breeding, modification, and selection for properties that suit the needs of consumers. Corn originated in the highlands of Mexico 7,000 to 10,000 years ago. European contact introduced corn to the rest of the world and has allowed it to become an essential crop for food, feed, and fuel (Goodman, 1988).

One of the most striking differences between cultivated corn and its wild relatives is the obvious emphasis that millennia of breeding and selection efforts have placed on grain yield. All modern corn varieties produce multi-rowed ears containing hundreds of kernels. Corn's closest wild relative, teosinte, produces a cluster of spikes, each with one or two rows of seeds. The transformation of spikes into a single enclosed ear is one of the key achievements in the development of corn as a crop (Wilkes, 2004). From the earliest cultivation efforts in Mexico until the early 20th century, corn existed primarily in the form of open pollinated varieties. The discovery of corn hybridization led to the development of modern-day dent corn, better adaptation to previously adverse environments, and significant yield increases (Duvick et al., 2004).

In the 1940s, when hybrid corn began to predominate, average U.S. yields were 30 – 40 bushels/acre (bu/ac, 2.2 metric tons/hectare (MT/ha)). By 2000, average U.S. yields were over 130 bu/ac (8.6 MT/ha). Improved breeding and the widespread availability of fertilizers and pesticides made significant contributions to these advancements (Troyer, 2004). Biotechnology provides additional avenues to improve productivity by reducing the inputs needed to control weeds and insects (Hicks and Thomison, 2004; Kaeppler, 2004).

In 2007, herbicide tolerance and insect resistance traits were grown on 73% of U.S. corn acres. Combined trait products, with combinations of herbicide tolerance and insect resistance, were the largest category at 28% of U.S. corn acres (NCGA, 2008). As a complement to these existing traits, MON 87460 provides yield stability under water-limited conditions that otherwise limit plant performance. Increasing demand for corn in the food, feed and fuel sectors places a premium on technologies that stabilize yield and allow more corn to be produced on existing acres.

SECTION 2. Characterization of the Vector Used in Transformation

Monsanto created plasmid PV-ZMAP595 to allow the *Agrobacterium*-mediated transformation of MON 87460. Vectors and transformation methods are selected to allow a high probability of obtaining the trait of interest and integration of the introduced

DNA into a single locus in the plant genome. This helps ensure that only the intended DNA encoding the desired traits is integrated into the plant genome and facilitates the molecular characterization of the product. Understanding the elements present in plasmid PV-ZMAP595 and the steps required for transformation provide context for the molecular analysis that Section 3 describes.

2.1. Method of transformation

MON 87460 was developed through *Agrobacterium*-mediated transformation of corn using the double-border, binary plasmid vector, PV-ZMAP595 (Figure IV-2). *Agrobacterium tumefaciens* strain ABI contains a modified Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell 1986). The vector, PV-ZMAP595, contains both the left and right border sequences flanking the transfer DNA (T-DNA) to facilitate transformation.

The *Agrobacterium*-mediated transformation to produce MON 87460 was based on the method described by Armstrong and Phillips (Armstrong and Phillips 1988). Briefly, freshly isolated immature corn embryos were used for the initiation of the callus. After co-culturing with *Agrobacterium* carrying the transformation vector, the calli were transferred from filter paper to callus initiation medium containing carbenicillin to eliminate *Agrobacterium*, and paromomycin to eliminate cells that were not transformed, so that only cells containing the T-DNA survived. The resulting transformed cells were then subcultured several times on a selection medium and regenerated into plants.

Plants generated through the above transformation (R_0 generation) were self-pollinated, and the subsequent R_1 plants were screened for the presence of CSPB protein, tolerance to kanamycin, and homozygosity of the inserted gene. Only the plants that were homozygous for the *cspB* insert and tolerant to kanamycin were advanced for development, and their progenies were subjected to further molecular (Southern blot) and phenotypic assessments. Regulatory studies on MON 87460 were initiated to further characterize the genetic insertion and the expressed proteins, and to establish the food, feed and environmental safety relative to conventional corn. The major steps involving the development of MON 87460 are depicted in Figure IV-3.

2.2. Plasmid PV-ZMAP595

Figure IV-2 presents a circular map of PV-ZMAP595 and Table IV-2 describes the elements the plasmid contains. In addition to the genes of interest, the plasmid is comprised of sections necessary for it to be maintained in *Agrobacterium*, sections necessary for plant transformation and sections necessary to express the genes of interest once transformation occurs. In this section, T-DNA refers to DNA that is transferred to the plant during transformation.

PV-ZMAP595 is approximately 9.4 kb and contains a single T-DNA delineated by left and right border regions that contains two expression cassettes: a *cspB* gene expression cassette, which contains coding sequence for CSPB from *Bacillus subtilis* and a neomycin phosphotransferase II (*nptII*) expression cassette, which confers resistance to kanamycin. An expression cassette is composed of a coding sequence and the regulatory elements necessary for the expression of the coding sequence. The T-DNA expected to

incorporate into the corn genome is approximately 4.6 kb and the DNA backbone region that is not incorporated into the corn genome is approximately 4.8 kb.

The *cspB* expression cassette contains the *cspB* coding sequence under the regulation of the *Ract1* promoter and leader, *Ract1* intron, and the *tr7* 3' nontranslated sequence. The *nptII* expression cassette contains the *nptII* coding sequence under the regulation of the *35S* promoter and the *nos* 3' nontranslated sequence.

The backbone region outside of the T-DNA contains two origins of replication for maintenance of plasmid in bacteria (OR-*oriV,* OR-*ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid copy number in *Escherichia coli* (*rop*). Table IV-2 describes the genetic elements and their prefixes (e.g. P-, I-, OR-, B-, CS-, and T-) in PV-ZMAP595.

2.2.1. The *cspB* gene and CSPB protein

MON 87460 expresses the CSPB protein, an RNA chaperone protein from *B. subtilis,* which is associated with enhanced stress acclimation and tolerance by unfolding misfolded RNA secondary structures, thereby facilitating RNA translation (Phadtare et al., 2002). The amino acid sequence of the CSPB protein produced in MON 87460 is identical to the native CSPB protein produced in *B. subtilis* with the exception of one amino acid change in the second position from leucine to valine, designated as CSPB-L2V. This amino acid change was implemented to facilitate the assembly of the plasmid vector PV-ZMAP595 for plant transformation. The deduced full-length amino acid sequence is shown in Figure IV-4.

2.2.2. The *cspB* regulatory sequences

The *cspB* expression cassette is adjacent to the right border region of plasmid PV-ZMAP595. The *cspB* coding sequence is under the regulatory control of the *Ract1* promoter and leader from the actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1990). Located between the *Ract1* promoter and the *cspB* coding sequence is the I-*Ract1* nontranslated intron from the actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1991). Following the *cspB* coding sequence is the 3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* (T-*tr7*) that directs polyadenylation (Dhaese et al., 1983).

2.2.3. The *nptII* gene and NPTII protein

The *nptII* cassette contains the *nptII* coding sequence flanked by *loxP* sites. The NPTII protein in MON 87460 confers resistance to kanamycin, which was used to facilitate the selection process. A *loxP* site consists of 34 nucleotides that are specifically recognized by CRE-recombinase. The deduced full-length amino acid sequence is shown in Figure IV-5.

2.2.4. The *nptII* regulatory sequences

The *nptII* expression cassette is adjacent to the left border region of plasmid PV-ZMAP595. The *nptII* coding sequence is under the regulatory control of the *35S* promoter from the Cauliflower Mosaic Virus (Odell et al., 1985). Following the *nptII* coding sequence is the 3' nontranslated sequence of the *nopaline synthase* gene from *Agrobacterium tumefaciens* (T-*nos*) which terminates transcription and directs polyadenylation (Bevan et al., 1983).

2.2.5. T-DNA borders

Plasmid PV-ZMAP595 contains right border and left border regions that delineate the T-DNA to be transferred into corn and are involved in the efficient transfer of the T-DNA into the corn genome. These border regions (Figure IV-2 and Table IV-2) were derived from *Agrobacterium tumefaciens* plasmids (Depicker et al., 1982; Barker et al., 1983).

2.2.6. Genetic elements outside the T-DNA borders

Four genetic elements exist outside of the T-DNA borders that are essential for the maintenance and selection of the vector PV-ZMAP595 in bacteria. They include: *OR-ori V,* origin of replication for the maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981); CS-*rop,* coding sequence of repressor of primer (ROP) protein for the maintenance of plasmid copy number in *E. coli* (Giza and Huang 1989); *OR-ori-pBR322,* origin of replication from pBR322 for the maintenance of the plasmid in *E. coli* (Sutcliffe, 1979); and *aadA*, a bacterial promoter and coding sequence of an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance for molecular cloning and selection purposes (Fling et al., 1985) prior to plant transformation. As these elements are outside of the border regions, they are not expected to be transferred into the corn genome. The absence of the backbone sequence in MON 87460 was confirmed by Southern blot analyses (see Part IV, Section 3.2.8).

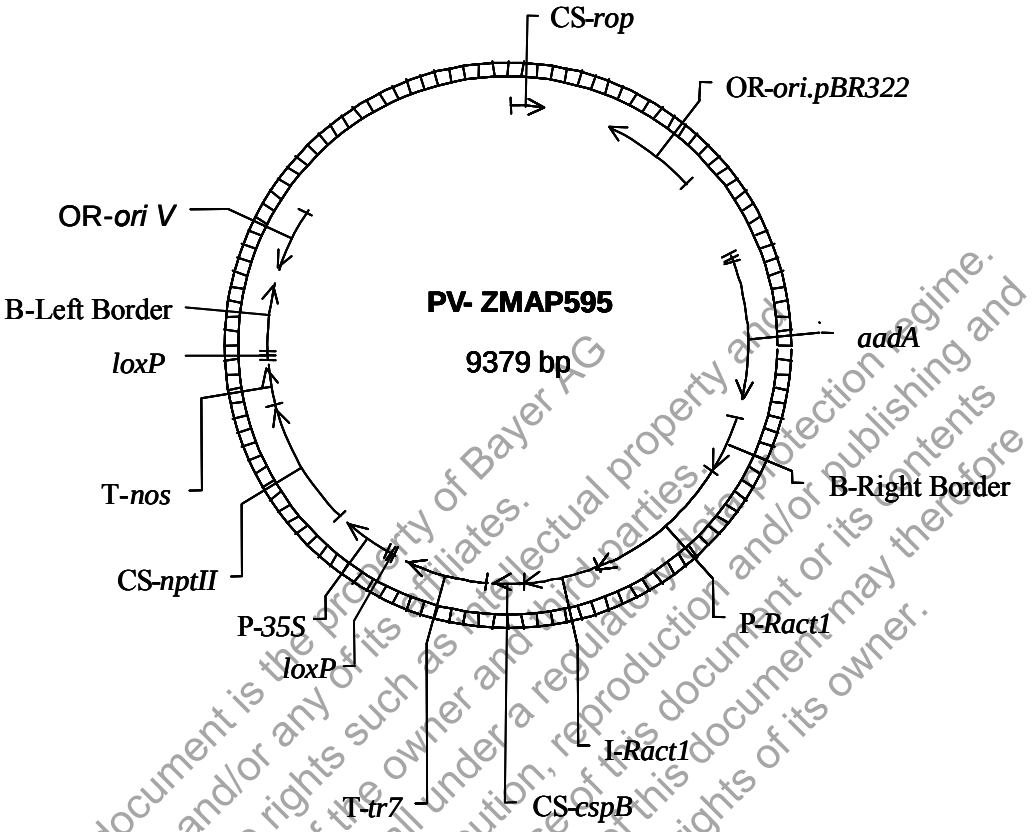


Figure IV-2. Circular Map of Plasmid PV-ZMAP595

Plasmid PV-ZMAP595 containing the T-DNA used in *Agrobacterium*-mediated transformation to produce MON 87460. Approximate locations of the genetic elements are depicted on the exterior of the map.

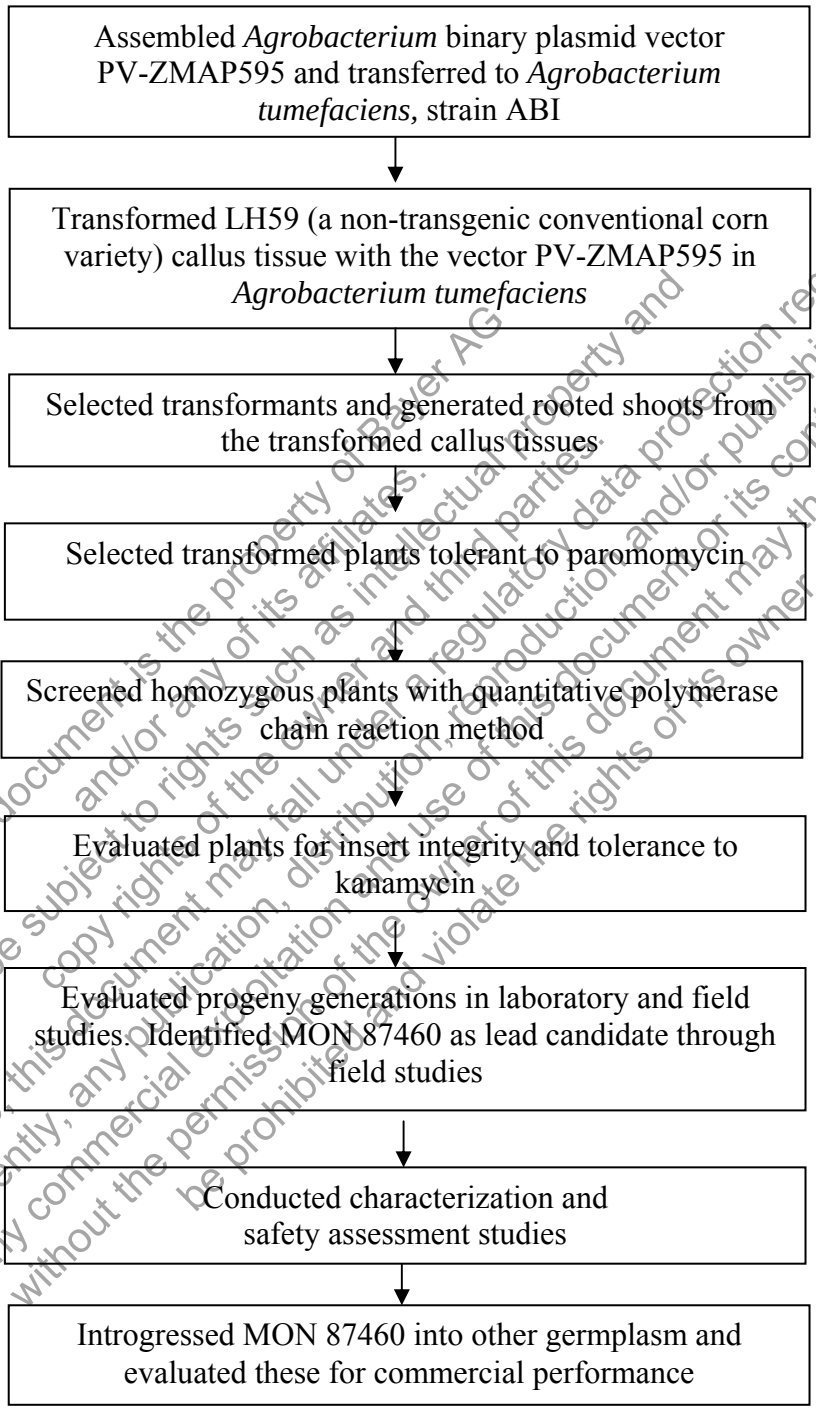


Figure IV-3. Process Map for Transformation, Selection, and Evaluation of MON 87460

Table IV-2. Summary of Genetic Elements in Vector PV-ZMAP595

Genetic Element	Location in Plasmid	Function (Reference)
Vector Backbone		
Intervening Sequence	1 – 52	Sequence used in DNA cloning
CS[1]-*rop*	53 – 244	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang 1989)
Intervening Sequence	245 – 671	Sequence used in DNA cloning
OR[2]-*ori.pBR322*	672 – 1260	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	1261 – 1790	Sequence used in DNA cloning
aadA	1791 – 2679	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 (Fling et al., 1985) (GenBank accession X03043)
Intervening Sequence	2680 – 2815	Sequence used in DNA cloning
		T-DNA
B[3]-Right Border	2816 – 3172	DNA from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	3173 – 3204	Sequence used in DNA cloning
P[4]-*Ract1*	3205 – 4128	Promoter and leader from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1990)
I[5]-*Ract1*	4129 – 4605	Intron from the rice actin gene, *act1,* of *Oryza sativa* (McElroy et al., 1991)
Intervening Sequence	4606 – 4607	Sequence used in DNA cloning
CS-*cspB*	4608 – 4811	Codon modified coding sequence of the *cspB* gene from *Bacillus subtilis* encoding CSPB (Willimsky et al., 1992)
Intervening Sequence	4812 – 4841	Sequence used in DNA cloning
T[6]-*tr7*	4842 – 5349	3' nontranslated sequence of *transcript* 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al., 1983)
Intervening Sequence	5350 – 5423	Sequence used in DNA cloning

Table IV-2 (cont.). Summary of Genetic Elements in Vector PV-ZMAP595

T-DNA (cont.)		
loxP	5424 – 5457	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992)
Intervening Sequence	5458 – 5483	Sequence used in DNA cloning
P-*35S*	5484 – 5776	Promoter for the 35S RNA of the Cauliflower Mosaic Virus (Odell et al., 1985)
Intervening Sequence	5777 – 5840	Sequence used in DNA cloning
CS-*nptII*	5841 – 6635	Coding sequence from *Tn5* (Beck et al., 1982) in *E. coli* encoding neomycin and kanamycin resistance (Fraley et al., 1983)
Intervening Sequence	6636 – 6666	Sequence used in DNA cloning
T-*nos*	6667 – 6919	3' nontranslated sequence of the *nopaline synthase* (NOS) gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation (Bevan et al., 1983)
Intervening Sequence	6920 – 6944	Sequence used in DNA cloning
loxP	6945 – 6978	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992)
Intervening Sequence	6979 – 6998	Sequence used in DNA cloning
B-Left Border	6999 – 7440	DNA from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Vector Backbone		
Intervening Sequence	7441 – 7526	Sequence used in DNA cloning
OR-*ori V*	7527 – 7923	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	7924 – 9379	Sequence used in DNA cloning

[1]CS – Coding Sequence
[2]OR – Origin of Replication
[3]B – Border
[4]P – Promoter
[5]I – Intron
[6]T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences

```
1           MVEGKVKWFN SEKGFGFIEV EGQDDVFVHF SAIQGEGFKT LEEGQAVSFE
51          IVEGNRGPQA ANVTKEA
```

Figure IV-4. Deduced Amino Acid Sequence of the Full Length CSPB Protein Present in MON 87460

The amino acid sequence of CSPB was deduced from the full-length *cspB* coding sequence present in PV-ZMAP595.

```
  1     MIEQDGLHAG SPAAWVERLF GYDWAQQTIG CSDAAVFRLS AQGRPVLFVK
 51     TDLSGALNEL QDEAARLSWL ATTGVPCAAV LDVVTEAGRD WLLLGEVPGQ
101     DLLSSHLAPA EKVSIMADAM RRLHTLDPAT CPFDHQAKHR IERARTRMEA
151     GLVDQDDLDE EHQGLAPAEL FARLKARMPD GEDLVVTHGD ACLPNIMVEN
201     GRFSGFIDCG RLGVADRYQD IALATRDIAE ELGGEWADRF LVLYGIAAPD
251     SQRIAFYRLL DEFF
```

Figure IV-5. Deduced Amino Acid Sequence of the Full Length NPTII Protein Present in MON 87460

The amino acid sequence of the NPTII was deduced from the full-length *nptII* coding sequence present in PV-ZMAP595

SECTION 3. Characterization of the Introduced Genetic Material

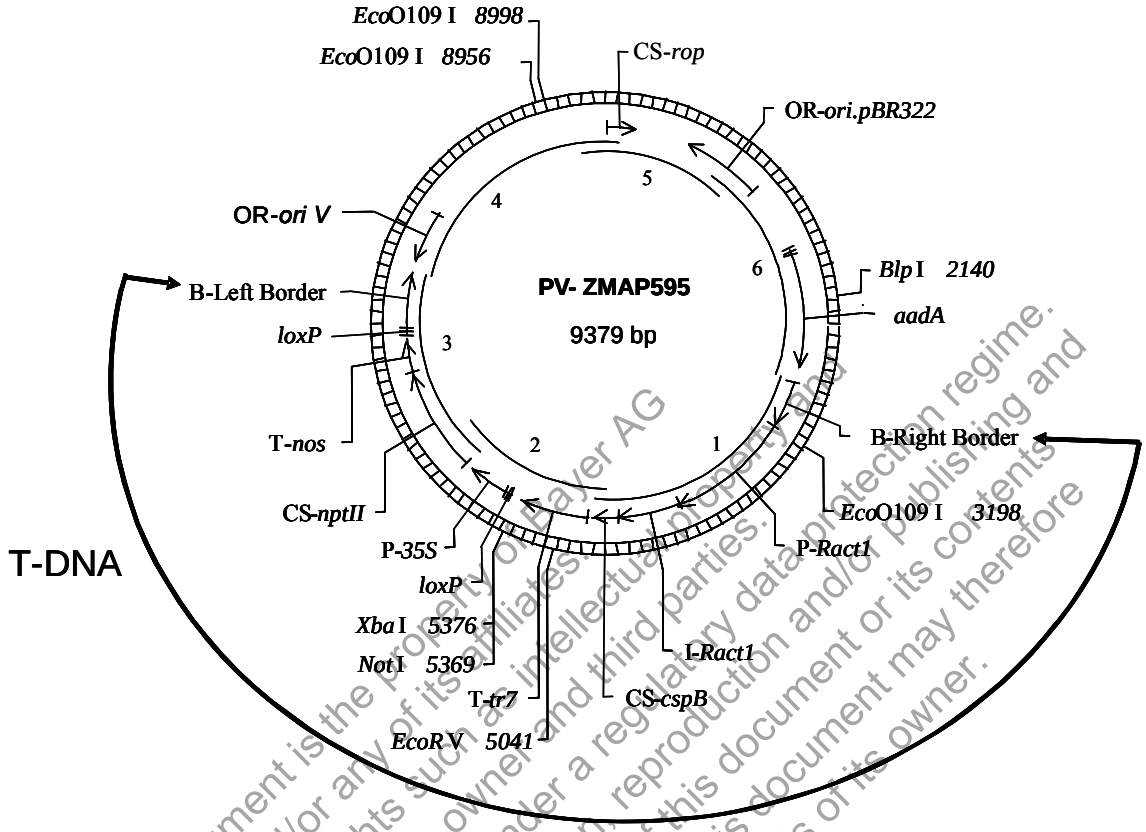
A multi-faceted molecular characterization confirmed that MON 87460 contains a single insert with a single copy of the intended sequence, backbone sequence is not present, and the insert is stably maintained over multiple generations. Genomic DNA from MON 87460 was digested using restriction enzymes and subjected to Southern blot analyses. Southern blot analyses confirm the number of inserts in the genome, the number of copies of the insert, the intactness of the genetic elements within the insert, the absence of backbone sequences, inserted DNA stability across multiple generations, and insert organization.

Figures IV-6 and IV-7 present maps of plasmid vector PV-ZMAP595 annotated with the probes used in the Southern analysis. Table IV-3 summarizes the expected DNA fragments using various restriction enzymes and probes. Figure IV-18 depicts the generations used in this study and Figures IV-9 – IV-17, IV-19 and IV-20 present the Southern blot analyses. The molecular size markers on the left of the figures were used to estimate the sizes of bands present in the long-run lanes of Southern blots. The molecular size markers on the right of the figures were used to estimate the sizes of bands present in the short-run lanes. Appendix A provides materials and methods for the molecular analysis.

Molecular analyses confirmed the presence of each genetic element at the insertion site and not at any region outside of the insert, confirmed the lack of plasmid backbone elements, and confirmed insert stability across generations. In addition, DNA sequencing analyses were performed and results confirmed the nucleotide sequence of the insert in MON 87460 as well as the organization of the genetic elements. Furthermore, segregation analysis confirmed that the insert segregated according to Mendelian genetics as expected. These results are consistent with a single chromosomal insertion of the *cspB* and *nptII* cassettes.

Genomic DNA from MON 87460 was digested with appropriate restriction enzymes and subjected to Southern blot analyses to characterize the T-DNA that was integrated into the corn genome. Genomic DNA samples from conventional corn were used as the negative controls for the blots to identify potential nonspecific hybridization signals. The positive controls for Southern blots were generated by digestions of plasmid DNA with enzyme combinations to produce the DNA banding patterns that were most relevant to the molecular assessment of MON 87460. Probe templates generated from the plasmid DNA were also used as positive controls. In addition, DNA markers were included to provide size estimation of the hybridized bands on Southern blots.

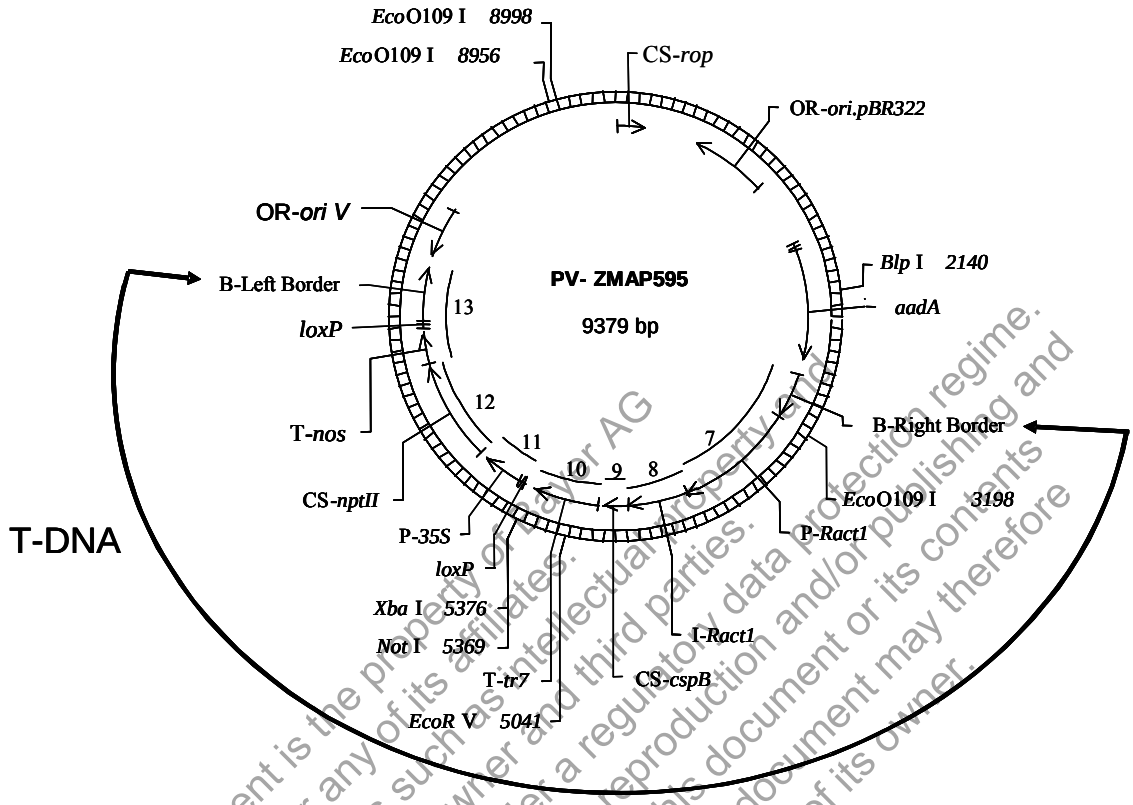
Table IV-4 lists the genetic elements detected in MON 87460. The insert matches the T-DNA sequence of PV-ZMAP595 starting with *Ract1* promoter and ending at Left Border. The information and results derived from the molecular analyses were used to construct a linear map of the insert in MON 87460. This linear map depicts restriction sites identified in the insert, the corn genomic DNA flanking the insert, and provides information on the expected banding patterns and sizes of the DNA fragments after restriction enzyme digestions. The linear map is shown in Figure IV-8.



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
1	T-DNA Probe 1	2816	4782	2.0
2	T-DNA Probe 2	4670	6085	1.4
3	T-DNA Probe 3	5839	7440	1.6
4	Backbone Probe 1	7441	66	2.0
5	Backbone Probe 2	9241	1245	1.4
6	Backbone Probe 3	1094	2815	1.7

Figure IV-6. Genetic Elements and Restriction Sites of Vector PV-ZMAP595 Used in Southern Blot Analyses (Probes 1-6)

Locations of the genetic elements are depicted by arrows on the interior of the map with their annotations shown on the exterior of the map. Restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The overlapping T-DNA and backbone probes used in the Southern analyses (labeled 1-6 within the interior of the map) are detailed in the accompanying table.



Probe	DNA Probe	Start Position	Stop Position	Total Length (~kb)
7	P-*Ract1* Probe	2816	4128	1.3
8	I-*Ract1* Probe	4129	4607	0.5
9	CS-*cspB* Probe	4608	4811	0.2
10	T-*tr7* Probe	4842	5354	0.5
11	*loxP* + P-*35S* Probe	5424	5785	0.36
12	CS-*nptII* Probe	5839	6635	0.8
13	T-*nos* + *loxP* + Left Border Probe	6667	7440	0.8

Figure IV-7. Genetic Elements and Restriction Sites of Vector PV-ZMAP595 Used in Southern Blot Analyses (Probes 7-13)

Locations of the genetic elements are depicted by arrows on the interior of the map with their annotations shown on the exterior of the map. Restriction sites for enzymes used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The genetic element probes used in the Southern analyses (labeled 7-13 within the interior of the map) are detailed in the accompanying table.

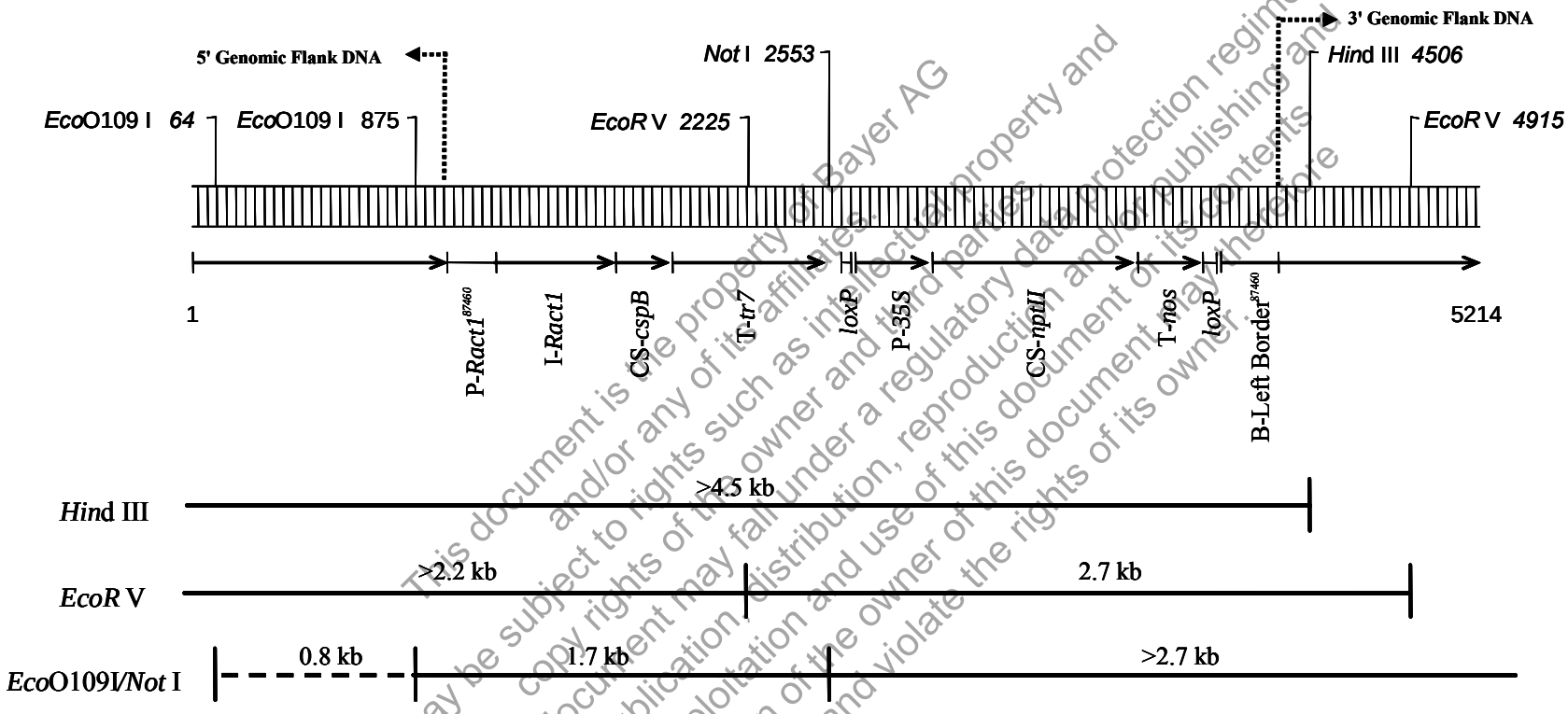


Figure IV-8. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 87460

A linear map of the insert and known genomic DNA flanking the insert in MON 87460 is shown. Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern analyses. The lower portion of the map shows the expected sizes of the DNA fragments after digestions with respective restriction enzyme or combination of enzymes. The dotted line indicates the additional DNA fragment that might be present if partial digestion of the internal *Eco*O109 I restriction site occurs. Arrows with dotted lines indicate the end of the insert and the beginning of corn genomic flanking sequence.

Table IV-3. Summary Chart of the Expected DNA Fragments Using Combinations of Restriction Enzymes and Probes

Probes used	**1, 2, 3**	**4, 5, 6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Southern blot in Figure	IV-9	IV-10	IV-11	IV-12	IV-13	IV-14	IV-15	IV-16	IV-17
Plasmid									
*Blp*I + *Xba* I	3.2 kb + 6.1 kb	3.2 kb + 6.1 kb	3.2 kb	3.2 kb	3.2 kb	3.2 kb	6.1 kb	6.1 kb	6.1 kb
Probe templates [1]	1.4 kb + 1.6 kb + 2.0 kb	1.4 kb + 1.7 kb + 2.0 kb	--[2]	--	--	--	--	--	--
MON 87460									
Hind III	> 4.5 kb	--	--	--	--	--	--	--	--
*Eco*R V	2.7 kb + > 2.2 kb	no band	> 2.2 kb	> 2.2 kb	> 2.2 kb	2.7 kb + > 2.2 kb	2.7 kb	2.7 kb	2.7 kb
*Eco*O109 I and *Not* I	--	no band	1.7 kb	1.7 kb	1.7 kb	1.7 kb	1.7 kb	> 2.7 kb	>2.7 kb

[1] probe templates were spiked when multiple probes are used in Southern blot analysis.
[2] '--' indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis.

Table IV-4. Summary of Genetic Elements in MON 87460

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
Sequence flanking 5' end of the insert	1-1121	Corn genomic DNA
P3-*Ract1*^{87460}	1122-1312	Truncated promoter and leader from the rice actin gene, *act1*, of *Oryza sativa* (McElroy et al., 1990)
I4-*Ract1*	1313-1789	Intron from the rice actin gene, *act1*, of *Oryza sativa* (McElroy et al., 1991)
Intervening Sequence	1790-1791	Sequence used in DNA cloning
CS5-*cspB*	1792-1995	Codon optimized coding sequence of the cspB gene from *B. subtilis* encoding CSPB (Willimsky et al., 1992)
Intervening Sequence	1996-2025	Sequence used in DNA cloning
T6-*tr7*	2026-2533	3' nontranslated sequence of transcript 7 gene from *Agrobacterium tumefaciens* that directs polyadenylation (Dhaese et al., 1983)
Intervening Sequence	2534-2607	Sequence used in DNA cloning
loxP	2608-2641	Sequence from Bacteriophage P1 for the recombination site recognized by Cre recombinase (Russell et al., 1992)
Intervening Sequence	2642-2667	Sequence used in DNA cloning
P-*35S*	2668-2960	Promoter for the 35S RNA of the Cauliflower mosaic virus (Odell et al., 1985)
Intervening Sequence	2961-3024	Sequence used in DNA cloning
CS-*nptII*	3025-3819	Coding sequence from Tn5 (Beck et al., 1982) in *E. coli* encoding neomycin and kanamycin resistance (Fraley et al., 1983)
Intervening Sequence	3820-3850	Sequence used in DNA cloning
T-*nos*	3851-4103	3' nontranslated sequence of the nopaline synthase gene from *Agrobacterium tumefaciens* which terminates and directs polyadenylation (Bevan et al., 1983)
Intervening Sequence	4104-4128	Sequence used in DNA cloning

Table IV-4 (continued). Summary of Genetic Elements in MON 87460

Genetic Element	Location in Sequence	Function (Reference)
loxP	4129-4162	Sequence from *Bacteriophage P1* for the recombination site recognized by Cre recombinase (Russell et al., 1992)
Intervening Sequence	4163-4182	Sequence used in DNA cloning
B[7]-Left Border[87460]	4183-4430	DNA from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Sequence flanking 3' end of the insert	4431-5214	Corn genomic DNA

[1] Flanking sequences and intervening sequences are not functional genetic elements
[2] Numbering includes the insert in MON 87460 and adjacent genomic DNA
[3]P – Promoter
[4]I – Intron
[5]CS – Coding Sequence
[6]T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences
[7]B – Border

3.1. Insert and copy number

The presence of a single T-DNA insert in the MON 87460 genome was confirmed by digesting the test and control DNA with *Hind* III, a restriction enzyme that does not cleave within the T-DNA. Therefore, *Hind* III releases a restriction fragment containing the entire T-DNA and adjacent plant genomic DNA (Figure IV-8). The number of restriction fragments detected indicates the number of inserts present in MON 87460. The presence of a single copy of the T-DNA integrated at a single locus was confirmed by digesting test and control genomic DNA samples with the restriction enzyme *EcoR* V, which cleaves once within the insert (Figure IV-8). A single copy of the T-DNA within MON 87460 will produce two bands, each representing a portion of the T-DNA along with adjacent plant genomic DNA.

The Southern blot used to determine insert and copy number of the T-DNA (Figure IV-9) contained several controls. To determine if any endogenous background hybridization bands were detected when probing with the T-DNA, the blot contained conventional corn genomic DNA digested with *Hind* III (Figure IV-9, lanes 1 and 8) or *EcoR* V (Figure IV-9, lanes 3 and 10). The conventional control DNA digested with *Hin*d III or *Eco*R V produced several hybridization signals. These hybridization signals result from the probes hybridizing to endogenous sequences residing in the corn genome and are not specific to the inserted DNA. These signals were produced in both test and control lanes, and therefore the bands are considered to be endogenous background.

To ensure that each of the T-DNA probes was able to hybridize to their respective targets, probe template spikes (Figure IV-6, probes 1-3) that were generated from plasmid PV-ZMAP595 and mixed at different concentrations with the control DNA pre-digested with *Eco*R V were included on the blot (Figure IV-9, lanes 5-6). The expected

hybridization bands at approximately 1.4, 1.6 and 2.0 kb were detected. The approximately 0.1 and 1 copies of the 1.4 kb band were faint in comparison to the 1.6 and 2.0 kb bands, but were clearly detectable. The detection of the probe template positive hybridization controls demonstrates that all three probes are hybridizing to the target DNA. To ensure that the T-DNA probes hybridize to the plasmid used for transformation, plasmid PV-ZMAP595 digested with a combination of *Blp* I and *Xba* I was spiked in the control DNA pre-digested with *Eco*R V. The expected hybridization bands of approximately 3.2 and 6.1 kb (Figure IV-9, lane 7) were detected (Figures IV-6 and IV-7; Table IV-3).

MON 87460 DNA digested with *Hin*d III (Figure IV-9, lanes 2 and 9) and hybridized with the T-DNA probes produced a single unique band of approximately 6.8 kb. This is consistent with the expected band being greater than 4.5 kb (Figure IV-8) and confirms that MON 87460 contains one insert located within a 6.8 kb *Hin*d III restriction fragment. MON 87460 DNA digested with *Eco*R V and hybridized with the T-DNA probes produced two bands (Figure IV-9, lanes 4 and 11) of approximately 2.7 and 7.2 kb. The approximately 2.7 kb band is the expected size for the border fragment containing the 3' end of the T-DNA along with the adjacent genomic DNA flanking the 3' end of the insert (Figure IV-8). The approximately 7.2 kb band is consistent with the expected band being greater than 2.2 kb (Figure IV-8). This band represents the 5' border fragment containing the 5' end of the inserted DNA along with the adjacent genomic DNA flanking the 5' end of the insert.

The results presented in Figure IV-9 show that MON 87460 contains only a single copy of the T-DNA that resides at a single locus of integration on an approximately 6.8 kb *Hin*d III restriction fragment.

The copy number of the inserted *cspB* and *nptII* coding sequences and each of the associated genetic elements were assessed by digesting MON 87460 genomic DNA with restriction enzyme *Eco*R V or a combination of *Eco*O109 I and *Not* I and hybridizing Southern blots with probes covering the inserted *cspB* and *nptII* cassettes. The size of the genomic fragments and the T-DNA elements expected to be contained in each of those fragments is indicated below and summarized in Table IV-3.

Digestion of MON 87460 genomic DNA with the combination of *Eco*O109 I and *Not* I was expected to generate two border fragments with expected sizes of 1.7 kb and greater than 2.7 kb (Figure IV-8). The 1.7 kb restriction fragment contains genomic DNA flanking the 5' end of the insert, *Ract1* promoter and leader, *Ract1* intron, *cspB* coding sequence, and the *tr7* 3' nontranslated sequence. The restriction fragment greater than 2.7 kb contains the 5' *loxP* sequence, *35S* promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border and genomic DNA flanking the 3' end of the insert.

Digestion of MON 87460 genomic DNA with *Eco*R V was expected to release two border fragments with expected sizes of 2.7 kb and greater than 2.2 kb (Figure IV-8). The restriction fragment greater than 2.2 kb contains genomic DNA flanking the 5' end of the insert, *Ract1* promoter and leader, *Ract1* intron, *cspB* coding sequence, and a portion of the *tr7* 3' nontranslated sequence. The approximately 2.7 kb restriction fragment contains the remaining portion of the *tr7* 3' nontranslated sequence, 5' *loxP* sequence, *35S*

promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border, and genomic DNA flanking the 3' end of the insert.

Individual Southern blots were hybridized with the following probes: Right Border + *Ract1* promoter and leader probe, *Ract1* intron probe, *cspB* coding sequence probe, *tr7* 3' nontranslated sequence probe, *loxP* + *35S* promoter probe, *nptII* coding sequence probe, or *nos* 3′ nontranslated + *loxP* + Left Border sequence probe (Figure IV-7, probes 7 – 13, respectively).

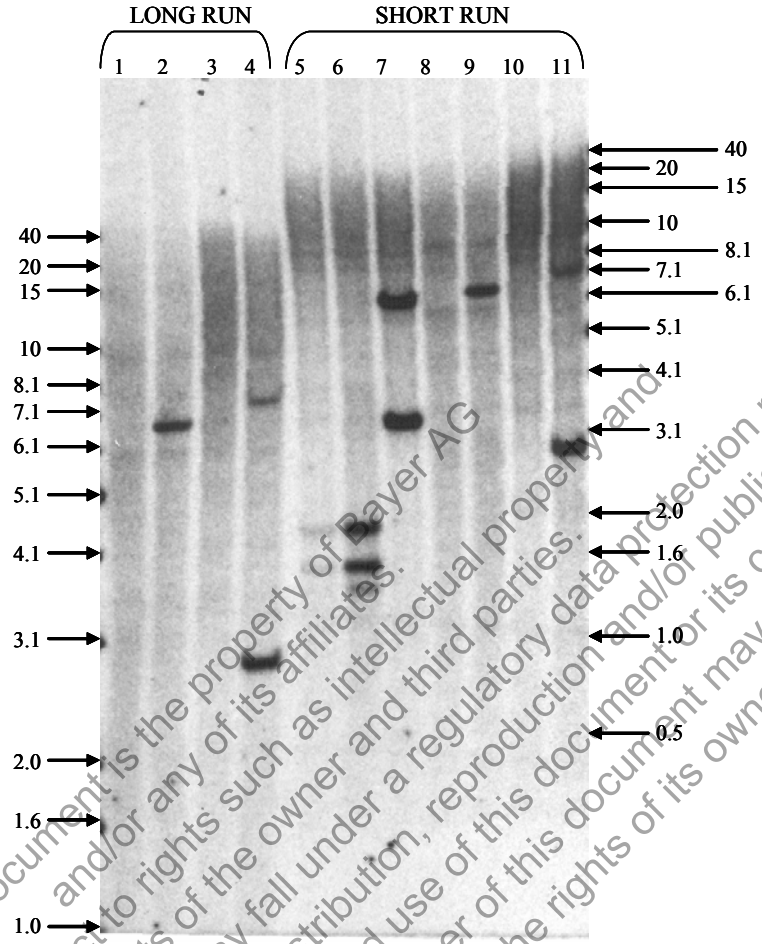


Figure IV-9. Southern Blot Analysis of MON 87460: Insert and Copy Number

The blot was hybridized simultaneously with three overlapping ^{32}P-labeled T-DNA probes that span the insert (Figure IV-6, probes 1-3). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Hin*d III)
2: MON 87460 (*Hin*d III)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
6: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
7: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
8: Conventional (*Hin*d III)
9: MON 87460 (*Hin*d III)
10: Conventional (*Eco*R V)
11: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from molecular weight (MW) markers on ethidium stained gel.

3.2. Intactness of the *cspB* and *nptII* expression cassettes

The presence and intactness of all the elements of the *cspB* and *nptII* expression cassettes was assessed by digestion of MON 87460 genomic DNA with the restriction enzyme *Eco*R V or a combination of *Eco*O109 I and *Not* I. This analysis confirmed that the expression cassettes are intact and a single copy is present.

Digestion of MON 87460 genomic DNA with the combination of *Eco*O109 I and *Not* I was expected to generate two border fragments with expected sizes of 1.7 kb and greater than 2.7 kb (Figure IV-8 and Table IV-3). The 1.7 kb restriction fragment contains genomic DNA flanking the 5' end of the insert, *Ract1* promoter and leader, *Ract1* intron, *cspB* coding sequence, and the *tr7* 3' nontranslated sequence. The restriction fragment greater than 2.7 kb contains the 5' *loxP* sequence, *35S* promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border and genomic DNA flanking the 3' end of the insert.

Digestion of MON 87460 genomic DNA with *Eco*R V was expected to release two border fragments with expected sizes of 2.7 kb and greater than 2.2 kb (Figure IV-8 and Table IV-3). The restriction fragment greater than 2.2 kb contains genomic DNA flanking the 5' end of the insert, *Ract1* promoter and leader, *Ract1* intron, *cspB* coding sequence, and a portion of the *tr7* 3' nontranslated sequence. The approximately 2.7 kb restriction fragment contains the remaining portion of the *tr7* 3' nontranslated sequence, 5' *loxP* sequence, *35S* promoter, *nptII* coding sequence, *nos* 3' nontranslated sequence, 3' *loxP* sequence, left border, and genomic DNA flanking the 3' end of the insert.

Individual Southern blots were hybridized with the following probes: Right Border + *Ract1* promoter and leader probe, *Ract1* intron probe, *cspB* coding sequence probe, *tr7* 3' nontranslated sequence probe, *loxP* + *35S* promoter probe, *nptII* coding sequence probe, or *nos* 3' nontranslated + *loxP* + Left Border sequence probe (Figure IV-7, probes 7 – 13, respectively).

3.2.1. Right border + *Ract1* promoter and leader

Figure IV-11 presents the results of this analysis and confirms no additional, detectable Right Border, *Ract1* promoter and leader elements other than those associated with the intact *cspB* cassette are present in MON 87460. Conventional corn control DNA digested with *Eco*O109 I and *Not* I (Figure IV-11, lanes 1 and 7) or *Eco*R V (Figure IV-11, lanes 3 and 9) probed with the P-*Ract1* probe served as a negative control and produced several hybridization signals. These signals result from the probes hybridizing to endogenous sequences residing in the corn genome and are not specific to the inserted DNA. The bands are considered to be endogenous background because they were produced in both test and control lanes.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this analysis showed the expected hybridization band of approximately 3.2 kb (Figure IV-11, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-11, lanes 2 and 8) produced the expected single unique band of approximately 1.7 kb

(Figure IV-8). The hybridization band in the long run (Figure IV-11, lane 8) appears slightly larger than the corresponding band in the short run (Figure IV-11, lane 2), most likely due to better resolution of the band in the longer run. MON 87460 DNA digested with *Eco*R V (Figure IV-11, lanes 4 and 10) produced a single unique band of approximately 7.5 kb. This is consistent with the expected band being greater than 2.2 kb (Figure IV-8). This band in the long run (Figure IV-11, lane 10) appears slightly larger than the corresponding band in the short run (Figure IV-11, lane 4), most likely due to better resolution of the band in the longer run. There were no additional bands detected using the Right Border, promoter and leader sequence probe. Based on the results presented in Figure IV-11, MON 87460 contains no additional, detectable Right Border, *Ract1* promoter and leader elements other than those associated with the intact *cspB* cassette.

3.2.2. *Ract1* intron

Figure IV-12 presents the results of the *Ract1* intron analysis and confirms that MON 87460 contains no additional, detectable *Ract1* intron elements other than those associated with the intact *cspB* cassette. To determine if any endogenous background hybridization bands were detected when probing with the I-*Ract1* probe (probe 8), the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-12, lanes 1 and 7) or *Eco*R V (Figure IV-12, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands.

As a positive control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with *Eco*R V pre-digested control DNA. Results of this experiment produced a band of approximately 3.1 kb (Figure IV-12, lanes 5 and 6). The expected size of this band is 3.2 kb (Table IV-3). The altered migrations may be due to the difference in salt concentrations between the DNA sample and the molecular weight marker (Sambrook and Russell 2001).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-12, lanes 2 and 8) that was electrophoresed, blotted, and hybridized with probe 8 produced the expected single unique band of approximately 1.7 kb (Figure IV-8). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. MON 87460 DNA digested with *Eco*R V (Figure IV-12, lanes 4 and 10) produced the single unique band of approximately 7.2 kb. This is consistent with the expected band being greater than 2.2 kb (Figure IV-8). There were no additional hybridization bands detected using the *Ract1* intron probe. Based on the results presented in Figure IV-12, MON 87460 contains no additional, detectable *Ract1* intron elements other than those associated with the intact *cspB* cassette.

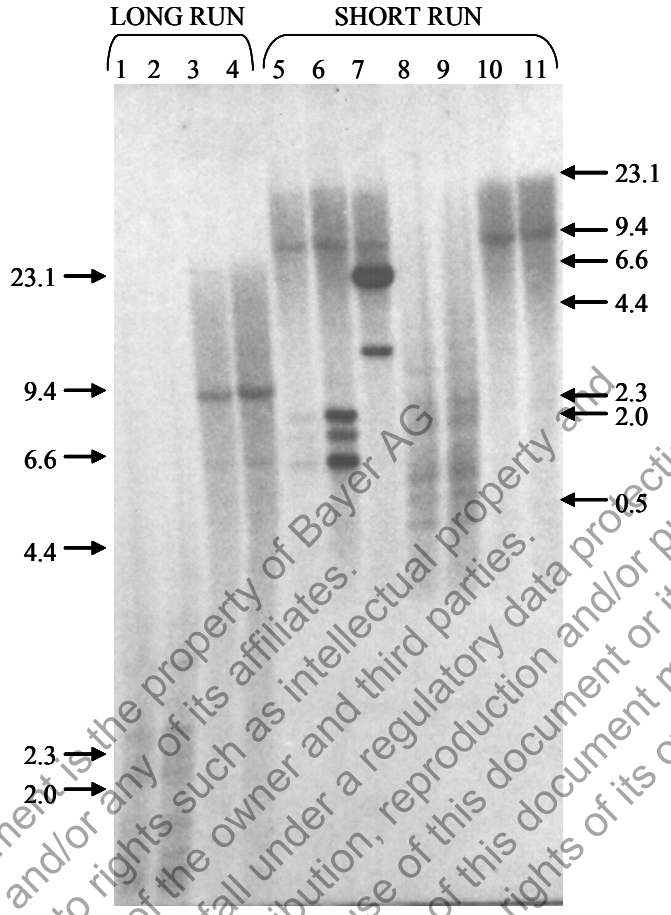


Figure IV-10. Southern Blot Analysis of MON 87460: PVZMAP595 Backbone

The blot was hybridized simultaneously with three overlapping ^{32}P-labeled probes that span the entire backbone sequence (Figure IV-6, probes 4-6) of plasmid PV-ZMAP595. Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
6: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
7: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
8: Conventional (*Eco*O109 I/*Not* I)
9: MON 87460 (*Eco*O109 I/*Not* I)
10: Conventional (*Eco*R V)
11: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

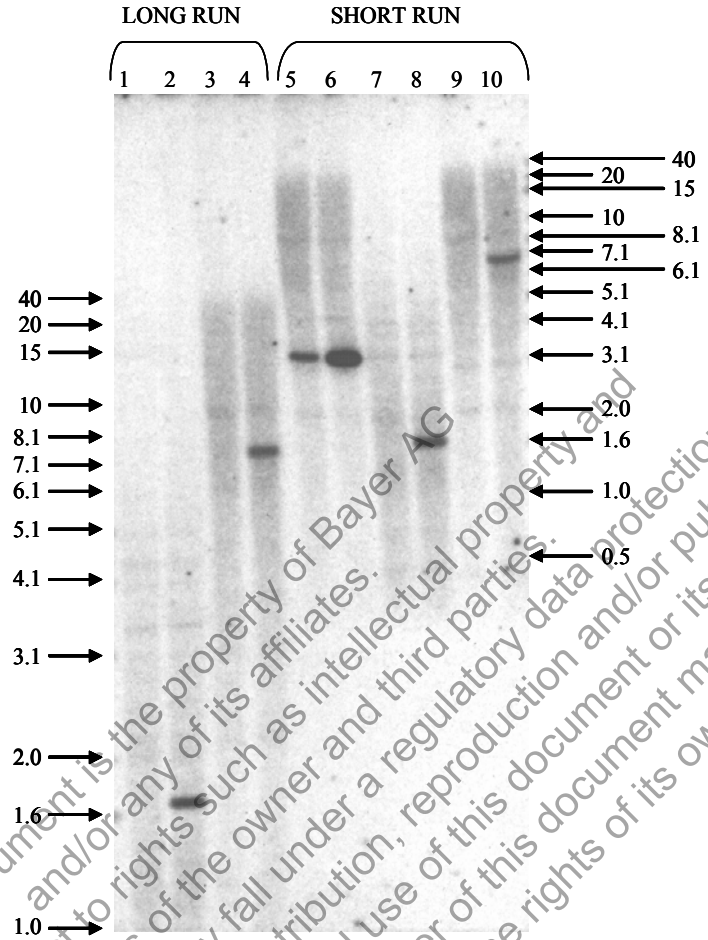


Figure IV-11. Southern Blot Analysis of MON 87460: P-*Ract1*

The blot was hybridized with a ^{32}P-labeled probe that spanned the Right Border, *Ract1* promoter and leader (Figure IV-7, probe 7). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

3.2.3. *cspB* coding sequence

Figure IV-13 presents the results of the *cspB* coding sequence analysis and confirms that MON 87460 contains no additional, detectable *cspB* coding sequence elements other than those associated with the intact *cspB* cassette. To determine if any endogenous background hybridization bands were detected when probing with the CS-*cspB* probe (probe 9), the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-13, lanes 1 and 7) or *Eco*R V (Figure IV-13, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands, as expected for the negative control (Figure IV-13, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced a band of approximately 3.1 kb (Figure IV-13, lanes 5 and 6). The expected size of this band is 3.2 kb. The altered migrations may be due to the difference in salt concentrations between the DNA sample and the molecular weight marker (Sambrook and Russell, 2001).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-13, lanes 2 and 8) and hybridized with probe 9 produced the expected single unique band of approximately 1.7 kb (Figure IV-8). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. MON 87460 DNA digested with *Eco*R V (Figure IV-13, lanes 4 and 10) and hybridized with probe 9 produced the single unique band of approximately 7.2 kb. This is consistent with the expected band being greater than 2.2 kb (Figure IV-8). There were no additional hybridization bands detected using the *cspB* coding sequence probe. Based on the results presented in Figure IV-13, MON 87460 contains no additional, detectable *cspB* coding sequence elements other than those associated with the intact *cspB* cassette.

Although difficult to observe in Figure IV-13, lanes 2 and 8, overexposure of the Southern blots showed a faint band of approximately 2.5 kb that is consistent with partial digestion of genomic DNA. An *Eco*O109 I site is present at position 875 in the 5' flanking genomic DNA (Figure IV-8) and the 2.5 kb band is therefore the product of partial digestion at this site.

3.2.4. *tr7* 3' nontranslated sequence

Figure IV-14 presents the results of the *tr7* 3' nontranslated sequence analysis and confirms that MON 87460 contains no additional, detectable *tr7* 3' nontranslated sequence elements other than those associated with the intact *cspB* cassette. To determine if any endogenous background hybridization bands were detected when probing with probe 10, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-14, lanes 1 and 7) or *Eco*R V (Figure IV-14, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands, as expected for the negative control (Figure IV-14, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control

DNA. Results of this experiment produced an expected band which migrated at approximately 3.1 kb (Figure IV-14, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-14, lanes 2 and 8) and hybridized with probe 10 produced the expected band of 1.7 kb (Figure IV-8). MON 87460 DNA digested with *Eco*R V (Figure IV-14, lanes 4 and 10) and hybridized with probe 10 produced the expected bands of approximately 2.7 and 7.5 kb. The approximately 7.5 kb band is consistent with the expected band being greater than 2.2 kb and the approximately 2.7 kb band is the expected size for the 3´ border fragment (Figure IV-8). The 2.7 kb band is less intense than the approximately 7.5 kb band probably due to a smaller portion of the *tr7* probe hybridizing to the 2.7 kb fragment. There were no additional bands detected using the *tr7* 3' nontranslated probe. Based on the results presented in Figure IV-14, MON 87460 contains no additional, detectable *tr7* 3' nontranslated sequence elements other than those associated with the intact *cspB* cassette.

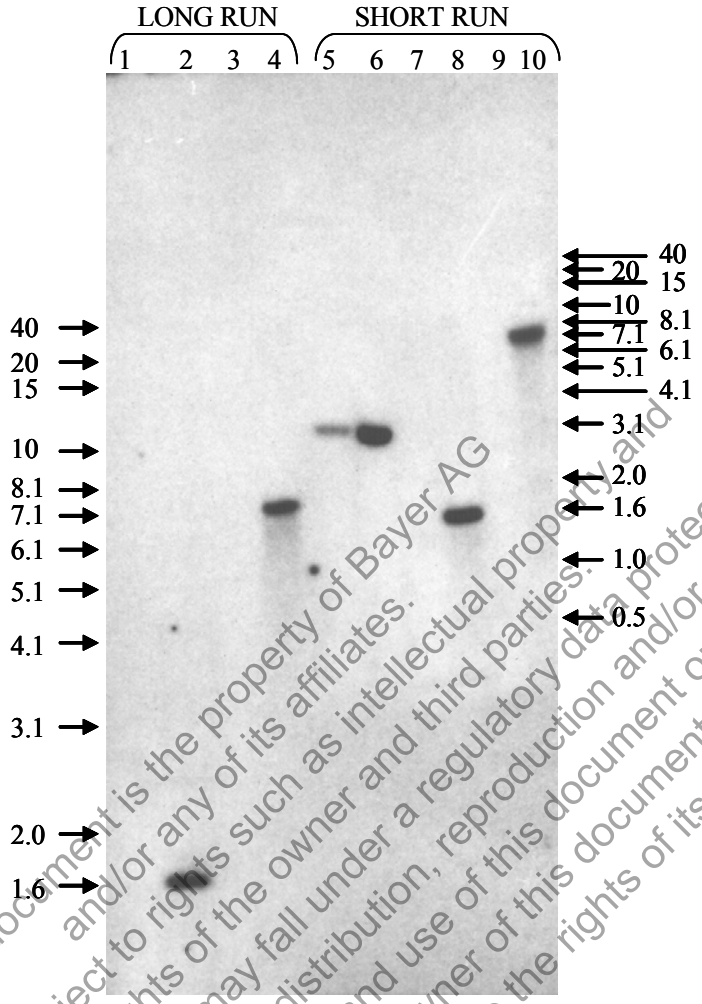


Figure IV-12. Southern Blot Analysis of MON 87460: I-*Ract1*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *Ract1* intron (Figure IV-7, probe 8). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

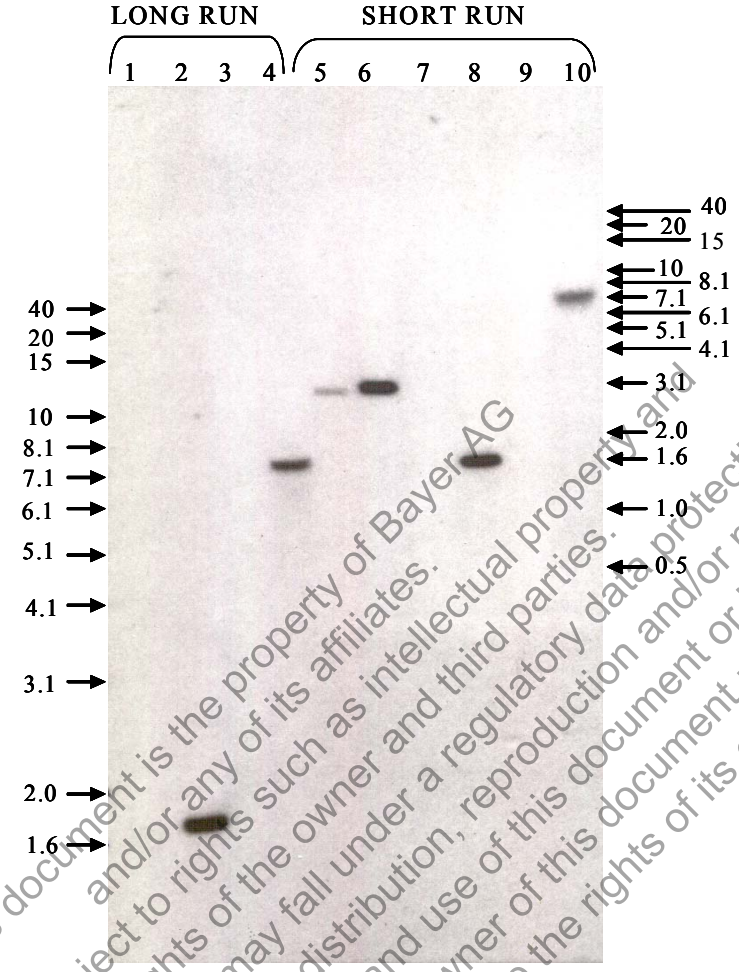


Figure IV-13. Southern Blot Analysis of MON 87460: CS-*cspB*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *cspB* coding sequence (Figure IV-7, probe 9). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

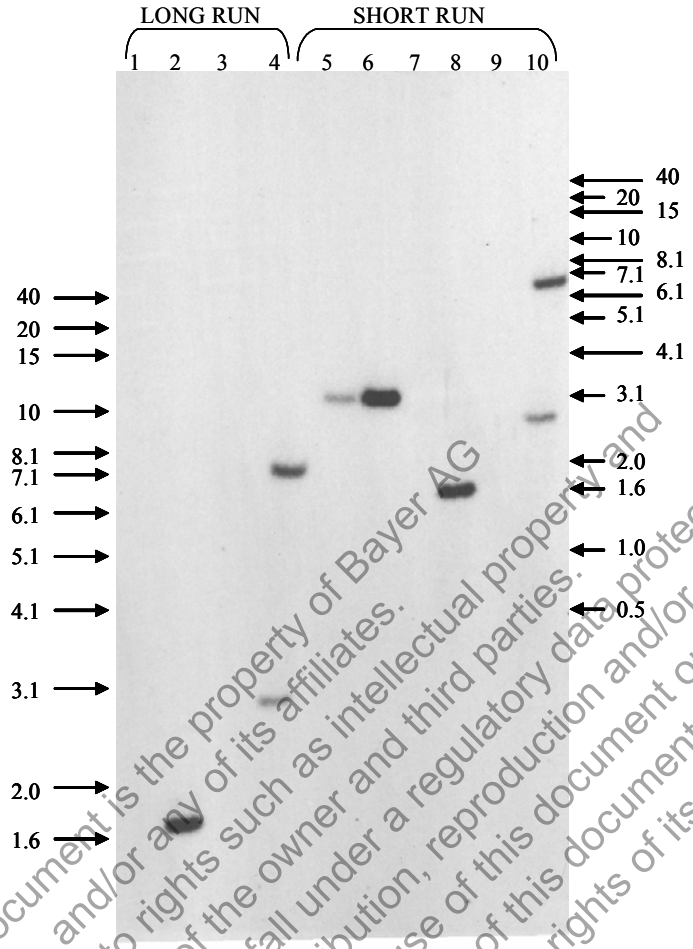


Figure IV-14. Southern Blot Analysis of MON 87460: T-*tr7*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *tr7* 3' nontranslated sequence (Figure IV-7, probe 10). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

3.2.5. *loxP* + *35S* promoter

Figure IV-15 presents the results of the loxP + *35S* promoter analysis and confirms that MON 87460 contains no additional, detectable *loxP* sequence or *35S* promoter elements other than those associated with the intact *nptII* cassette. To determine if any endogenous background hybridization bands were detected when probing with probe 11, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-15, lanes 1 and 7) or *Eco*R V (Figure IV-15, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands, as expected for the negative control (Figure IV-15, lanes 1, 3, 7, and 9).

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced the expected size band at 6.1 kb (Figure IV-15, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-15, lanes 2 and 8) and hybridized with probe 11 produced the expected single unique band of approximately 3.2 kb. This is consistent with the expected band being greater than 2.7 kb (Figure IV-8). MON 87460 DNA digested with *Eco*R V (Figure IV-15, lanes 4 and 10) and hybridized with probe 11 produced the expected single unique band of 2.7 kb (Figure IV-8). As there were no unexpected bands detected, the results presented in Figure IV-15 show that MON 87460 contains no additional, detectable *loxP* sequence or *35S* promoter elements other than those associated with the intact *nptII* cassette.

3.2.6. *nptII* coding sequence

Figure IV-16 presents the results of the *nptII* coding sequence analysis and confirms that MON 87460 contains no additional, detectable *nptII* coding sequence elements other than those associated with the intact *nptII* cassette. To determine if any endogenous background hybridization bands were detected when probing with probe 12, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-16, lanes 1 and 7) or *Eco*R V (Figure IV-16, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA and hybridized with probe 12. Results of this experiment produced an expected band which migrated at approximately 5.5 kb (Figure IV-16, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-16, lanes 2 and 8) and hybridized with probe 12 produced the expected single unique band of approximately 3.2 kb (Figure IV-8). This band in the long run appears slightly larger than the corresponding band in the short run, most likely due to better resolution of the band in the long run. This band size is consistent with the expected band being greater than 2.7 kb (Figure IV-8). MON 87460 DNA digested with *Eco*R V (Figure IV-16, lanes 4 and 10) that and hybridized with probe 12 produced the expected single unique band of 2.7 kb (Figure IV-8). There were no additional bands detected using the *nptII* coding sequence probe. Based on the results presented in Figure IV-16, MON 87460 contains no

additional, detectable *nptII* coding sequence elements other than those associated with the intact *nptII* cassette.

3.2.7. *nos* 3' nontranslated sequence + *loxP* + left border sequence

Figure IV-17 presents the results of the *nos* 3' Nontranslated sequence + *loxP* + Left Border Sequence analysis and confirms that MON 87460 contains no additional, detectable *nos* 3' nontranslated sequence, *loxP* sequence or left border sequence elements other than those associated with the intact *nptII* cassette. To determine if any endogenous background hybridization bands were detected when probing with probe 13, the blot contained conventional corn genomic DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-17, lanes 1 and 7) or *Eco*R V (Figure IV-17, lanes 3 and 9). The results of this analysis showed no detectable hybridization bands.

As a positive hybridization control, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with pre-digested control DNA. Results of this experiment produced the expected size band at 6.1 kb (Figure IV-17, lanes 5 and 6).

MON 87460 DNA digested with a combination of *Eco*O109 I and *Not* I (Figure IV-17, lanes 2 and 8) and hybridized with probe 13 produced the expected single unique band of approximately 3.2 kb. This is consistent with the expected band being greater than 2.7 kb (Figure IV-8). MON 87460 DNA digested with *Eco*R V (Figure IV-17, lanes 4 and 10) and hybridized with probe 13 produced the expected single unique band of 2.7 kb (Figure IV-8). As there were no unexpected bands detected, the results presented in Figure IV-17 show that MON 87460 contains no additional, detectable *nos* 3' nontranslated sequence, *loxP* sequence or left border sequence elements other than those associated with the intact *nptII* cassette.

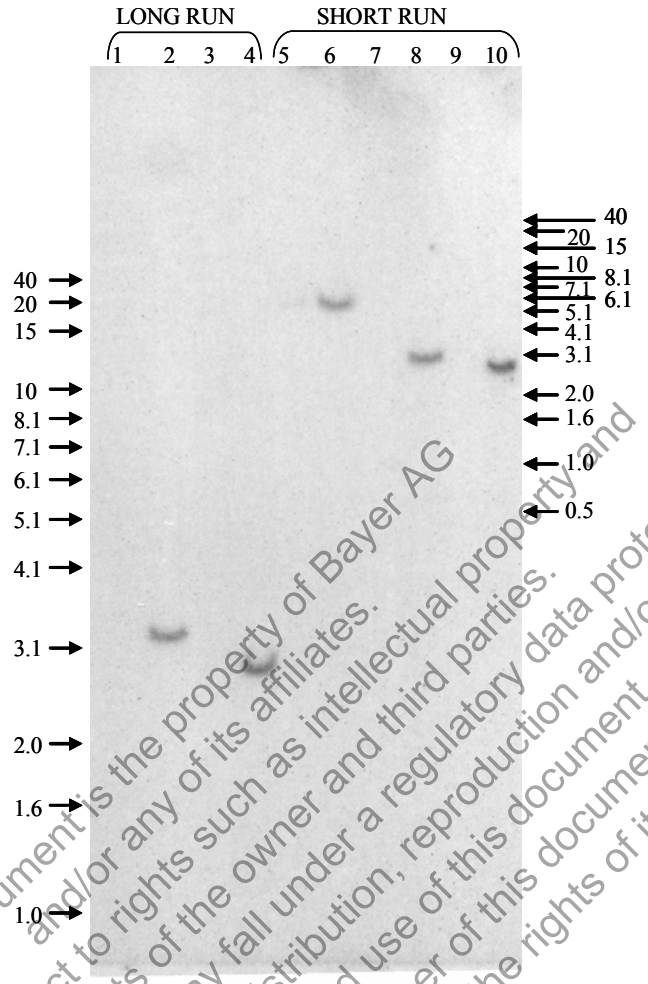


Figure IV-15. Southern Blot Analysis of MON 87460: loxP + P-*35S*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *loxP* sequence and *35S* promoter (Figure IV-7, probe 11). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

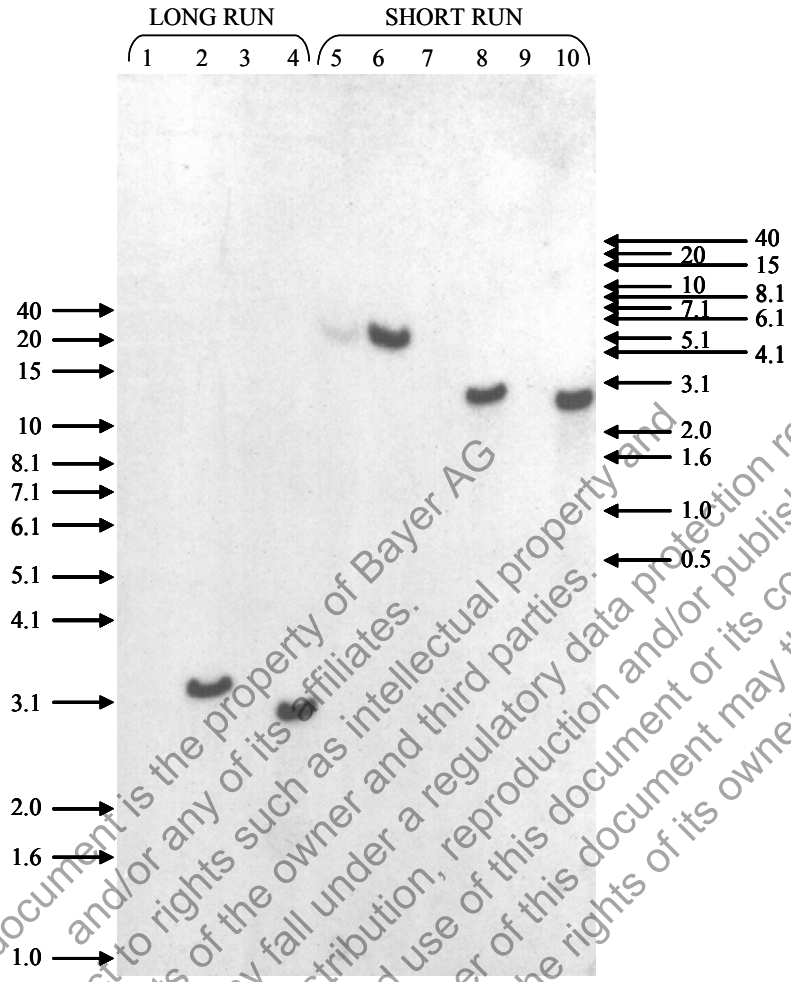


Figure IV-16. Southern Blot Analysis of MON 87460: CS-*nptII*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *nptII* coding sequence (Figure IV-7, probe 12). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

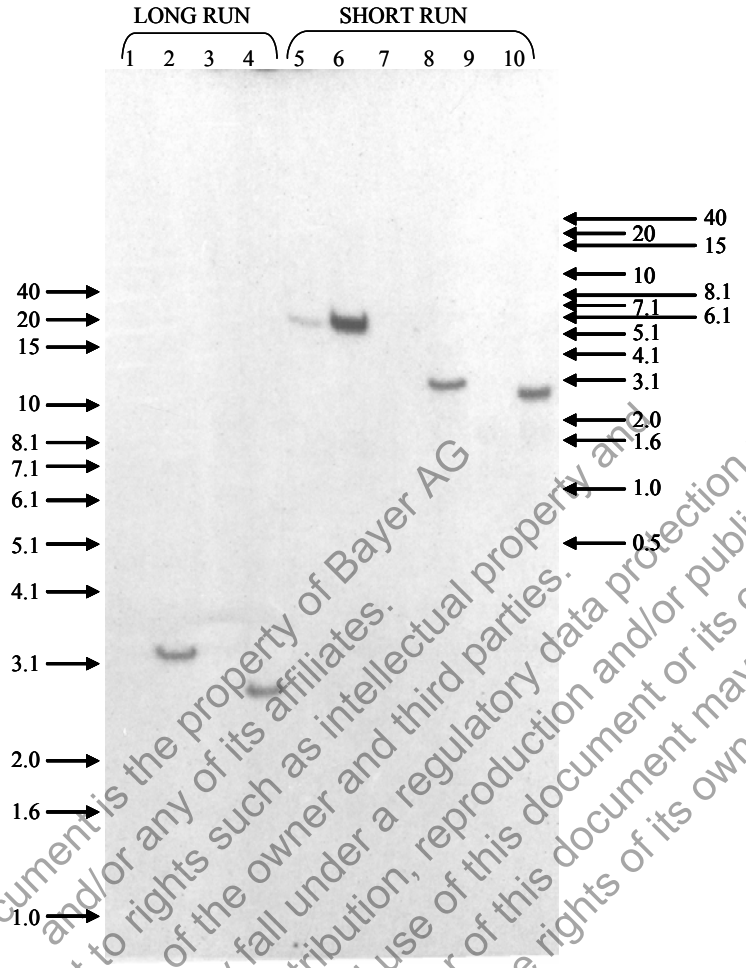


Figure IV-17. Southern Blot Analysis of MON 87460: T-*nos* + *loxP* + B-Left Border

The blot was hybridized with a ^{32}P-labeled probe that spanned the *nos* 3' nontranslated sequence, *loxP* sequence and Left Border sequence (Figure IV-7, probe 13). Each lane contains ~10 µg of digested genomic DNA isolated from seed. Lane designations are as follows:

1: Conventional (*Eco*O109 I/*Not* I)
2: MON 87460 (*Eco*O109 I/*Not* I)
3: Conventional (*Eco*R V)
4: MON 87460 (*Eco*R V)
5: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~0.1 copy]
6: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
7: Conventional (*Eco*O109 I/*Not* I)
8: MON 87460 (*Eco*O109 I/*Not* I)
9: Conventional (*Eco*R V)
10: MON 87460 (*Eco*R V)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

3.2.8. Analysis to confirm the absence of plasmid PV-ZMAP595 backbone

The generations of MON 87460 utilized to confirm stability of the insert were also tested for the presence of backbone sequence by Southern blot analysis. Test and control DNA samples were digested with *Eco*R V and the blot was hybridized simultaneously with three radiolabeled probes that span the entire backbone sequence of plasmid PV-ZMAP595 (Figure IV-6, probes 4-6). Results demonstrate that the generations tested do not contain any detectable backbone sequence from the transformation vector PV-ZMAP595.

To determine if any endogenous background hybridization bands were detected when hybridizing with the three radiolabeled backbone probes, the blot contained conventional control DNA digested with *Eco*R V (Figure IV-20, lane 1). Several hybridization bands were detected. These signals were produced in all lanes, including those lanes containing the conventional control DNA material, and therefore they are considered endogenous background. These hybridization signals result from the probes hybridizing to endogenous targets residing in the corn genome and are not specific to the inserted DNA.

To ensure that each of the backbone probes was capable of hybridizing to its respective target, the blot contained probe template spikes (Figure IV-6, probes 4-6) that were generated from plasmid PV-ZMAP595 and mixed with the control DNA pre-digested with *Eco*R V (Figure IV-20, lanes 2 and 3). The expected sizes of the three bands are 1.4, 1.7, and 2.0 kb; however, the migrations of the approximately 1.7, 2.1 and 2.5 kb fragments are slightly higher than indicated by the molecular weight marker. The altered migrations may be due to the difference in salt concentrations between the DNA sample and the molecular weight marker (Sambrook and Russell, 2001). The results show that the three probes hybridized to the target DNA. To ensure the probes were capable of hybridizing to the plasmid used for transformation, the blot contained plasmid PV-ZMAP595 that was digested with a combination of *Blp* I and *Xba* I and mixed with control DNA pre-digested with *Eco*R V (Figure IV-20, lane 4). Hybridization with probes 4-6 produced the expected size bands at approximately 3.2 and 6.1 kb, in addition to the endogenous background produced by the conventional control DNA.

MON 87460 DNA isolated from multiple generations of MON 87460 (Figure IV-18), digested with restriction enzyme *Eco*R V, and hybridized with three overlapping ^{32}P-labeled probes that span the backbone sequences of PV-ZMAP595 (Figure IV-6, probes 4-6) showed no detectable hybridization signals, besides the endogenous background bands (Figure IV-20, lanes 5-11). Consistent with results depicted in Figure IV-10 (lanes 4 and 11), these results demonstrate that the generations tested do not contain any detectable backbone sequence from the transformation vector PV-ZMAP595.

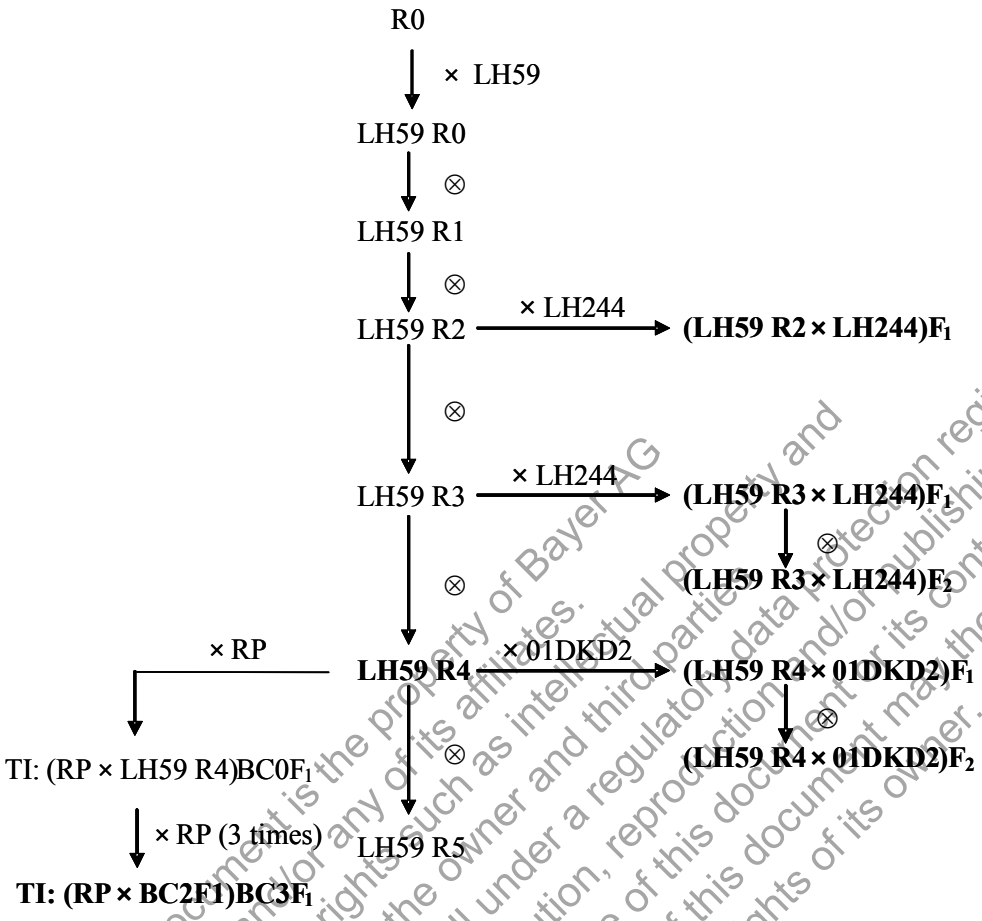
3.3. Insert stability across generations of MON 87460

DNA samples from seven generations of MON 87460 were isolated and subjected to digestion with EcoR V (refer to generations indicated in bold in Figure IV-18) to confirm that the T-DNA is stable across multiple generations. Digestion of the test materials with EcoR V was expected to release two border fragments with expected sizes of 2.7 kb and >2.2 kb (Figure IV-8). The blot was hybridized simultaneously with three radiolabeled probes that span the entire T-DNA sequence of plasmid PV-ZMAP595 (Figure IV-6, probes 1-3). The hybridization bands detected in each generation are compared to the fully characterized R3F1 [(LH59 R3×LH244)F_1] generation to determine insert stability. Results of this analysis confirm that the single copy of T-DNA in MON 87460 is stable across the selected generations.

The Southern blot used to confirm generational stability of the T-DNA (Figure IV-19) contained several controls. To determine if any endogenous background hybridization bands were detected when hybridizing with the three radiolabeled probes that span the entire T-DNA, the blot contained conventional control DNA digested with *Eco*R V (Figure IV-19, lane 1). This analysis detected several hybridization bands. These hybridization signals result from the probes hybridizing to endogenous targets residing in the corn genome and are not specific to the inserted DNA.

To ensure that each probe was capable of hybridizing to its respective target, the blot contained probe template spikes (Figure IV-6, probes 1-3) that were generated from plasmid PV-ZMAP595 and mixed at different concentrations with control DNA pre-digested with *Eco*R V (Figure IV-19, lanes 2 and 3). When hybridized with three overlapping ^{32}P-labeled probes that span the entire T-DNA (Figure IV-6, probes 1-3), the expected hybridization bands at approximately 1.4, 1.6, and 2.0 kb were detected. The 0.1 and 1 copies of the 1.4 kb band are faint in comparison to the 1.6 and 2.0 kb bands, but were clearly detectable. The detection of the probe template positive hybridization controls demonstrates that all three probes are hybridizing to the target DNA. To ensure that the probes were capable of hybridizing to the plasmid used for transformation, the blot contained plasmid PV-ZMAP595 digested with a combination of *Blp* I and *Xba* I and mixed with control DNA pre-digested with *Eco*R V (Figure IV-19, lane 4). Hybridization with probes 1-3 produced the expected size bands at approximately 3.2 and 6.1 kb in addition to the endogenous background.

DNA isolated from multiple generations of MON 87460 (Figure IV-18), digested with restriction enzyme *Eco*R V, and hybridized with three overlapping ^{32}P-labeled probes that span the entire T-DNA (Figure IV-6, probes 1-3) produced two hybridization bands at 2.7 kb and approximately 7.2 kb (Figure IV-19, lanes 5-11). The approximately 7.2 kb band is consistent with the 5' border fragment which was expected to be greater than 2.2 kb (Figure IV-8). The 2.7 kb band is the expected size for the border fragment containing the 3' end of the insert and adjacent flanking genomic DNA (Figure IV-8). This is the same restriction pattern observed for the F_1 generation (LH59 R3 x LH244) shown in Figure IV-9 (lanes 4 and 11). There were no additional unexpected bands detected, demonstrating that the single copy of T-DNA in MON 87460 is stable across the selected generations.



R0 = transformed plant; F(#) = filial generation; ⊗ = self-pollination; BC(#) = backcross generation; RP = recurrent parent for commercial seed development; TI = trait integration for commercial seed development.

Figure IV-18. MON 87460 Breeding Diagram

The (LH59 R3 × LH244)F_1 generation was used for the molecular characterization of MON 87460. The (LH59 R2 × LH244) F1, (LH59 R3 × LH244)F_1, (LH59 R3 × LH244)F_2, LH59 R4, (LH59 R4 × 01DKD2)F_1, (LH59 R4 × 01DKD2)F_2, and (RP×BC2F1)BC3F_1 generations were used for generational stability (indicated in bold). The (LH59 R3 × LH244)F_2 and (LH59 R4 × 01DKD2)F_2 generations were used for expression and composition analyses.

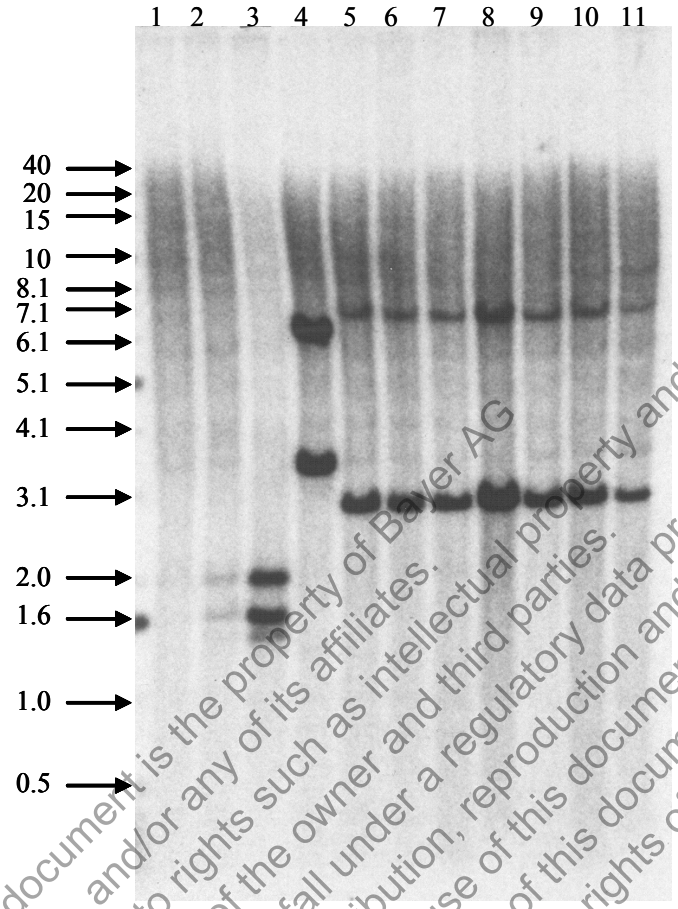


Figure IV-19. Generational Stability of MON 87460: Insert and Copy Number

The blot was hybridized with three overlapping ^{32}P-labeled probes that spanned the T-DNA (Figure IV-6, probes 1-3). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. The breeding history of MON 87460 is illustrated in Figure IV-18.

Lane designations are as follows:

1: Conventional (*Eco*R V)
2: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
3: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
4: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
5: MON 87460 [(LH59 R2 x LH244)F_1, *Eco*R V]
6: MON 87460 [(LH59 R3 x LH244)F_1, *Eco*R V]
7: MON 87460 [(LH59 R3 x LH244)F_2, *Eco*R V]
8: MON 87460 (LH59 R4, *Eco*RV)
9: MON 87460 [(LH59 R4 x 01DKD2)F_1, *Eco*R V]
10: MON 87460 [(LH59 R4 x 01DKD2)F_2, *Eco*R V]
11: MON 87460 [(RP x BC2F1)BC3F_1, *Eco*R V]

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

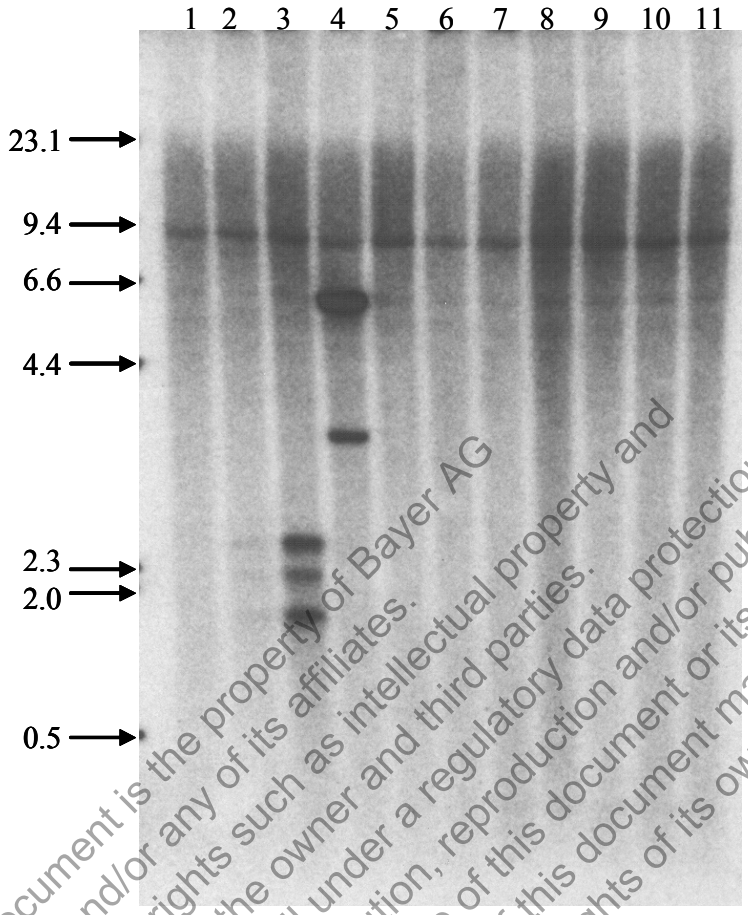


Figure IV-20. Generational Stability of MON 87460: PV-ZMAP595 Backbone

The blot was hybridized with three overlapping ^{32}P-labeled probes that spanned the T-DNA (Figure IV-6, probes 4-6). Each lane contains approximately 10 µg of digested genomic DNA isolated from seed. The breeding history of MON 87460 is illustrated in Figure IV-18. Lane designations are as follows:

1: Conventional (*Eco*R V)
2: Conventional (*Eco*R V) spiked with probe templates [~0.1 copy]
3: Conventional (*Eco*R V) spiked with probe templates [~1 copy]
4: Conventional (*Eco*R V) spiked with PV-ZMAP595 (*Blp* I/*Xba* I) [~1 copy]
5: MON 87460 [(LH59 R2 x LH244)F_1, *Eco*R V]
6: MON 87460 [(LH59 R3 x LH244)F_1, *Eco*R V]
7: MON 87460 [(LH59 R3 x LH244)F_2, *Eco*R V]
8: MON 87460 (LH59 R4, *Eco*RV)
9: MON 87460 [(LH59 R4 x 01DKD2)F_1, *Eco*R V]
10: MON 87460 [(LH59 R4 x 01DKD2)F_2, *Eco*R V]
11: MON 87460 [(RP x BC2F1)BC3F_1, *Eco*R V]

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium stained gel.

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3.4. Organization and sequence of the insert DNA in MON 87460

The organization of the genetic elements within the insert of MON 87460 was confirmed by DNA sequence analyses. Several polymerase chain reaction (PCR) primers were designed to amplify overlapping DNA fragments spanning the entire length of the insert. The amplified DNA fragments were subjected to DNA sequence analyses. The DNA sequence of the insert contains 3309 base pairs beginning at base 3938 of PV-ZMAP595 located in the P-*Ract1* element region, and ending at base 7246 in the Left Border region of PV-ZMAP595. There are 733 base pairs of the P-*Ract1* element region of PV-ZMAP595 (base 3205-3937) absent in the MON 87460 insert presumably resulting from double-strand break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta 1998). In addition to the insert DNA sequence, 1121 base pairs of corn genomic DNA flanking the 5' end of the insert and 784 base pairs of corn genomic DNA flanking the 3' end of the insert were also determined. Results confirm the presence and that the organization of the insert genetic elements is as depicted in Table IV-4.

3.5. Trait inheritance in MON 87460

During the development of the MON 87460, trait segregation data were generated and analyzed. Chi-square analysis was performed over two generations to confirm the segregation and stability of the *csp*B gene in MON 87460. The Chi-square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles. The R_0 plant was self-pollinated to produce R_1 seed, which is expected to segregate 1:2:1 (1 homozygote:2 hemizygous:1 null segregant) for the gene. A homozygous selection (R_1 plant) was identified from the segregating population by using an NPTII based Invader assay. The selected R_1 plant was self-pollinated again to produce R_2 seed, which was expected to be fixed for the trait, meaning, all seed are homozygous for the gene.

In additional tests, plants were backcrossed to produce BC3F1, BC3F2, BC3F3, BC4F1, and BC5F1 seed. These generations are derived from the R4 generation identified in Figure IV-18. The BC3F1, BC4F1, and BC5F1 generations were expected to segregate 1:1 (1 positive:1 negative). The BC3F2 generation was expected to segregate 1:2:1 (1 homozygote:2 hemizygous:1 null segregant) for the gene. The BC3F3 generation was expected to be fixed for the trait.

The Chi-square test was computed as:

$$x^2 = \sum \frac{(o-e)^2}{e}$$

where o = observed frequency of the genotype and e = expected frequency of the genotype. The critical Chi-square value at $\alpha = 0.05$ and 1 degree of freedom is 3.841.

The segregation patterns reported in Table IV-5 are based on PCR-based assays. The Chi-square values for the R1, BC3F1, BC3F2, BC4F1, and BC5F1 generations indicated no significant differences between the observed and expected segregation ratios. The data

for the R2 and BC3F3 generations confirmed that the populations were fixed and that all plants tested positive for the *csp*B gene. These results are consistent with molecular characterization data indicating single insertion site of the gene and confirm that the *cspB*/*nptII* cassette within MON 87460 follows the expected Mendelian pattern of segregation.

Table IV-5. Segregation Patterns of *csp*B Between Generations of MON 87460

Generation	Number of Plants	Observed Positives	Observed Negatives	Expected Positives	Expected Negatives	Chi-Square*	Probability ($\alpha = 0.05$)
R1	36	26	10	27	9	0.1481	NS
R2	89	89	0	89	0	Fixed	—
BC3F1	178	84	94	89	89	0.562	NS
BC3F2	154	124	30	115.5	38.5	2.502	NS
BC3F3	474	474	0	474	0	Fixed	—
BC4F1	80	44	36	40	40	0.800	NS
BC5F1	82	44	38	41	41	0.439	NS

* The critical Chi-square value at $\alpha = 0.05$ and 1 degree of freedom is 3.841.
*csp*B – Gene encoding cold shock protein B from *B. subtilis*.
NS – not significant.

3.6. Conclusion

Molecular analyses demonstrated that one intact copy of the *cspB* and *nptII* expression cassette was integrated at a single chromosomal locus contained within a ~6.8 kb *Hind* III restriction fragment. No additional elements from the transformation vector PV-ZMAP595, linked or unlinked to the intact DNA insert, were detected in the genome of MON 87460. Additionally, backbone sequence from PV-ZMAP595 was not detected. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 87460 has been maintained across seven generations of breeding, thereby confirming the stability of the DNA insert over multiple generations. These generations were also shown not to contain any detectable backbone sequence from plasmid PV-ZMAP595. In addition, DNA sequence analyses confirmed the sequence identity between the MON 87460 insert and the portion of the T-DNA from PV-ZMAP595 that was integrated into the corn genome. These results also confirmed the organization of the genetic elements within the *cspB* and *nptII* expression cassettes of MON 87460, which was identical to that in plasmid PV-ZMAP595. Analysis of the T-DNA insertion site indicates that there is a 22-base pair (bp) deletion of genomic DNA at the insert-to-plant DNA junction. Segregation analyses show heritability and stability of the *cspB* and *nptII* genes occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes that the DNA insert is present at a single chromosomal locus.

SECTION 4. Other Data and Information about the Development of MON 87460

In addition to the preceding studies contained in Part IV, open reading frame bioinformatic analyses were performed on the 5′ and 3′ insert junctions and on the *cspB* and *nptII* coding sequences. These analyses examine the potential for any putative proteins that could be produced in MON 87460 Results from all analyses demonstrate that any putative MON 87460 is unlikely to produce proteins that exhibit allergenic, toxic or otherwise biologically adverse properties.

4.1. Insert junction open reading frame analysis

Analyses of putative polypeptides encoded by DNA spanning the 5′ and 3′ junctions of the MON 87460 inserted DNA were performed using a bioinformatic comparison strategy. The purpose of the assessment is to evaluate the potential for novel open reading frames that may have concerns for similarity to known allergens and toxins. DNA sequence spanning the 5′ and 3′ junctions of the MON 87460 insertion site was analyzed for translational stop codons (TGA, TAG, TAA) and all open reading frames originating or terminating within the MON 87460 insertion site were translated using the standard genetic code from stop codon to stop codon. Five sequences of eight amino acids or greater in length spanning the 5′ junction, and four sequences of eight amino acids or greater in length spanning the 3′ junction were identified and used as search sequences for FASTA comparisons against the AD8 (version AD8; www.allergenonline.com), TOXIN6 and PROTEIN databases. In addition, the nine sequences were searched for eight amino sequences that match proteins in the AD8 database.

Results of the FASTA sequence alignments demonstrated a lack of structurally relevant similarity between any known allergens, toxins, or bioactive proteins and the nine putative polypeptides. Results from the eight amino acid search demonstrated the lack of immunologically relevant matches between any of the putative polypeptides and the AD8 database. Bioinformatic analyses performed using the nine query sequences support the conclusion that even in the highly unlikely event that any of the putative junction polypeptides were translated they would not share a sufficient degree of sequence similarity with known allergens or toxins. Therefore, there is no evidence for concern regarding health implications of the cross-junction putative polypeptides in MON 87460.

4.2. Assessment of open reading frames contained in the *cspB* and *nptII* coding sequences

Although DNA replication, DNA transcription and mRNA translation are of extremely high fidelity, mutation may in certain rare circumstances lead to the potential translation of mRNA on reading frames other than those defined by the intended translation start codon. In such instances, a novel protein may be produced. Due to the spontaneous nature of mutations, it is not possible to determine when or where in a coding sequence such an event may occur. In order to assess potential risks, bioinformatic analyses were performed using the AD8, TOXIN6 and PROTEIN databases on alternative open reading frame translation products that could be derived from the *cspB* and *nptII* coding sequences in MON 87460. Results demonstrate that putative proteins derived from alternative open reading frames of the *cspB* and *nptII* coding sequences are unlikely to be allergenic, toxic or otherwise exhibit adverse biological activity.

Using the translation of frames 2 through 6 of the *cspB* coding sequence for a FASTA search query in the AD8 database, no alignment met or exceeded the Codex Alimentarius (Codex, 2003) FASTA alignment threshold of 35% identity over 80 amino acids as the maximum length of query sequence derived from the translation of frames 2 through 6 is 68 amino acids. Furthermore, when frames 2 through 6 of the *cspB* coding sequence were used to perform FASTA searches of the TOXIN6 and PROTEIN databases, no significant sequence alignments were observed with toxins or other biologically active proteins.

Using the translation of frames 2 through 6 of the *nptII* coding sequence for a FASTA search query in the AD8 database, no alignment met or exceeded the Codex Alimentarius (Codex, 2003) FASTA alignment threshold of 35% identity over 80 amino acids. Likewise, no FASTA alignments with the TOXIN6 database displayed *E*-scores less than 1×10^{-5}. When used to search the PROTEIN database, translations of four of the five frames (2, 4, 5 and 6) yielded alignments with *E*-scores less than 1×10^{-5}. Inspection of these alignments revealed that none were with proteins known to display adverse biological activity when consumed in food or feed.

None of the possible open reading frames for the *cspB* coding sequence produced significant sequence alignments with the AD8, TOXIN6 or PROTEIN databases, confirming that any putative proteins are unlikely to be allergenic, toxic or biologically active. Data for the five putative peptides derived from the *nptII* coding sequence also demonstrate no significant sequence alignments with allergenic or toxic proteins. In the cases where putative proteins from the *nptII* coding sequence produced a significant alignment, it was with proteins that do not exhibit adverse biological activity when consumed in food and feed.

References

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PART V: PRESENCE OF GENES THAT ENCODE RESISTANCE TO ANTIBIOTICS

NPTII was used as an antibiotic resistance marker in the initial selection process. The presence of *nptII* does not pose any safety concerns. The NPTII protein expressed in MON 87460 is the same as the NPTII protein expressed in a number of other transgenic crops that has been found to be safe by multiple governmental agencies and scientific bodies throughout the world. Safety issues associated with use of *nptII* and the protein it expresses have previously been examined by FDA in a ruling that authorizes use of this gene product as a processing aid food additive for the development of transgenic crops (FDA, 1994). This ruling was reviewed by a panel of scientific experts who concluded that the approach taken by FDA in evaluating the safety of *nptII* and the protein it expresses was scientifically sound and included all relevant parameters (FDA, 1998). The safety of NPTII has been addressed in multiple publications (Fuchs et al., 1993a and 1993b; Flavel et al., 1992; and Nap et al., 1992). In 2007, the European Food Safety Authority (EFSA) affirmed its conclusion that the presence of *nptII* does not pose a threat to human health or the environment (EFSA, 2007). Lastly, EPA has established an exemption from the requirement of a tolerance for NPTII and the genetic material necessary for its expression in or on raw agricultural commodities (40 CFR Part 180.1134). Collectively, these regulatory actions confirm the safety of the NPTII protein and the genetic material necessary for its expression.

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PART VI: CHARACTERIZATION OF THE PROTEINS INTRODUCED INTO MON 87460

SECTION 1. Identity and Characterization of the CSPB and NPTII Proteins Produced in MON 87460

A multistep approach is necessary to assess the safety of proteins introduced into plants using biotechnology. These steps include: 1) characterization of the physicochemical and functional properties of the protein; 2) quantifying protein expression in plant tissues; 3) examining the similarity of the protein to known allergens, toxins or other biologically active proteins known to have adverse effects on animals; 4) evaluating the digestibility of the protein in simulated gastrointestinal fluids; 5) documenting the history of safe consumption of the protein or its structural and functional homology to proteins that lack adverse effects on human or animal health; and 6) investigating potential mammalian toxicity through animal assays and calculating margins of exposure.

This part is organized into five sections that describe the safety assessment of the CSPB and NPTII proteins. The first section describes the biochemical characterization of the plant-produced CSPB and NPTII proteins and the equivalence of these proteins to their respective *E. coli*-produced proteins used in subsequent laboratory studies. The second section describes CPSB and NPTII protein expression levels in MON 87460. The third section provides an allergenicity assessment for the CSPB protein and updated bioinformatic data for the NPTII protein. The fourth section provides an evaluation of potential protein toxicity. Section 5 presents human and animal dietary risk assessments for CSPB and NPTII.

All data indicate that the CSPB and NPTII proteins are safe for human and animal consumption. Both proteins have histories of safe consumption and expression levels in MON 87460 are low, particularly in grain. Both proteins lack similarity to known allergens, toxins and anti-nutritional proteins known to have adverse effects to humans and animals. Additionally, both proteins are readily digestible in simulated gastric and simulated intestinal fluids and are not immunodetectable following heat treatment. Neither protein exhibits any toxicity in a mouse gavage assay even when doses are several orders of magnitude greater than would be experienced under the most conservative exposure scenarios. Ultimately, the safety assessment supports the conclusion that there are no meaningful risks to human health from dietary exposure to either the CSPB or NPTII proteins produced in MON 87460.

1.1. Identity and function of the CSPB protein

Cold shock proteins confer environmental stress tolerance in bacteria

CSPB produced in MON 87460 belongs to the cold-shock protein (CSP) family, which has been extensively studied in bacteria. Early investigations of bacterial responses to cold-induced stress led to the discovery of CSPs, a group of small proteins that contain a highly conserved RNA-binding sequence identified as a cold shock domain (CSD).

In bacteria, a variety of environmental stresses are known to disrupt normal cell physiology, in part due to the production of RNA secondary structures which leads to a reduction in protein synthesis. Under environmental stress, CSD-containing proteins

have been shown to bind to a broad array of RNA, including RNA secondary structures (Cristofari and Darlix, 2002), leading to maintenance of mRNA levels, sustainable translation, and improved cellular function. While some members of the bacterial CSP family accumulate strictly in response to temperature shifts (Etchegaray et al., 1996), others, including the *B. subtilis* CSPB protein, are also involved in maintaining normal cellular functions at both optimal temperatures (Graumann et al., 1997) and under nutrient limitation (Anderson et al., 2006).

In actively transcribing *B. subtilis* cells, CSPB is localized around the nucleoid, co-localizing with the ribosomes (Mascarenhas et al., 2001; Weber et al., 2001). In stationary-phase cells CSPB is distributed throughout the cell, indicating that specific localization of CSPB depends on cell development stage (Weber et al., 2001). Accumulation of the CSPB protein in *B. subtilis* cells occurs after transition from exponential growth to stationary phase (Graumann et al., 1997; Graumann and Marahiel, 1999), indicating that CSPB accumulation in cells can be triggered under several stress conditions that share a common signal such as inactivation of ribosomes (Schindler et al., 1999; Graumann et al., 1997). Stability of the protein both *in vivo* and *in vitro* depends on the protein's ability to form a complex with nucleic acids, most likely mRNAs (Schindler et al., 1999). In the absence of polynucleic acids, the CSPB protein has a very low thermodynamic stability and is susceptible to rapid proteolytic degradation (Schindler et al., 1999).

The amino acid sequence of the CSPB protein produced in MON 87460 is identical to that of the native CSPB protein produced in *B. subtilis* with the exception of one amino acid change (in the second position from leucine to valine L2V) that was necessary for cloning purposes. Bacterial CSPs are composed of approximately 67-73 amino acid residues (Graumann et al., 1997) and contain several positively charged amino acid residues that may facilitate binding to the negatively charged backbones of polynucleotides.

The structure of CSPB from *B. subtilis* has been previously described (PDB accession number 1NMF) (Schindelin et al., 1993; Schindelin et al., 1994). The CSPB protein in MON 87460 consists of 66 amino acids and has an isoelectric point of 4.31. The protein is composed of five antiparallel β-strands forming a five-strand β-barrel similar to the structure of CSPA protein from *E. coli* (PDB accession number 1MJC) (Schindelin at al, 1993; Newkirk at al, 1994). Experimental evidence indicates that CSPs bind at the single-stranded portions of RNA loops and then progressively cover this region forcing the double-stranded portion to open (Phadtare et al., 2002). It has been suggested that CSPs bind to single stranded nucleic acids, RNA and ssDNA, but do not appear to bind to dsDNA (Max et al, 2006). The stable association of CSPs with nucleic acids has been confirmed by co-crystallization of the *B. subtilis* CSPB protein in a complex with single stranded polynucleotides (Bienert et al., 2004; Max et al., 2006). The crystal structure data revealed the stoichiometry and sequence determinants of the binding of single-stranded nucleic acids to a preformed site on CSPB. All CSPs possess binding sites for single stranded nucleic acids called ribonucleoprotein (RNP) binding motifs (Newkirk et al, 1994; Schröder et al., 1995). CSPB protein, like other CSPs, contains two conserved RNP motifs: RNP1 and RNP2. Within the CSPB RNP domains four aromatic amino acids, phenylalanines 15, 27, and 30 (F15, F27, and F30) and histidine 29 (H29) are required for the double-stranded polynucleotide "melting" capability (Figure VI-1).

These amino acids are conserved in CSPs and are thought to be essential for their function in bacteria (Phadtare et al., 2002). *In vitro* studies suggest that by binding to RNA secondary structures, CSPs reduce the free energy required for misfolded RNA to unfold and adopt the correct configuration (Herschlag, 1995). These findings together with the described mechanism of RNA unfolding led to the classification of CSPs as RNA chaperones. RNA chaperones are a class of nucleic acid binding proteins responsible for maintaining RNA function and processing (Cristofari and Darlix, 2002).

Figure VI-1. Protein Sequence of the *Bacillus subtilis* CSPB Variant

The figure shows the relative position of the β−sheets and the four aromatic amino acids (in bold, red) required for double-stranded polynucleotide "melting" capability.

CSD-containing proteins also confer environmental stress tolerance in plants

Similar to bacteria, CSD-containing proteins in plants also bind RNA, unfold RNA secondary structures caused by environmental stress, and help maintain cellular functions under stress. These plant CSD-containing proteins share a high level of similarity to the bacterial CSPs and have been shown to share *in vitro* and *in vivo* functions with bacterial CSPs (Karlson and Imai, 2003; Kim et al, 2007; Nakaminami et al., 2005 and 2006; Chaikam and Karlson, 2008; Fusaro et al., 2007). Plant CSD-containing proteins have been reported to respond to abiotic stresses in *Arabidopsis* (Fusaro et al., 2007), wheat (Karlson et al., 2002), and rice (Chaikam and Karlson, 2008), and to play an important role in various aspects of plant development (Fusaro et al. 2007; Chaikam and Karlson, 2008). Direct relationships between the ability of CSD-containing proteins to bind RNA and/or ssDNA and stress tolerance have been established (Nakaminami et al., 2006; Castiglioni et al., 2008) and results of *in vitro* experiments show that plant CSD-containing proteins can bind RNA, synthetic mRNA, and ssDNA (Sasaki et al., 2007). The apparent absence of binding sequence specificity indicates that plant CSD-containing proteins could be involved in a more general response to stress by binding RNAs and, therefore, helping cells to maintain cellular functions following the stress. CSD-containing proteins from rice and *Arabidopsis* have been shown to be highly expressed in apical meristems, ovules, embryos, and seeds (Fusaro et al. 2007; Chaikam and Karlson, 2008) and, therefore, could potentially affect growth rate, flowering time, and seed development. The CSD-containing proteins have been localized both in the cytoplasm and the nuclei (Sasaki et al., 2007; Fusaro et al. 2007) indicating that these proteins can potentially be involved in multiple aspects of RNA function including localization, translation and stability.

Appendix B provides supplemental information on the function of CSPB in MON 87460. The data confirm that CSPB in MON 87460, like CSD-containing proteins found in plants, interacts with RNA to unfold secondary structures, localizes to the cytoplasm and

nucleus and accumulates in rapidly growing tissues. The data also demonstrate that MON 87460 exhibits key physiological advantages under water-limited conditions such as a more normal level of photosynthesis than the control. Finally, the data demonstrate that the yield advantage exhibited by MON 87460 under water limitation results primarily from increased numbers of kernels per plant, consistent with the current understanding of the effect of drought stress on corn yield potential.

1.2. Characterization of the CSPB protein

Low CPSB levels in MON 87460 necessitated production of CSPB in *E. coli* for further safety studies. A number of analyses were performed to characterize the purified CSPB protein produced in MON 87460 and to demonstrate its equivalence to the *E. coli*-produced CSPB protein. Appendix C provides materials and methods for these analyses. The analyses employed for the characterization of MON 87460-produced CSPB protein included:

1. N-terminal sequence analysis;
2. matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry (MS) to generate a tryptic peptide map;
3. immunoblot analysis to establish protein identity through immunoreactivity with CSPB-specific antibody;
4. sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to assess the apparent molecular weight of the protein;
5. functional polynucleotide-unfolding ("melting") assay to demonstrate biological activity of the CSPB protein.

The equivalence of the MON 87460-produced and *E. coli*-produced CSPB proteins was established by demonstrating identity in the following characteristics:

1. immunoreactivtiy with CSPB-specific antibody;
2. protein molecular weights;
3. glycosylation status;
4. functional activity.

1.2.1. CSPB protein N-terminal sequence analysis

Sequencing of the first 15 amino acids comprising the N-terminal of MON 87460-produced CSPB protein produced the expected result (Table VI-1). The N-terminal methionine was not detected. This result is expected as removal of the N-terminal methionine, catalyzed by methionine aminopeptidase, is a common modification that occurs co-translationally before completion of the nascent protein chain and has no effect on protein structure or activity (Arfin and Bradshaw, 1988; Polevoda and Sherman, 2000). The N-terminal sequence information, therefore, confirms the identity of the CSPB protein isolated from MON 87460 and the intactness of its N-terminus.

1.2.2. CSPB protein MALDI-TOF mass spectrometry analysis

The identity of the MON 87460-produced CSPB protein was further confirmed by tryptic peptide mass mapping analysis using MALDI-TOF MS. Protein identification made by peptide mapping is reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al., 1997). Observed tryptic

peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one Dalton (Da) were found between the observed and predicted fragment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation. The protein sample was heat-denatured, chemically reduced, alkylated, digested with trypsin, guanidinated, and the masses of the tryptic peptides were measured.

CSPB is a small protein with a limited number of the trypsin-digested peptides that are amenable to identification by MALDI-TOF. There were four unique peptide fragments identified that matched expected masses of the CSPB trypsin-digested peptides. The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CSPB protein (Figure VI-2), resulting in an 88% (58 out of 66 amino acids) coverage of the total protein. This analysis confirmed the identity of the MON 87460-produced CSPB protein. Appendix D presents the tryptic masses of CSPB.

1.2.3. CSPB protein immunoreactivity

A western blot analysis using goat anti-CSPB serum was conducted to determine the relative immunoreactivity of the MON 87460-produced CSP protein and the *E. coli*-produced CSPB reference standard. The results demonstrated that the anti-CSPB antibody recognized the MON 87460-produced CSPB that migrated identically to the *E. coli*-produced reference standard protein (Figure VI-3). Furthermore, the immunoreactive signal increased with increasing levels of CSPB loading. Immunoreactivities between the MON 87460- and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot. Based on the analysis, the MON 87460- and *E. coli*-produced CSPB proteins demonstrated equivalent immunoreactive properties, which confirmed the identity and equivalence of the two proteins.

Table VI-1. N-terminal Amino Acid Sequence Analysis of the CSPB Protein Purified from Grain Tissue of MON 87460

Amino acid[1] residue # from the N-terminus →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Predicted CSPB Sequence[2] →	M	V	E	G	K	V	K	W	F	N	S	E	K	G	F	G
	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Observed Sequence→	-	V	E	G	K	V	K	W	F	N	S	E	K	G	F	G

1 The single letter amino acid code is: E, Glutamic acid; F, Phenylalanine; G, glycine; K, Lysine; M, methionine; N, Asparagine, S, serine; V valine and W, tryptophan.

2 The predicted amino acid sequence of the CSPB protein was deduced from the coding region of the full length *cspB* gene present in MON 87460.

0001 VEGKVKWFNS EKGFGFIEVE GQDDVFVHFS AIQGEGFKTL EEGQAVSFEI

0051 VEGNRGPQAA NVTKEA

Figure VI-2. MALDI-TOF MS Coverage Map of the CSPB Protein Isolated From MON 87460

The amino acid sequence of the plant-produced CSPB protein was deduced from the coding region of the full-length *cspB* gene present in MON 87460 (see Part IV, Section 2). Boxed regions correspond to tryptic peptide masses that were identified from the protein sample using MALDI-TOF MS. In total, 88% (58 of 66 total amino acids) of the expected protein sequence were identified.

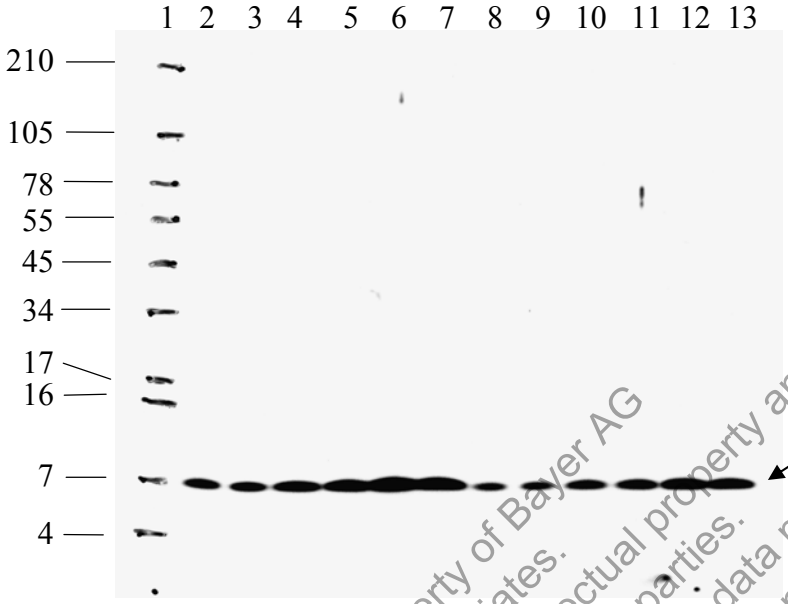

Figure VI-3. Western Blot Analysis of MON 87460- and *E. coli*-Produced CSPB Proteins

Aliquots of the purified, MON 87460- and *E. coli*-produced CSPB proteins were separated by SDS-PAGE, and electrotransferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was probed with goat anti-CSPB serum and developed using an enhanced chemiluminescense (ECL) system (GE Healthcare). Approximate molecular weights (kDa) of markers loaded in Lane 1 are shown on the left side of the blot.

Lane	Sample	Amount Loaded (ng)
1	See Blue® Plus2 Pre-Stained molecular weight markers	—
2	*E. coli*-produced CSPB reference standard	3
3	*E. coli*-produced CSPB reference standard	3
4	*E. coli*-produced CSPB reference standard	6
5	*E. coli*-produced CSPB reference standard	6
6	*E. coli*-produced CSPB reference standard	9
7	*E. coli*-produced CSPB reference standard	9
8	MON 87460-produced CSPB protein	3
9	MON 87460-produced CSPB protein	3
10	MON 87460-produced CSPB protein	6
11	MON 87460-produced CSPB protein	6
12	MON 87460-produced CSPB protein	9
13	MON 87460-produced CSPB protein	9

1.2.4. CSPB protein molecular weight equivalence

The equivalence in apparent molecular weight of the purified MON 87460- and the *E. coli*-produced CSPB proteins was demonstrated using SDS-PAGE (Figure VI-4). The MON 87460-produced CSPB protein migrated with a molecular weight indistinguishable to that of the *E. coli*-produced protein standard analyzed concurrently (Figure VI-4). Based on comparable electrophoretic mobilities, the MON 87460- and *E. coli*-produced CSPB proteins were determined to have equivalent apparent molecular weights.

The predicted mass of the MON 87460-produced CSPB protein was also confirmed by MALDI-TOF MS. The average mass obtained for CSPB was 7220 Da. This experimentally obtained mass differs from the theoretical mass calculated for the CSPB reference standard protein by 131 Da. The difference between the expected and the observed mass for MON 87460-produced CSPB corresponds to the mass of methionine (131 Da). The absence of the N-terminal methionine was confirmed by N-terminal sequencing (Section 1.2.1).

1.2.5. CSPB protein glycosylation equivalence

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher *et al.*, 1988). These carbohydrate moieties may be complex, branched polysaccharide structures, simple oligosaccharides or monosaccharides. In contrast, prokaryotic organisms such as non-virulent *E. coli* strains used for cloning and expression purposes, lack the necessary biochemical synthetic capacity required for protein glycosylation. An investigation of glycosylation status therefore is necessary to confirm that the MON 87460-produced CSPB protein is equivalent to the *E. coli*-produced CSPB protein. Results of this analysis confirm that the proteins are equivalent in this respect.

To assess whether potential post-translational glycosylation of the MON 87460-produced CSPB protein occurred, the purified protein sample was subjected to glycosylation analysis. The *E. coli*-produced CSPB reference standard represented a negative control. The positive controls were the transferrin and horseradish peroxidase (HRP) proteins which are known to have multiple covalently-linked carbohydrate modifications. The transferrin protein and HRP, as well as the purified CSPB protein isolated from MON 87460 and *E. coli* were separated on SDS-PAGE, transferred to a PVDF membrane, and glycosylation analysis was performed to detect carbohydrate moieties on the proteins. The results of this analysis are shown in Figure VI-5. The positive controls, transferrin and HRP, were detected at the expected molecular weights of ~75 and ~50 kDa, respectively, in a concentration-dependent manner (Figure VI-5, Panel A, Lanes 4-5 and 2-3). No detectable signal was observed for the MON 87460- and *E. coli*-produced CSPB proteins (Figure VI-5, Panel A, Lanes 6-7 and 8-9). To confirm that sufficient MON 87460- and *E. coli*-produced CSPB proteins were present for carbohydrate detection and glycosylation analysis, the membrane was stained with SYPRO® Ruby stain to detect proteins (Figure VI-5, Panel B). Both MON 87460- and *E. coli*-produced CSPB were clearly detected on the membrane (Figure VI-5, Panel B, Lanes 6-9).These results demonstrate that the MON 87460-produced CSPB protein is not glycosylated and, thus is equivalent to the *E. coli*-produced CSPB reference standard.

1.2.6. CSPB protein functional activity equivalence

The functional activities of the *E. coli*- and MON 87460-produced CSPB proteins were measured using an assay where protein unfolds or "melts" a DNA-hairpin structure. Results confirm the two proteins exhibit similar functional activity. The DNA-hairpin structure is labeled with a fluorophore at the 5'- and quencher at the 3'-terminus. Due to the close proximity of the fluorescent tag and quencher in the hairpin conformation, the fluorescence is efficiently quenched. When a CSPB protein "melts" the hairpin conformation, the fluorescent tag and quencher are spatially separated which permits fluorescence. This assay has been broadly utilized to characterize the specificity of a variety of CSPs including CSD-containing proteins identified in bacteria and plants (Karlson et al, 2002; Kim et al, 2007; Phadtare et al, 2002).

In this assay protein specific activity is expressed as the amount (pmol) of open Dual Labeled Probe (DLP) that is induced by a microgram (µg) of CSPB. The *E. coli*- and MON 87460-produced CSPB proteins were considered functionally equivalent if the specific activity of one protein was within 25% of the other.

The DLP consists of a custom synthesized 35-base oligonucleotide DNA fragment with a fluorescein amidite derived from 6-carboxyfluorescein (6-FAM) label at the 5' end and a black hole quencher at the 3' end. The oligonucleotide probe forms a double strand stem of six base pairs due to the complementary bases located at the 5' and 3' ends. The 23 nucleotides (dT) in the middle form a loop. CSPB has been shown to have a high affinity for poly dT sequences and its binding to the loop will separate the double strands of the probe, which separates the fluorophore from the quencher, allowing fluorescence to be emitted and measured.

MON 87460-produced CSPB had a specific activity of 0.660 pmole open DLP/µg protein and the *E. coli*-produced reference standard had a specific activity of 0.757 pmole open DLP/µg protein. The difference in specific activities was 12.8% (Table VI-2). These results clearly demonstrate that the CSPB proteins derived from MON 87460 and *E. coli* have equivalent functional activities.

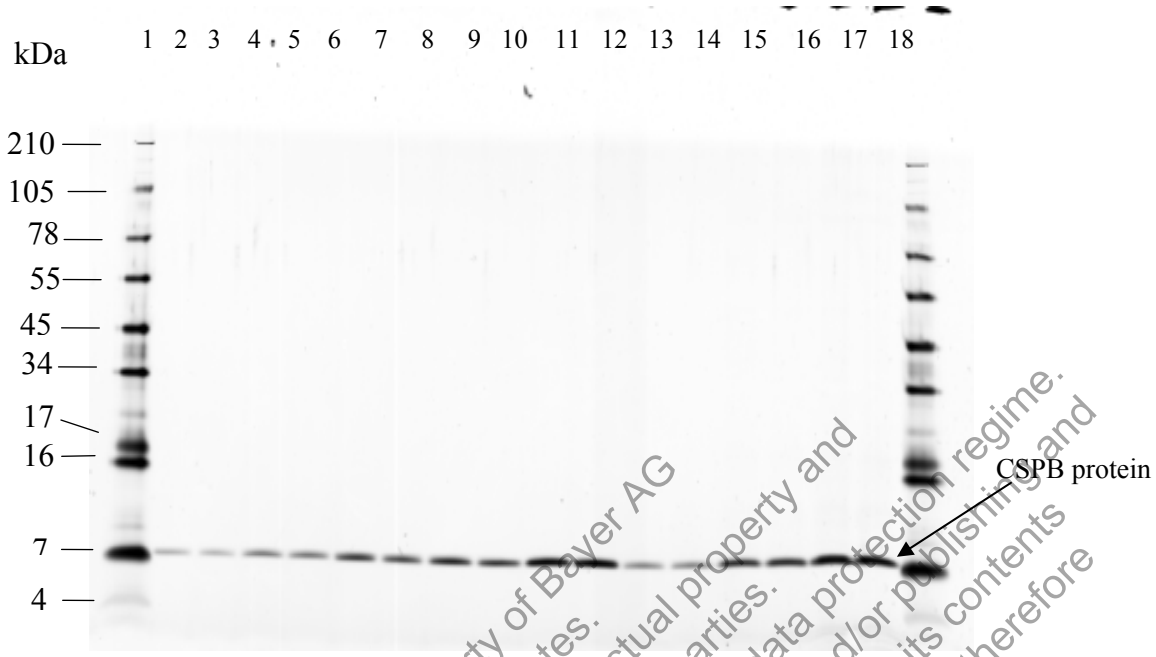

Figure VI-4. SDS-PAGE of *E. coli*- and MON 87460-Produced CSPB Proteins

Aliquots of the MON 87460-produced CSPB and the *E. coli*-produced CSPB reference standard were separated by a tricine 10-20% polyacrylamide gradient gel and stained with an Owl Silver Staining kit. Approximate molecular weights (kDa) of markers loaded in Lanes 1 and 18 are shown on the left side of the gel.

Lane	Sample	Amount Loaded (ng)	Amount Loaded (µl)
1	See Blue® Plus2 Pre-Stained molecular weight markers	—	15
2	*E. coli*-produced CSPB standard	10	—
3	*E. coli*-produced CSPB standard	10	—
4	*E. coli*-produced CSPB standard	20	—
5	*E. coli*-produced CSPB standard	20	—
6	*E. coli*-produced CSPB standard	30	—
7	*E. coli*-produced CSPB standard	30	—
8	*E. coli*-produced CSPB standard	40	—
9	*E. coli*-produced CSPB standard	40	—
10	*E. coli*-produced CSPB standard	60	—
11	*E. coli*-produced CSPB standard	60	—
12	MON 87460-produced CSPB protein	—	10
13	MON 87460-produced CSPB protein	—	10
14	MON 87460-produced CSPB protein	—	20
15	MON 87460-produced CSPB protein	—	20
16	MON 87460-produced CSPB protein	—	30
17	MON 87460-produced CSPB protein	—	30
18	See Blue® Plus2 Pre-Stained molecular weight markers	—	15

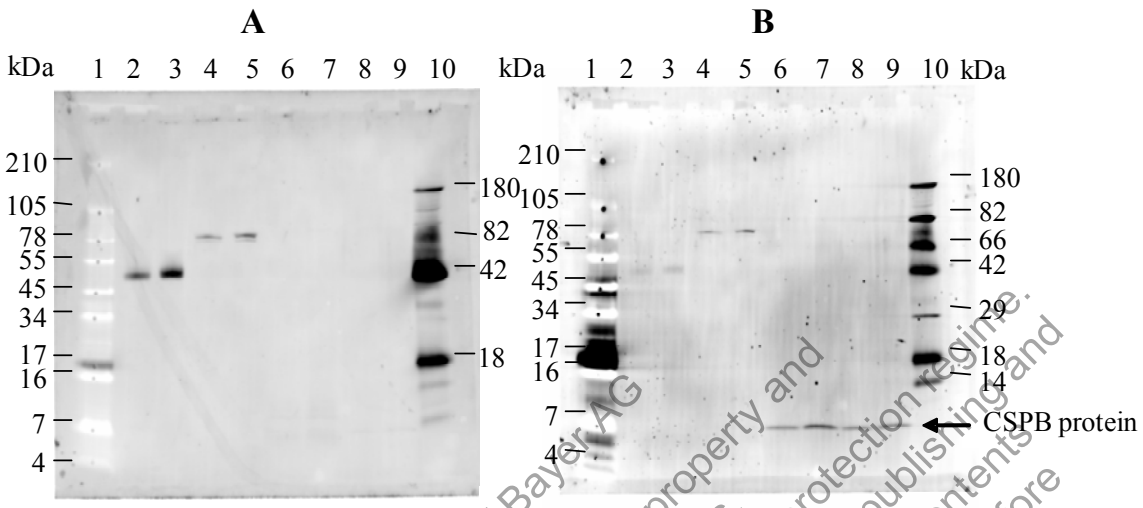

Figure VI-5. Glycosylation Analysis of the MON 87460-Produced CSPB Protein

Aliquots of the MON 87460-produced CSPB protein, *E. coli*-produced CSPB reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (10-20% gradient) and electrotransferred to a PVDF membrane. (**A**) Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm (Lanes 1-5). (**B**) The same blot was stained with SYPRO Ruby. The signal was captured using a Bio-Rad Molecular Imager FX. Approximate molecular weights (kDa) correspond to the See Blue® Plus2 pre-stained dual color molecular weight marker loaded in Lane 1 and CandyCane glycosylated markers loaded in Lane 10.

Lane	Sample	Amount (ng)
1	See Blue® Plus2 Pre-Stained molecular weight markers	—
2	Horseradish Peroxidase (positive control)	25
3	Horseradish Peroxidase (positive control)	50
4	Transferrin (positive control)	25
5	Transferrin (positive control)	50
6	MON 87460-produced CSPB	25
7	MON 87460-produced CSPB	50
8	*E. coli*-produced CSPB (negative control)	25
9	*E. coli*-produced CSPB (negative control)	50
10	CandyCane Glycoprotein molecular weight standards	—

Table VI-2. CSPB Functional Assay Results

Assay activity is expressed as the amount (pmol) of open Dual Labeled Probe (DLP) that is induced by a microgram (µg) of CSPB. The probe consists of a custom synthesized 35-base oligonucleotide DNA fragment with a 6-FAM fluorescent label at the 5' end and a black hole quencher at the 3' end. Fluorescence was determined with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The amount of open probe was determined relative to the standard curve prepared from serial dilutions of 6-FAM. The opening of the DLP was measured for both proteins, using 3 µg of CSPB for each replicate. The activity represents the average of three replicates.

Specific Activity (pmoles opened DLP/µg CSPB)		**% Difference[1] (MON 87460 vs. *E. coli*) CSPB**
MON 87460 – CSPB	***E. coli* -CSPB**	
0.660	0.757	12.8

[1] Percent difference was calculated as follows:

$$\frac{\left|ActivityEcoli - ActivityPlant\right|}{ActivityEcoli} \times 100 = \%Difference$$

1.2.7. Conclusions of the CSPB protein characterization

A comparison of the MON 87460-produced CSPB to the *E. coli*-produced CSPB reference protein standard confirmed the identity of the MON 87460-produced CSPB protein and established the equivalence of the plant produced protein to the *E. coli*-produced CSPB reference protein standard. The molecular weight of the MON 87460- and *E. coli*-produced CSPB proteins was estimated by SDS-PAGE. SDS-PAGE demonstrated that the proteins migrated at the same molecular weight indicating that the CSPB proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87460-produced CSPB protein were equivalent to those of the *E. coli*-produced CSPB reference standard. The N-terminus of the MON 87460-produced CSPB was consistent with the predicted amino acid sequence translated from the *cspB* coding sequence, and the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated *cspB* coding sequence. The MON 87460- and the *E. coli*-produced CSPB reference standard were also equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CSPB protein isolated from MON 87460 and establish its equivalence to the *E. coli*-produced CSPB reference protein standard.

1.3. Identity and function of the NPTII protein

The NPTII protein functions as a selectable marker in the initial laboratory stages of plant cell selection following transformation (Horsch at al., 1984; DeBlock et al., 1984). NPTII uses adenosine triphosphate (ATP) to phosphorylate neomycin and related aminoglycoside antibiotics, thereby inactivating them. Cells that produce the NPTII enzyme selectively survive exposure to these aminoglycosides. The *nptII* coding sequence is derived from the prokaryotic *E. coli* transposon *Tn5* (Beck et al., 1982). The purpose of inserting the gene encoding the NPTII protein into corn cells along with CSPB was to have an effective method for selecting cells after transformation. In general, the frequency of plant cells that are transformed is often low, ranging from $1x10^{-5}$ to $lx10^{-4}$ of cells treated (Fraley et al., 1983). Therefore, the selectable marker, NPTII, facilitates the screening process.

1.4. Characterization of the NPTII protein

The NPTII protein produced in MON 87460 was characterized and its equivalence to a previously characterized *E. coli*-produced NPTII reference substance was demonstrated. Demonstration of the equivalence between *E. coli*- and MON 87460-produced NPTII proteins allows utilization of previous safety assessment data to confirm the safety of the NPTII protein in MON 87460. The analyses employed for the characterization of MON 87460-produced NPTII protein and establishment of the equivalence between MON 87460- and *E. coli*-produced proteins included:

1. immunoblot analysis to establish protein identity through immunoreactivity with NPTII–specific antibody and demonstrate immuno-equivalence between MON 87460 and *E. coli*-produced NPTII proteins,
2. SDS-PAGE to assess the apparent molecular weight of the protein and establish equivalence of the apparent molecular weight between MON 87460- and *E. coli*-produced proteins.

1.4.1. NPTII protein immunoreactivity

Immunoblot analysis established that MON 87460-produced NPTII and *E. coli*-produced NPTII have equivalent immunoreactive properties. The expression levels of NPTII protein in MON 87460 leaf tissue allowed detection of the protein with an NPTII-specific antibody directly in leaf extracts without additional enrichment. An extract was also prepared from a leaf sample of conventional corn with a similar genetic background as MON 87460 to serve as a negative control for the presence of the NPTII protein. To ensure that the electromobility of the NPTII protein had not been altered as a result of matrix effects, the reference substance was spiked into the leaf extract from conventional corn and analyzed alongside the leaf extract from MON 87460. The leaf extract from MON 87460, *E. coli*-produced NPTII protein, and NPTII-spiked conventional corn leaf extract were subjected to a reducing and denaturing SDS-PAGE and then transferred to a nitrocellulose membrane for detection using an anti-NPTII antibody. A co-migrating immunoreactive band was observed in the leaf extract from MON 87460 (Figure VI-6, Lanes 4-6), leaf NPTII-spiked conventional corn leaf extract (Figure VI-6, Lanes 7-9), and pure *E. coli*-produced NPTII protein (Figure VI-6, Lane10). As expected, the immunoreactive signal increased with increased loading levels of the leaf extract from MON 87460 and increased amount of the leaf extract from conventional corn spiked with the *E. coli*-produced NPTII protein. No immunoreactive bands were observed in the leaf extract from conventional corn (Figure VI-6, Lane 3). Based on this analysis, the MON 87460- and *E. coli*-produced NPTII proteins demonstrated equivalent immunoreactive properties, which confirmed both the identity and equivalence of the two proteins.

1.4.2. NPTII protein molecular weight equivalence

The molecular weight of the MON 87460-produced NPTII protein and its equivalence to the molecular weight of the *E. coli*-produced NPTII reference standard was confirmed using densitometric analysis of the western blot (Figure VI-6). The electromobility of the MON 87460-produced protein was indistinguishable from the electromobility of the *E. coli*-produced NPTII protein. The estimated molecular weight of the MON 87460-produced NPTII protein was 27.4 kDa, which was similar to the previously determined molecular weight of the *E. coli*-produced NPTII reference standard (27.1 kDa). Based on the identical electrophoretic mobility and apparent molecular masses, the MON 87460- and *E. coli*-produced NPTII proteins have equivalent molecular weights.

1.4.3. Conclusions of the NPTII protein characterization

MON 87460-produced and *E. coli*-produced NPTII proteins have equivalent immunoreactivities and apparent molecular weights. The results of this analysis confirmed the identity of the MON 87460-produced NPTII protein and established the equivalence of the plant produced protein to the *E. coli*-produced NPTII reference protein standard. A western blot analysis was utilized to compare the immunoreactivity and apparent molecular weight of the MON 87460-produced NPTII protein to that of the previously characterized *E. coli*-produced NPTII reference protein standard. The MON 87460- and *E. coli*-produced NPTII proteins displayed similar immunoreactivity with NPTII-specific antibody and had identical electromobility on SDS-PAGE. Taken together, these data establish equivalence between the MON 87460-produced and *E. coli*-produced NPTII reference protein standard.

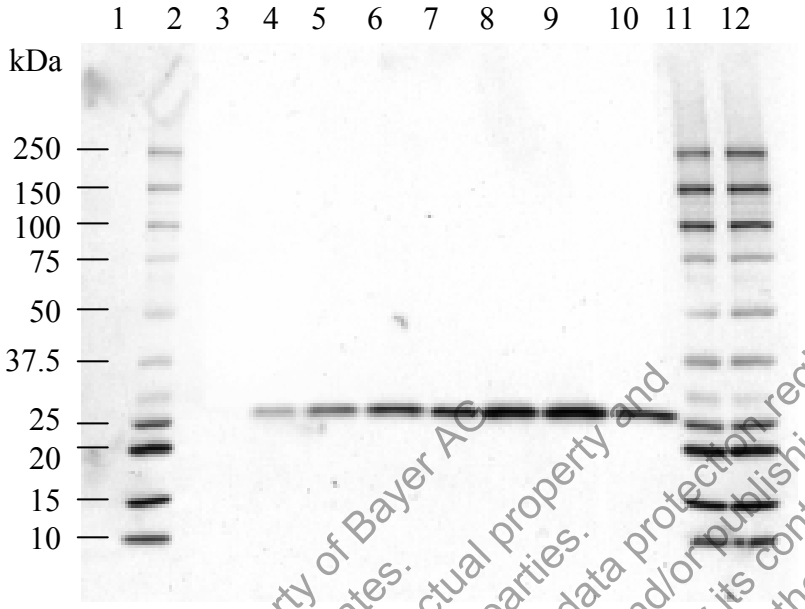

Figure VI-6. Western Blot Analysis of the MON 87460- and *E. coli*-Produced NPTII Protein

Corn leaf extracts from MON 87460 and conventional corn. *E. coli*-produced NPTII and *E. coli*-produced NPTII spiked into leaf extract of conventional corn were separated by SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membrane was probed with rabbit anti-NPTII antibody and an HPR-conjugated secondary antibody and visualized using an ECL system. Approximate molecular weights (kDa) are shown on the left and correspond to the protein marker loaded in Lanes 2, 11 and 12. The 10 s exposure is shown and is representative of the bands observed in the other exposures.

Lane	Sample	Amount Loaded (ng)	Amount Loaded (µl)
1	Empty	—	—
2	Precision Plus Protein WesternC markers	—	5
3	Leaf extract from conventional corn	—	10
4	Leaf extract from corn MON 87460	—	5
5	Leaf extract from corn MON 87460	—	10
6	Leaf Extract from corn MON 87460	—	15
7	*E. coli*-produced NPTII spiked*	0.25	5
8	*E. coli*-produced NPTII spiked*	0.5	10
9	*E. coli*-produced NPTII spiked*	0.75	15
10	*E. coli*-produced NPTII	0.5	10
11	Precision Plus Protein WesternC markers	—	5
12	Precision Plus Protein WesternC markers	—	5

* *E. coli*-produced NPTII spiked in leaf extract from conventional corn

SECTION 2. Levels of CSPB and NPTII Proteins in MON 87460

2.1. Results from U.S. 2006 field production

Levels of CSPB and NPTII proteins in MON 87460 tissues that are relevant to the risk assessment were quantified with a validated Enzyme-Linked Immunosorbent Assay (ELISA). Tissue samples were collected from six field trials conducted in the U.S. during 2006. The trials were located in Iowa, Illinois, Indiana, Kansas, and Nebraska which represent the major corn-growing regions of the U.S. and provide a range of environmental conditions that would be encountered in the commercial production of corn. At each site, three replicated plots of MON 87460 and a conventional control hybrid were planted using a randomized complete block field design. Over-season leaf (OSL), over-season whole plant (OSWP), over-season root (OSR), pollen, silk, forage, forage root, grain, stover, and senescent root tissues were collected from each replicated plot at all field sites.

Leaves were randomly collected from plants in each plot at each site. Twenty leaves were combined to form the leaf sample for each plot. There were 18 leaf samples across all sites for OSL-1, OSL-3, and OSL-4 and 17 leaf samples for OSL-2. OSL samples were collected as follows:

Over-season leaf (OSL)	*Corn development stage*	*Days after planting (DAP)*
OSL-1	V2-V4	15-22
OSL-2	V6-V8	27-38
OSL-3	V10-V12	41-56
OSL-4	pre-VT (pre-tasseling)	49-63

The aerial portion of the plant was collected from four plants in each plot at each site at the V2-V4 stage and combined to form the whole plant sample. Two plants were collected and combined to form the whole plant samples for the later growth stages. OSWP samples were collected as follows:

Overseason whole plant (OSWP)	*Corn development stage*	*DAP*
OSWP-1	V2-V4	15-22
OSWP-2	V6-V8	27-38
OSWP-3	V10-V12	41-56
OSWP-4	pre-VT (pre-tasseling)	49-63

Roots remaining after collection of whole plants from each plot were combined to form the root sample. Therefore, roots from four plants at the V2-V4 stage and roots from two plants at later stages were combined. OSR samples were collected as follows.

Overseason root (OSR)	*Corn development stage*	*DAP*
OSR-1	V2-V4	15-22
OSR-2	V6-V8	27-38
OSR-3	V10-V12	41-56
OSR-4	pre-VT (pre-tasseling)	49-63
Forage root	early dent stage (R4-R6)	90-103
Senescent root	after harvest	135-151

Approximately 10 ml of pollen was collected from multiple tassels in each plot at each site at pollination, approximately 59-68 days after planting. Silks were collected approximately 58-74 days after planting from five plants, except for the Indiana and Iowa

sites, where silks were collected from ten plants. Silks were only collected from ears of plants that were covered with shoot bags to preserve their genetic identity.

Two whole plants in each plot at each site were cut above the soil surface at an early dent stage, at approximately 96-109 days after planting, and then combined to form the forage sample. The roots of these plants were combined to form the forage root sample.

Grain was harvested at maturity from all plants in each plot at each site and dried to a moisture content of 11-17%. Following harvest, approximately 136-158 days after planting, two whole plants in each plot at each site were cut above the soil surface and combined to form the stover sample. The roots of these plants were also removed, washed and combined to form a senescent root sample.

All tissue samples, except grain, were stored and shipped on dry ice for processing and analysis. Grain was stored and shipped at room temperature. All tissue samples were stored in a –80ºC freezer upon receipt. Tissue samples were extracted and analyzed by a validated ELISA (Appendix E).

CSPB expression levels were determined in all 19 tissue types described above. Because of the extensive historical safety data for NPTII, the number of tissues evaluated for NPTII expression was fewer than those evaluated for CSPB protein. The NPTII levels were evaluated in four of the 19 tissue types including OSL-1 (V2-V4), OSR-1 (V2-V4), forage and grain. These four tissues were selected to span the life cycle of corn.

Tables VI-3 and Table VI-4 summarize results obtained from ELISA analysis of the various tissue types. The levels of CSPB protein were determined in over season leaf (OSL-1through OSL-4), over season root (OSR-1 through OSR-4), over season whole plant (OSWP-1 through OSWP-4), forage, forage root, senescent root, stover, silk, pollen, and grain (Table VI-3). The levels of NPTII protein were determined in OSL-1, OSR-1, forage and grain tissues (Table VI-4). Moisture content was measured for all tissue types. Protein levels for all tissue types are provided in µg/g fresh weight tissue (fwt) and µg/g dry weight tissue (dwt) basis.

The mean CSPB protein levels across the six sites were highest in pollen (13 µg/g dwt), followed by silk (1.2 µg/g dwt), young leaf (OSL1, 3.1 µg/g dwt), forage (0.10 µg/g dwt), grain (0.072 µg/g dwt), stover (0.042 µg/g dwt), senescent root (0.041 µg/g dwt), and forage root (0.029µg/g dwt). In tissues harvested throughout the growing season, mean CSPB protein levels in MON 87460 across all sites ranged from 0.47 – 3.1 µg/g dwt in leaf, 0.24 – 1.4 µg/g dwt in root, and 0.67 – 2.8 µg/g dwt in whole plant.

The mean NPTII protein levels across the six sites were highest in young leaf (OSL1, 2.6 µg/g dwt), followed by roots (OSR1, 0.47 µg/g dwt), and forage (0.13 µg/g dwt). The levels of NPTII protein in grain were below the NPTII assay Limit of Quantitation (LOQ), which was 0.0047 µg/g fwt for grain. NPTII protein levels in MON 87460 were lower than NPTII levels determined for the equivalent tissue types in MON 863, a previously-approved corn product that also relied on NPTII as a selectable marker. The range of NPTII protein levels in MON 863 leaf, forage, and grain were 0.74 – 1.4, 0.17 – 0.23 and <LOQ µg/g fwt, respectively. The range in NPTII protein levels for MON 87460 in leaf, forage, and grain were 0.21 – 0.63, 0.017 – 0.053, and <LOQ µg/g fwt, respectively (Table VI-4).

Table VI-3. Summary of CSPB Protein Levels in Tissue Collected from MON 87460 Produced in the 2006 U.S. Growing Season

Tissue Type	Mean (SD) (µg/g fwt)[1]	Range[2] (µg/g fwt)[3]	Mean (SD) (µg/g dwt)[4]	Range (µg/g dwt)	LOQ / LOD (µg/g fwt)
OSL-1	0.45 (0.14)	0.24 – 0.77	3.1 (0.93)	2.0 – 5.1	0.0150 / 0.0069
OSL-2	0.40 (0.21)	0.18 – 0.80	2.2 (1.2)	1.0 – 4.4	0.0150 / 0.0069
OSL-3	0.21 (0.067)	0.10 – 0.29	1.0 (0.30)	0.54 – 1.5	0.0150 / 0.0069
OSL-4	0.10 (0.047)	0.034 – 0.19	0.47 (0.25)	0.16 – 0.96	0.0150 / 0.0069
OSR-1	0.13 (0.052)	0.060 – 0.24	1.4 (0.55)	0.55 – 2.4	0.0020 / 0.0018
OSR-2	0.11 (0.052)	0.030 – 0.21	1.0 (0.43)	0.25 – 1.6	0.0020 / 0.0018
OSR-3	0.059 (0.026)	0.015 – 0.11	0.43 (0.20)	0.077 – 0.84	0.0020 / 0.0018
OSR-4	0.035 (0.015)	0.012 – 0.063	0.24 (0.10)	0.098 – 0.42	0.0020 / 0.0018
OSWP-1	0.30 (0.13)	0.11 – 0.46	2.8 (1.4)	1.1 – 5.1	0.0045 / 0.0043
OSWP-2	0.18 (0.075)	0.096 – 0.31	1.9 (0.87)	0.86 – 3.4	0.0045 / 0.0043
OSWP-3	0.11 (0.041)	0.067 – 0.22	0.88 (0.29)	0.45 – 1.4	0.0045 / 0.0043
OSWP-4	0.091 (0.049)	0.015 – 0.18	0.67 (0.36)	0.11 – 1.3	0.0045 / 0.0043
Forage Root	0.0055 (0.0034)	0.0020 – 0.015	0.029 (0.013)	0.014 – 0.055	0.0020 / 0.0018
Senescent Root	0.0040 (0.0013)	0.0022 – 0.0068	0.041 (0.084)	0.014 – 0.36	0.0020 / 0.0018
Forage	0.027 (0.0095)	0.011 – 0.046	0.10 (0.032)	0.041 – 0.17	0.0045 / 0.0043
Stover	0.018 (0.013)	0.0056 – 0.047	0.042 (0.024)	0.013 – 0.090	0.0045 / 0.0043
Silk	0.099 (0.024)	0.031 – 0.13	1.2 (0.32)	0.31 – 1.8	0.0075 / 0.0047
Pollen	7.3 (1.6)	5.3 – 10	13 (2.3)	10 – 17	0.0500 / 0.0450
Grain	0.063 (0.014)	0.040 – 0.089	0.072 (0.015)	0.045 – 0.10	0.0038 / 0.0017

[1]The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=18 for all tissues except OSL 2, OSWP 2 and forage where n=17, and senescent root where n=16).
[2]Minimum and maximum values were determined for each tissue type across all sites.
[3]Protein quantities are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
[4]Protein quantities are expressed as "µg/g" of tissue on a dry weight (dwt) basis.

Table VI-4. Summary of NPTII Protein Levels in Tissue Collected from MON 87460 Produced in the 2006 U.S. Growing Season

Tissue Type	Mean (SD) (μg/g fwt)[1]	Range[2] (μg/g fwt)[3]	Mean (SD) (μg/g dwt)[4]	Range (μg/g dwt)	LOQ / LOD (μg/g fwt)
OSL-1	0.37 (0.12)	0.21 – 0.63	2.6 (0.92)	1.3 – 4.2	0.0470/0.0090
OSR-1	0.041 (0.011)	0.024 – 0.068	0.47 (0.12)	0.30 – 0.85	0.0075/0.0043
Forage	0.034 (0.011)	0.017 – 0.053	0.12 (0.049)	0.053 – 0.20	0.0056/0.0024
Grain	<LOQ		N/A[5]		0.0047/0.0024

[1]Protein quantities are expressed as microgram (μg) of protein per gram (g) of tissue on a fresh weight (fwt) basis. The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=18).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels quantities are expressed as μg/g of tissue on a dry weight (dwt) basis.
[4]The dry weight (dwt) values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A - Not applicable

2.2. Results from Chile 2006/2007 field production

The second season field trial was designed to assess protein levels in MON 87460 under a range of typical environmental conditions relevant to its commercial production. This trial was conducted in Chile during 2006/2007 using two water treatment levels (well-watered and water-limited) to assess the impact of different soil moisture conditions on protein expression. The levels of the CSPB and NPTII proteins in various tissues of MON 87460 that are relevant to the risk assessment were assessed by a validated ELISA. OSL, OSWP, OSR, pollen, silk, forage, forage root, grain, stover, and senescent root tissues were collected from four field sites. The trial locations were Calera de Tango (CT), Colina (CL), Lumbreras (LUM) and Quillota (QUI), covering a range of environmental conditions representative of commercial corn production areas for MON 87460. At each site, three replicated plots of MON 87460, as well as the conventional control, were planted using a strip-plot design with three replicates per site. The whole-plot for each replicate was an irrigation treatment (well-watered or water-limited). The sub-plot for each irrigation treatment was substance type (test and control substances), which was randomized in strips across the irrigation treatments to assess for any changes in protein levels under different soil moisture conditions. Well-watered plots were irrigated to achieve optimal yield, whereas water-limited plots were managed to impose a drought stress by withholding irrigation during the late vegetative through early grain fill growth stages (i.e., approximately V10 through R2). For a site to be included in the combined-site analysis, commercial reference varieties had to exhibit phenotypic responses indicative of a treatment effect. Specifically, reference varieties in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to the same reference varieties planted in the well-watered plots. Moderate water deficits result in approximately a 15% yield loss annually for corn grown in both temperate and tropical regions (Barker et al., 2005). Assessments for plant height, ear height and days to 50% silking were also made as reduced height and a delay in silking are indicators of moisture deficit in corn (Campos et al., 2006). Reference varieties at CT, CL and LUM exhibited the expected phenotypic response. (Appendix F). Reference varieties at QUI did not and it was therefore not possible to include this site in the combined-site analysis. The QUI data are treated separately and presented in Appendix G.

The youngest leaves were randomly collected from plants in each plot at each site. Forty leaves were combined to form the leaf sample for each plot. Over-season leaf samples were collected as follows:

Over-season leaf (OSL)	*Corn development stage*	*Days after planting (DAP)*
OSL-1	V2-V4	13-20
OSL-2	V6-V8	32-38
OSL-3	V10-V12	48-52
OSL-4	~VT	62-65

The aerial portion of the plant was collected from four plants in each plot at each site at the V2-V4 stage and combined to form the whole plant sample. Overseason whole plant samples were collected as follows:

Overseason whole plant (OSWP)	*Corn development stage*	*DAP*
OSWP-1	V2-V4	13-20
OSWP-2	V6-V8	32-38
OSWP-3	V10-V12	48-52
OSWP-4	~VT	62-65

Roots remaining after collection of whole plants from each plot were combined to form the root sample. Four whole plants in each plot at each site were cut above the soil surface at an early dent stage, approximately 106-113 days after planting, and then combined to form the forage sample. The roots of these plants were also removed, washed and combined to form the forage root sample. Grain was harvested at maturity from all plants in each plot at each site and dried to a moisture content of 11-17%. Following harvest, approximately 151-153 days after planting, four whole plants in each plot at each site were cut above the soil surface and combined to form the stover sample. The roots of these plants were also removed, washed and combined to form a senescent root sample.

Overseason root samples were collected as follows:

Overseason root (OSR)	*Corn development stage*	*DAP*
OSR-1	V2-V4	13-20
OSR-2	V6-V8	32-38
OSR-3	V10-V12	48-52
OSR-4	~VT	62-65
Forage root	early dent stage	106-113
Senescent root	after harvest	151-153

Quantities of pollen ranging from 0.5-40 ml and averaging approximately 10 ml were collected from multiple tassels in each plot at each site at pollination, approximately 67-74 days after planting. Silks were collected approximately 64-72 days after planting from five plants. Silks were only collected from ears of plants that were covered with shoot bags to preserve the genetic identity.

All tissue samples, except grain, were stored and shipped on dry ice for processing and analysis. Grain was stored and shipped at room temperature. All tissue samples were stored in a -80 ºC freezer upon receipt. Tissue samples were extracted and analyzed by a validated ELISA according to applicable standard operating procedures (SOPs).

CSPB expression levels were determined in all 19 tissue types described above. Given the extensive historical safety data for NPTII, the number of tissues evaluated for NPTII expression was fewer than those evaluated for CSPB protein. The NPTII levels were evaluated in four of the 19 tissue types including OSL1 (V2-V4), OSR1 (V2-V4), forage, and grain. These four tissues were selected to span the life cycle of corn.

The results obtained from ELISA analysis are summarized in Tables VI-5 and VI-6 for the various tissue types, including the tissues collected throughout the growing season. Table VI-5 presents the levels of CSPB protein determined in over season leaf (OSL1-4), over season root (OSR1-4), over season whole plant (OSWP1-4), forage, forage root, senescent root, stover, silk, pollen and grain harvested from the CT, CL and LUM sites. Table VI-6 presents the levels of NPTII determined in OSL1, OSR1, forage and grain

harvested from the CT, CL and LUM sites. Moisture content was measured for all tissue types. Protein levels for all tissue types are provided in µg/g fwt and µg/g dwt basis.

Mean CSPB protein levels across the three sites that were subjected to either well-watered or water-limited conditions were highest in pollen (18 µg/g dwt well-watered and 17 µg/g dwt water-limited conditions), followed by young leaf (OSL-1, 2.8 µg/g dwt for both well-watered and water-limited conditions), young root (OSR-1, 1.3 µg/g dwt well-watered and 1.5 µg/g dwt water-limited conditions), silk (0.82 µg/g dwt well-watered and 1.1 µg/g dwt µg/g dwt water-limited conditions), forage (0.11 µg/g dwt µg/g dwt well-watered and 0.15 µg/g dwt water-limited conditions), grain (0.048 µg/g dwt µg/g dwt well-watered and 0.038 µg/g dwt water-limited conditions), stover (0.033 µg/g dwt well-watered and 0.072 µg/g dwt water-limited conditions), senescent root (0.031 µg/g dwt well-watered and 0.052 µg/g dwt water-limited conditions), and forage root (0.039 µg/g dwt µg/g dwt well-watered and 0.076 µg/g dwt water-limited conditions). In tissues harvested throughout the growing season, mean CSPB protein levels in MON 87460 across all sites ranged from 0.39 – 2.8 µg/g dwt in leaves harvested from plants grown under well-watered and from 0.44 – 2.8 µg/g dwt in leaves harvested from plants grown under water-limited conditions. The mean CSPB protein levels in overseason roots harvested from MON 87460 plants across all sites ranged from 0.31 – 1.3 µg/g dwt in well-watered and 0.40 – 1.5 µg/g dwt in water-limited conditions. In whole MON 87460 plants, the mean CSPB protein levels were 0.67 – 3.2 µg/g dwt in well-watered and 0.70 – 2.9 µg/g dwt in water-limited conditions.

Mean NPT II protein levels across the three sites were highest in young leaf (OSL-1, 2.4 µg/g dwt well-watered and 2.6 µg/g dwt water-limited), followed by root (OSR-1, 0.51 µg/g dwt in well-watered and 0.48 µg/g dwt water-limited conditions), and forage (0.16 µg/g dwt in well-watered and 0.17 µg/g dwt water-limited conditions). The levels of NPT II protein in grain were below the NPT II assay LOQ (0.0047 µg/g fwt for grain) for tissue collected from plants under both well-watered and water-limited conditions. The NPT II protein levels in MON 87460 were lower than NPT II levels determined for the equivalent tissue types in MON 863 that also relied on NPT II as a selectable marker. The range of NPT II protein levels in MON 863 leaf, forage, and grain were 0.74 – 1.4, 0.17 – 0.23 and <LOQ µg/g fwt, respectively. The range in NPT II protein levels for MON 87460 in leaf, forage, and grain were 0.84-5.0 µg/g dwt well-watered and 0.98-4.0 µg/g dwt water-limited, 0.13-0.19 µg/g dwt well-watered and 0.14-0.22 µg/g dwt water-limited, and LOD/LOQ µg/g fresh weight for well-watered and water limited, respectively

Table VI-5. Summary of CSPB Protein Levels in Tissue Collected from MON 87460 Grown at the CT, CL and LUM Sites During the 2006/2007 Chilean Growing Season under Well-Watered and Water-Limited Conditions

Tissue Type	Well-Watered		Water-Limited		LOQ / LOD (µg/g fwt)
	Mean (SD)[1] Range[2] (µg/g fwt)[3]	Mean (SD) Range (µg/g dwt)[4]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)	
OSL-1	0.50 (0.19) 0.28 - 0.80	2.8 (1.0) 1.7 - 4.5	0.50 (0.20) 0.26 - 0.80	2.8 (0.95) 1.7 - 4.2	0.015/0.0069
OSL-2	0.48 (0.18) 0.21 - 0.69	2.6 (1.2) 0.96 - 3.8	0.47 (0.15) 0.23 - 0.62	2.6 (1.0) 1.1 - 3.6	0.015/0.0069
OSL-3	0.13 (0.10) 0.023 - 0.33	0.56 (0.48) 0.10 - 1.5	0.11 (0.073) 0.023 - 0.25	0.45 (0.32) 0.086 - 1.1	0.015/0.0069
OSL-4	0.10 (0.041) 0.040 - 0.14	0.39 (0.13) 0.18 - 0.58	0.11 (0.054) 0.050 - 0.20	0.44 (0.17) 0.22 - 0.69	0.015/0.0069
OSR-1	0.13 (0.029) 0.079 - 0.18	1.3 (0.29) 0.79 - 1.8	0.14 (0.034) 0.10 - 0.20	1.5 (0.43) 0.95 - 2.2	0.0020/0.0018
OSR-2	0.086 (0.025) 0.070 - 0.13	0.86 (0.25) 0.70 - 1.4	0.10 (0.015) 0.082 - 0.12	0.82 (0.092) 0.74 - 0.95	0.0020/0.0018
OSR-3	0.061 (0.012) 0.035 - 0.075	0.49 (0.12) 0.27 - 0.62	0.054 (0.012) 0.036 - 0.076	0.41 (0.13) 0.24 - 0.63	0.0020/0.0018
OSR-4	0.045 (0.012) 0.032 - 0.067	0.31 (0.076) 0.22 - 0.45	0.058 (0.016) 0.036 - 0.084	0.40 (0.087) 0.28 - 0.52	0.0020/0.0018
OSWP-1	0.32 (0.11) 0.18 - 0.52	3.2 (0.98) 1.8 - 4.8	0.30 (0.092) 0.20 - 0.42	2.9 (0.84) 1.8 - 3.8	0.0045/0.0043
OSWP-2	0.19 (0.036) 0.12 - 0.24	2.3 (0.54) 1.4 - 3.0	0.18 (0.046) 0.12 - 0.25	2.2 (0.61) 1.4 - 3.1	0.0045/0.0043
OSWP-3	0.10 (0.042) 0.065 - 0.17	0.89 (0.34) 0.59 - 1.4	0.091 (0.032) 0.067 - 0.15	0.71 (0.25) 0.44 - 1.1	0.0045/0.0043
OSWP-4	0.11 (0.026) 0.076 - 0.17	0.67 (0.16) 0.48 - 0.98	0.13 (0.037) 0.10 - 0.20	0.70 (0.16) 0.55 - 1.0	0.0045/0.0043
Forage Root	0.0052 (0.0018) 0.0026 - 0.0088	0.039 (0.015) 0.017 - 0.068	0.011 (0.0039) 0.0056 - 0.016	0.076 (0.029) 0.035 - 0.12	0.0020/0.0018
Senescent Root	0.0040 (0.0017) 0.0026 - 0.0073	0.031 (0.015) 0.020 - 0.061	0.0067 (0.0051) 0.0026 - 0.017	0.052 (0.040) 0.019 - 0.14	0.0020/0.0018
Forage	0.026 (0.0041) 0.018 - 0.034	0.11 (0.018) 0.077 - 0.14	0.035 (0.0078) 0.022 - 0.047	0.15 (0.040) 0.087 - 0.22	0.0045/0.0043
Stover	0.011 (0.0023) 0.0071 - 0.014	0.033 (0.0070) 0.018 - 0.040	0.021 (0.010) 0.011 - 0.036	0.072 (0.033) 0.035 - 0.12	0.0045/0.0043
Silk	0.073 (0.019) 0.050 - 0.12	0.82 (0.28) 0.50 - 1.5	0.13 (0.048) 0.054 - 0.22	1.1 (0.38) 0.49 - 1.8	0.0075/0.0047
Pollen	18 (5.6) 7.0 - 24	25 (7.4) 8.9 - 33	18 (6.5) 12 - 31	27 (10) 18 - 48	0.050/0.045
Grain	0.041 (0.012) 0.028 - 0.065	0.048 (0.014) 0.033 - 0.075	0.033 (0.0067) 0.021 - 0.045	0.038 (0.0079) 0.024 - 0.053	0.0038/0.0017

[1] The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites (n=9 for well-watered and n=9 for water-limited, except OSR-2 where n=6 for under both well-watered and water-limited conditions and senescent root where n=6 for well-watered).
[2]Minimum and maximum values were determined for each tissue type across all sites.
[3]Protein quantities are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
[4]Protein quantities are expressed as "µg/g" of tissue on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

Table VI-6. Summary of NPTII Protein Levels in Tissue Collected from MON 87460 Grown at the CT, CL and LUM Sites During the 2006/2007 Chilean Growing Season under Well-Watered and Water-Limited Conditions

Tissue Type	Well-Watered		Water-Limited		LOQ[5] / LOD[6] (µg/g fwt)
	Mean (SD)[1] Range[2] (µg/g fwt)[3]	Mean (SD) Range (µg/g dwt)[4]	Mean (SD) Range (µg/g fwt)	Mean (SD) Range (µg/g dwt)	
OSL-1	0.42 (0.23) 0.15 - 0.85	2.4 (1.3) 0.84 - 5.0	0.46 (0.18) 0.16 - 0.68	2.6 (0.98) 0.98 - 4.0	0.047/0.0090
OSR-1	0.051 (0.0083) 0.041 - 0.064	0.51 (0.083) 0.41 - 0.64	0.046 (0.0075) 0.035 - 0.057	0.48 (0.097) 0.39 - 0.64	0.0075/0.0043
Forage	0.037 (0.0041) 0.031 - 0.044	0.16 (0.020) 0.13 - 0.19	0.039 (0.0048) 0.034 - 0.048	0.17 (0.028) 0.14 - 0.22	0.0056/0.0024
Grain	<LOQ(N/A[7]) <LOD-0.0057	N/A (N/A) N/A	<LOQ (N/A) <LOD-0.0051	N/A (N/A) N/A	0.0047/0.0024

[1]The mean and standard deviation (SD) were calculated across sites (n=9 for well-watered and n=9 for water-limited, except OSL-1 where n=8 for water-limited).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
[4]Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fresh weight by the dry weight conversion factors obtained from moisture analysis data.
[5]LOQ – Limit of quantitation
[6]LOD-Limit of detection
[7]N/A – Not applicable

2.3. Conclusions on the levels of CSPB and NPTII proteins in MON 87460

In MON 87460, *cspB* gene expression is driven by the rice actin constitutive promoter and thus is expected to occur in all plant tissues at various levels. The protein was detected in all tissue types with highest level of expression in pollen, followed by silk, leaf, forage, grain, stover, senescent root and forage root. In general, the levels of the CSPB protein declined over the growing season.

In MON 87460, *nptII* gene expression is driven by the constitutive CaMV 35S promoter, which dictates expression across all plant tissues at various levels. The NPTII protein was detected in three out of four analyzed tissue types with highest level determined in leaf, followed by roots and forage. The level of NPTII protein in grain was below the LOQ of the method.

SECTION 3. Assessment of the Potential for Allergenicity of the CSPB and NPTII Proteins Produced in MON 87460

3.1. Approach to the assessment of allergenicity

Assessments of a protein's allergenic potential compare the biochemical characteristics of the protein to characteristics of known allergens (Codex, 2003a). A protein is not likely to be associated with allergenicity if:

1. The protein is from a non-allergenic source;
2. The protein represents only a very small portion of the total protein in the grain;
3. The protein does not share structural similarities to known allergens based on the amino acid sequence;
4. The protein is rapidly digested in simulated gastric fluid.

A detailed assessment of these factors for CSPB confirmed that this protein is not likely to be associated with allergenicity. Information on the methods used to evaluate the similarity to known allergens and stability in simulated digestive fluids is provided below.

The safety of the *E. coli* NPTII protein was previously evaluated and it was concluded that the NPTII protein poses no allergenic risk when ingested (FDA, 1998). The bioinformatic analysis utilizing an updated allergen database confirmed that the NPTII protein does not share structurally or immunologically relevant amino acid sequence similarities with known allergens. Furthermore, the NPTII protein was not detected in MON 87460 grain.

3.1.1. Rationale for studying structural similarity to known allergens

In 2003, the Codex Alimentarius Commission (Codex) published guidelines for the evaluation of the potential allergenicity of novel proteins based on amino acid sequence similarity (Codex, 2003a). The guidelines are based on the comparison of amino acid sequences between introduced proteins and allergens, where potential allergenic cross-reactivity may exist if the introduced protein is found to have at least 35% amino acid identity with an allergen over any segment of at least 80 amino acids. The Codex guidelines also recommended that a sliding window search with a scientifically justified peptide size could be used to identify immunologically relevant peptides in otherwise unrelated proteins. The extent of sequence similarities between the CSPB protein present in MON 87460 and known allergens, gliadins and glutenins was assessed using the FASTA sequence alignment tool and an eight-amino acid sliding window search (Thomas et al., 2004; Codex, 2003a). The data generated from these analyses confirm that the CSPB protein does not share any amino acid sequence similarities with known allergens, gliadins, or glutenins.

3.1.2. Rationale for studying stability in simulated digestive fluids

Stability to gastrointestinal digestion is a factor that increases the likelihood of allergic oral sensitization to proteins. Many allergens can withstand proteolytic digestion by enzymes of the gastrointestinal tract (Astwood et al., 1996; Metcalfe et al., 1996; Vieths et al., 1999; Moreno et al., 2005; Vassilopoulou et al., 2006). When resistant to digestion, allergens, or their fragments, are presented to the intestinal immune system,

which can lead to a variety of gastrointestinal and systemic manifestations of immune-mediated allergy. Conversely, rapid digestion of proteins is strongly correlated with a reduced likelihood of sensitization or allergic reaction when consumed.

A simulated gastric fluid (SGF) assay containing pepsin is a key step in assessments of protein digestibility. A relationship between the digestibility in SGF and the likelihood of being an allergen has been previously reported with a group of proteins consisting of both allergens and non-allergens (Astwood et al., 1996). Recently, the SGF assay protocol was standardized by the International Life Sciences Institute (ILSI) based on results obtained from an international, multi-laboratory ring study (Thomas et al., 2004). The study showed that the results of *in vitro* pepsin digestion assays are reproducible when standard protocols were followed. Using the ILSI protocol, the pepsin digestion assay confirmed the susceptibility of CSPB to pepsin digestion *in vitro.*

The complete digestion of protein by pepsin in the gastric system reduces the possibility that an intact protein or protein fragment will reach the absorptive epithelium of the small intestine where antigen processing cells reside. Absorption of antigen by cells of the mucosal immune system might lead to the production of antigen specific IgE and, thus, sensitization of susceptible individuals to the dietary protein. To reach the intestinal mucosa, proteins or protein fragments must first pass through the stomach where they are exposed to pepsin and then the duodenum where they are exposed to pancreatic fluid containing a mixture of proteolytic enzymes called pancreatin. In instances where transient stability of the protein or protein fragments are observed in SGF, further degradation of these fragments in simulated intestinal fluid (SIF) containing pancreatin and neutral pH should be evaluated. If a protein or protein fragments are readily digested by pancreatic fluid enzymes, such fragments would not reach absorptive cells of the intestinal mucosa and, thus, would not pose a potential allergenic risk. Susceptibility of CSPB protein to digestion with pepsin alone as well as to digestion with pepsin and pancreatin in sequential reactions was evaluated according to the methods described by Thomas et al. (2004) and by the United States Pharmacopeia (USP, 1995).

Finally, digestibility of protein in SIF is also used as a stand alone test system to assess the digestibility of food components *in vitro* (Yagami et al., 2000; Okunuki et al., 2002). The relationship between protein allergenicity and protein stability in the *in vitro* stand alone SIF study is limited, because the protein has not been first exposed to the acidic, denaturing conditions of the stomach, as would typically be the case *in vivo* (FAO/WHO, 2001).

3.2. Assessment of the potential for allergenicity of the CSPB protein

3.2.1. Source of the CSPB protein

The gene encoding the CSPB protein is derived from *B. subtilis*, a soil microorganism that is both ubiquitous and abundant in the environment (deBoer and Diderichsen, 1991). FDA has recognized the safety of *B. subtilis* by designating this organism as generally recognized as safe (GRAS) for use in the manufacturing of enzyme preparations to be used in food (FDA, 1999). There are no known reports of allergies to *B. subtilis* or to the proteins produced by *B. subtilis*.

3.2.2. Bioinformatics analyses of sequence similarity of the CSPB protein produced in MON 87460 to allergens

The allergen database (version AD8; www.allergenonline.com) was used to evaluate sequence similarities between the CSPB protein and known protein allergens, gliadins, and glutenins. This evaluation did not produce any meaningful sequence similarities. Using the FASTA sequence alignment tool, known allergens were ranked according to their degree of similarity to CSPB. Because the CSPB protein contains only 66 amino acids, none of the potential alignments with proteins in allergen database can meet or exceed the threshold of 35% shared amino acid identity over 80 or more amino acids. Although none of the obtained alignments satisfied minimum Codex standards, all alignments were thoroughly evaluated. One low -quality alignment between CSPB and the major allergen Mal d 1.06C from *Malus x domestica* (GI number 60280827) was identified, where four gaps were needed to align a stretch of 58 amino acids with 32.8% shared identity. This alignment had an *E*-score of 0.7. The *E*-score (expectation score) is a statistical measure of the likelihood that the observed similarity score could have occurred by chance in a search. A larger *E*-score indicates a lower degree of similarity between the query sequence and the sequence from the database. Typically, alignments between two sequences will need to have an *E*-score of 1×10^{-5} or smaller to be considered to have significant homology. The *E*-score of 0.7 is very near the *E*-scores of ~1, which are expected to occur for alignments between random, non-homologous sequences (Pearson, 2000). Therefore, this low quality alignment is not considered relevant from an allergenic assessment perspective. There were no other alignments with the AD8 database.

A second bioinformatics tool, an eight-amino acid sliding window search, was used to specifically identify short linear polypeptide matches to known or suspected allergens and confirmed the results of the AD8 analysis. It is possible that proteins structurally unrelated to allergens, gliadins, and glutenins may still contain smaller immunologically significant epitopes. An amino acid sequence may be considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Metcalfe et al., 1996; Hileman et al., 2002). Using a sliding window of fewer than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich et al., 2006) and are not useful to the allergy assessment process (Thomas et al., 2005).

A sliding eight- amino acid window search (ALLERGENSEARCH) was performed to identify whether or not a linearly contiguous match (exact identity matches) of eight amino acids exists between the CSPB amino acid sequence and any amino acid sequences contained within the allergen database (AD8). Results indicate that no alignments of eight contiguous amino acid identities were detected when the CSPB protein sequence was compared to known allergen sequences in the AD8 database.

3.2.3. Digestibility of the CSPB protein in simulated gastric fluid

Digestibility of the CSPB protein in SGF was assessed by SDS-PAGE and western blot methods and demonstrated that CSPB is completely digested by the 60 min time point. The extent of CSPB protein digestion was evaluated by visual analysis of stained polyacrylamide gels (Figure VI-7) or by visual analysis of developed X-ray film (Figure VI-8). The LOD of the CSPB protein by Colloidal Brilliant Blue G staining was

0.005 µg or approximately 0.6% of the total CSPB protein loaded (0.005 µg divided by 0.8 µg of the protein loaded in each lane of the gel; Figure VI-7, panel B). The limit of detection of the CSPB protein by western blotting was 0.1 ng or approximately 1% of the total CSPB protein loaded (0.1 ng divided by 10 ng of the protein loaded in each lane of the gel; Figure VI-8, panel B).

Visual examination of the Colloidal Brilliant Blue G stained gel (Figure VI-7, panel A) showed that the full-length CSPB protein was digested below the LOD within 30 s of digestion in SGF (Figure VI-7, panel A, Lane 5). Therefore, at least 99.4% (100% - 0.6% = 99.4%) of the full-length CSPB was digested within 30 s of incubation in SGF. A fragment with an apparent molecular weight of ~2.5 kDa was observed between the 30 s and 30 min digestion time points. No protein fragments were visible at the 60 min digestion time point (Figure VI-7, Lane 11).

Western blot analysis demonstrated that the CSPB protein was digested below the LOD within 30 s of incubation in SGF (Figure VI-8, panel A, Lane 5). Based on the western blot LOD for the CSPB protein in SGF and the observation that no full-length protein or immunoreactive bands were observed on the western blot at the 30-s digestion time point, it was concluded that at least 99% (100% – 1% = 99%) of the full-length CSPB protein was digested within 30 s of incubation in SGF based on the western blot analysis.

Because the transiently stable CSPB fragment (~2.5 kDa) was not cross-reactive with CSPB-specific antibodies, the identity of this fragment was established by N-terminal sequencing. The resulting sequence matched the predicted N-terminal sequence of the CSPB protein.

3.2.4. Digestibility of the CSPB protein in simulated intestinal fluid

To better understand the digestive fate of the transiently stable CSPB fragment, it was exposed to digestion with pancreatin in SIF following the digestion in SGF. Results confirm that the transiently stable fragment is completely degraded by SIF.

After digestion in SGF for 2 min, the reaction was quenched and the transiently stable CSPB protein fragment was exposed to further digestion in SIF. The digestibility in SIF of the CSPB protein fragment was evaluated by visual analysis of stained polyacrylamide gels (Figure VI-9). The gel was loaded with ~0.8 µg total protein (based on concentration of the protein prior to the digestion in SGF) for each of the SIF digestion time points. Visual examination of the stained gel demonstrated that the fragment of ~2.5 kDa was rapidly digestible (< 30 s) in SIF (Figure VI-9, Lane 7).

SIF is also used as a stand alone test system for *in vitro* studies to assess the digestibility of food components (Yagami et al., 2000; Okunuki et al., 2002). The relationship between protein allergenicity and protein stability in the SIF study is limited, because the protein has not been first exposed to the acidic, denaturing conditions of the stomach, as would be the case *in vivo* (FAO/WHO, 2001). Susceptibility of the CSPB protein in SIF was assessed according to methods described in the United States Pharmacopeia (1995).

The digestion of the CSPB protein in SIF was measured by western blot method (Figure VI-10). A western blot to determine the LOD (Figure VI-10, panel B) of the CSPB protein was performed concurrently with the western blot used to assess digestibility in SIF (Figure VI-9, panel A). The LOD was estimated to be 0.1 ng, which represented 1% of the total protein loaded in this experiment (0.1 ng divided by 10 ng of loaded protein).

Western blot analysis demonstrated that the full-length CSPB protein was digested below the LOD within 5 min of incubation in SIF (Figure VI-9, panel A, Lane 5). Therefore, at least 99% (100% - 1% = 99%) of the full-length CSPB protein was digested within 5 min. No proteolytic fragments were observed at any of the digestion time points. The digestion of CSPB protein in SIF was not evaluated using a stained gel because the results of the CSPB digestion in the sequential enzymatic digestion assay clearly demonstrated that no stable fragments can be observed after 30 s of incubation in SIF.

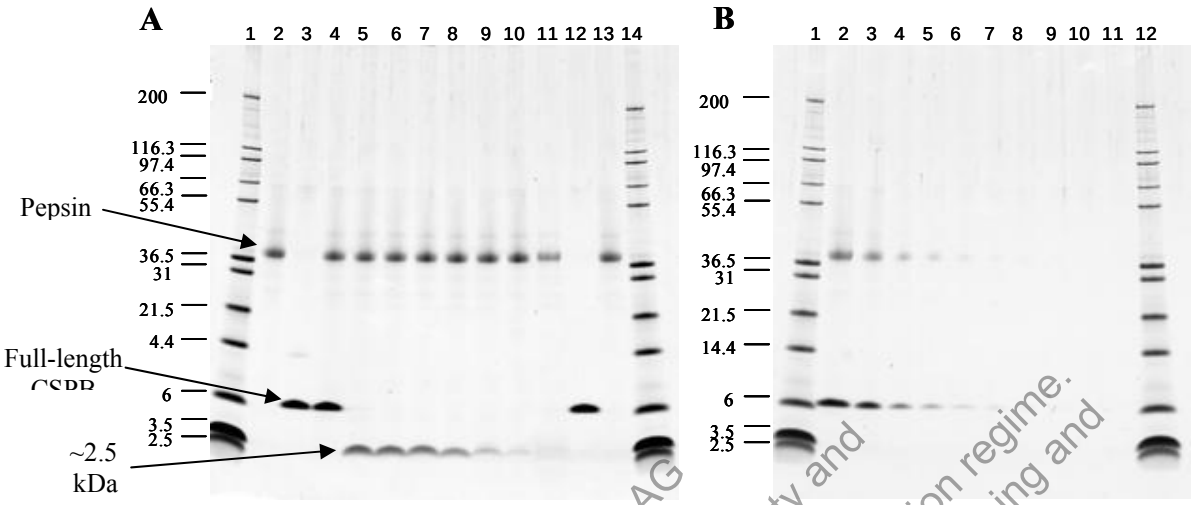

Figure VI-7. Colloidal Brilliant Blue G stained SDS-gels of CSPB protein digestion in SGF

Panel A corresponds to CSPB protein digestion in SGF. Based on pre-digestion protein concentrations, 0.8 µg (total CSPB protein) was loaded in lanes containing CSPB protein. The incubation times are indicated. **Panel B** corresponds to the limit of detection (LOD) of CSPB protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in each gel.

Lane assignment for Panel A:

Lane	Sample	Incubation Time (min)
1	Molecular weight marker	—
2	SGF N0[1]	0
3	SGF P0	0
4	SGF T0	0
5	SGF T1	0.5
6	SGF T2	2
7	SGF T3	5
8	SGF T4	10
9	SGF T5	20
10	SGF T6	30
11	SGF T7	60
12	SGF P7	60
13	SGF N7	60
14	Molecular weight marker	—

Lane assignment for Panel B:

Lane	Sample	Amount (µg)
1	Molecular weight marker	—
2	T0, protein+SGF	0.8
3	T0, protein+SGF	0.4
4	T0, protein+SGF	0.1
5	T0, protein+SGF	0.05
6	T0, protein+SGF	0.02
7	T0, protein+SGF	0.01
8	T0, protein+SGF	0.005
9	T0, protein+SGF	0.0025
10	T0, protein+SGF	0.001
11	T0, protein+SGF	0.0005
12	Molecular weight marker	—

[1]A numerical code using the numbers 0 through 7 was used to distinguish incubation time points. N0, N7- negative controls (no test protein), P0, P7- protein control (no pepsin), T0-T7- incubation time point in SGF.

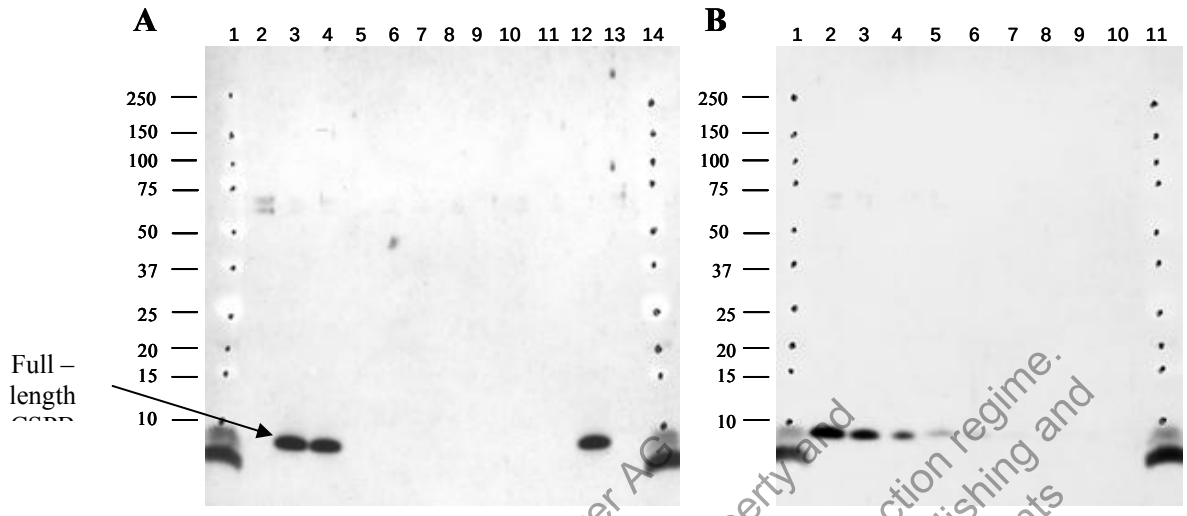

Figure VI-8. Western Blot Analysis of the Digestion of CSPB protein in SGF

Panel A corresponds to CSPB protein digestion in SGF. Based on pre-digestion protein concentrations, 10 ng (total protein) was loaded in the lanes containing CSPB protein. **Panel B** corresponds to the limit of detection of CSPB protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in each gel. In both gels, CSPB protein migrated to approximately 7 kDa. A 2 min exposure is shown. Blank or empty lanes are cropped and lanes renumbered.

Lane assignment for Panel A:

Lane	Sample	Incubation Time (min)[1]
1	Molecular weight marker	—
2	SGF N0	0
3	SGF P0	0
4	SGF T0	0
5	SGF T1	0.5
6	SGF T2	2
7	SGF T3	5
8	SGF T4	10
9	SGF T5	20
10	SGF T6	30
11	SGF T7	60
12	SGF P7	60
13	SGF N7	60
14	Molecular weight marker	—

Lane assignment for Panel B:

Lane	Sample	Amount (ng)
1	Molecular weight marker	—
2	T0, protein+SGF	10
3	T0, protein+SGF	5
4	T0, protein+SGF	2.5
5	T0, protein+SGF	1
6	T0, protein+SGF	0.5
7	T0, protein+SGF	0.2
8	T0, protein+SGF	0.1
9	T0, protein+SGF	0.05
10	T0, protein+SGF	0.025
11	Molecular weight marker	—

[1]A numerical code using the numbers 0 through 7 was used to distinguish incubation time points. N0, N7- negative controls (no test protein), P0, P7- protein control (no pepsin), T0-T7- incubation time point in SGF

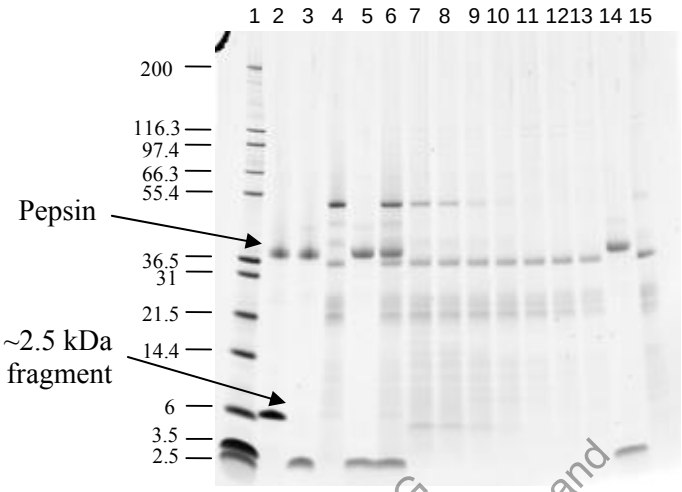

Figure VI-9. Colloidal Brilliant Blue G stained SDS-gel and Western Blot Analysis of the CSPB protein in SGF followed by SIF

After digestion of the CSPB protein in SGF for 2 min, the reaction was quenched and the transiently stable CSPB protein fragment was exposed to further digestion in SIF.
Based on protein concentrations before digestion in SGF, 0.8 µg of total protein was loaded per lane containing CSPB protein.

Lane	Sample	Incubation Time
1	Molecular weight marker	—
2	SEQ 0min	0
3	SEQ 2min	2 min
4	SEQ N0[1]	0
5	SEQ P0	0
6	SEQ T0	0
7	SEQ T1	0.5 min
8	SEQ T2	2 min
9	SEQ T3	5 min
10	SEQ T4	10 min
11	SEQ T5	30 min
12	SEQ T6	1 h
14	SEQ P7	2 h
15	SEQ N7	2 h

[1]A numerical code using the numbers 0 through 7 was used to distinguish incubation time points. N0, N7- negative controls (no test protein), P0, P7- protein control (no pepsin), T0-T7- incubation time point in sequential digestion assay (SEQ).

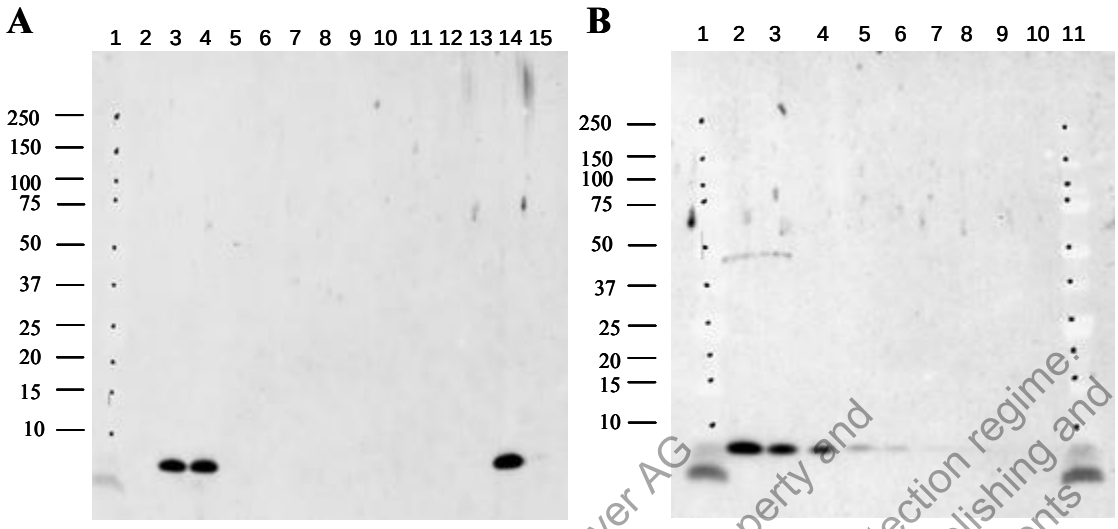

Figure VI-10. Western Blot Analysis of the Digestion of CSPB protein in SIF

Panel A corresponds to CSPB protein digestion in SIF. Based on pre-digestion protein concentrations, 10 ng (total protein) was loaded in the lanes containing CSPB protein. **Panel B** corresponds to the limit of detection of CSPB protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in each gel. One min exposure is shown.

Lane assignment for Panel A:

Lane	Sample	Incubation Time
1	Molecular weight marker	—
2	SIF N0	0
3	SIF P0	0
4	SIF T0	0
5	SIF T1	5 min
6	SIF T2	15 min
7	SIF T3	30 min
8	SIF T4	1 h
9	SIF T5	2 h
10	SIF T6	4 h
11	SIF T7	8 h
12	SIF T8	12 h
13	SIF T9	24 h
14	SIF P9	24 h
15	SIF N9	24 h

Lane assignment for Panel B:

Lane	Sample	Amount (ng)
1	Molecular weight marker	—
2	T0, protein+SIF	10
3	T0, protein+SIF	5
4	T0, protein+SIF	2.5
5	T0, protein+SIF	1
6	T0, protein+SIF	0.5
7	T0, protein+SIF	0.2
8	T0, protein+SIF	0.1
9	T0, protein+SIF	0.05
10	T0, protein+SIF	0.025
11	Molecular weight marker	—

[1]-A numerical code using the numbers 0 through 9 was used to distinguish incubation time points. N0, N9- negative controls (no test protein), P0, P9- protein controls (no pepsin), T0-T7- incubation time point in SIF.

3.2.5. Proportion of CSPB protein to the total protein in MON 87460 grain

CSPB was detected at low levels in various plant tissues at a number of time points during the growing season. Among these tissues, corn grain is the most relevant to the assessment of food allergenicity. The mean level of CSPB in MON 87460 grain is 0.072 µg/g dwt. The mean percent dry weight of total protein in MON 87460 grain is 10.50% (or 105,000 µg/g). The percent of CSPB protein in MON 87460 grain is calculated as follows:

(0.072 µg/g ÷ 105,000 µg/g) x 100% ≈ 0.00007% of total corn grain protein

Therefore, the CSPB protein represents a small portion of the total protein in MON 87460 grain.

3.3. Assessment of the potential for allergenicity of the NPTII protein

3.3.1. Source of the NPTII protein

The gene encoding the NPTII protein is derived from a non-virulent strain of *E. coli*, a bacterium that is ubiquitous in the environment and found in the digestive tracts of vertebrate species, including humans. Safety of the donor organism, *E. coli*, has been previously assessed by the FDA as part of the consultation process for other transformed crops that contain the same *nptII* gene (FDA, 1998). There are no known reports of allergies to *E. coli* or to the proteins produced by *E. coli*.

3.3.2. Bioinformatics analysis of sequence similarity of the NPTII protein produced in MON 87460 to allergens

The updated allergen database (version AD8; www.allergenonline.com) was also used for the evaluation of sequence similarities shared between the NPTII protein and known protein allergens, gliadins, and glutenins. Using the FASTA sequence alignment tool, known allergen proteins were ranked according to their degree of similarity to NPTII. None of the proteins in the AD8 database met or exceeded the threshold of 35% shared amino acid identity over 80 or more amino acids. In addition, there were no alignments that had an *E*-score below 1.0. The *E*-score (expectation score) is a statistical measure of the likelihood that the observed similarity score could have occurred by chance in a search. A larger *E*-score indicates a lower degree of similarity between the query sequence and the sequence from the database. Typically, alignments between two sequences will need to have an *E*-score of 1×10^{-5} or smaller to be considered to have significant homology. *E*-scores of ~1 are expected to occur for alignments between random, non-homologous sequences (Pearson, 2000).

A second bioinformatics tool, an eight-amino acid sliding window search, was used to specifically identify short linear polypeptide matches to known or suspected allergens. No such matches were detected. It is possible that proteins structurally unrelated to allergens, gliadins, and glutenins may still contain smaller immunologically significant epitopes. An amino acid sequence may be considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Metcalfe et al., 1996; Hileman et al., 2002). Using a sliding window of less than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich et al., 2006) and are not useful to the allergy assessment process (Thomas et al., 2005).

A sliding eight- amino acid window search (ALLERGENSEARCH) was performed to identify whether or not a linearly contiguous match (exact identity matches) of eight amino acids exists between the NPTII amino acid sequence and any amino acid sequences contained within the allergen database (AD8). The eight-amino acid sliding window search showed no immunologically relevant sequences (eight contiguous amino acid identities) detected when the NPTII protein sequence was compared to the allergen database.

3.3.3. Proportion of NPTII protein to the total protein in MON 87460 grain

The level of the NPTII protein in corn grain was below the LOD of the validated ELISA. The LOD of the ELISA method for corn is 0.0031 µg/g fwt. The mean % dry weight of total protein in grain of MON 87460 is 10.5% (or 105,000 µg/g). The percent of NPTII protein in grain of MON 87460 is calculated as follows:

(0.0031 µg/g ÷ 105,000 µg/g) x 100% = 0.000003% of total corn grain protein

Therefore, the NPTII protein, if present, represents an extremely small portion of the total protein in grain of MON 87460.

3.4. Conclusions

Allergenicity studies for CSPB and NPTII confirm that these proteins do not pose a meaningful allergenic risk. Both proteins were assessed for their potential allergenicity according to the recommendations of the Codex Alimentarius Commission. The proteins are from non-allergenic sources, lack structural similarity to known allergens, are rapidly digested in simulated gastric and simulated intestinal fluids and constitute a very small portion of the total protein present in the grain of MON 87460.

The CSPB protein is from *B. subtilis*, an organism that is not an allergenic source and is designated as GRAS by the FDA for use in enzyme preparations (FDA, 1999). Bioinformatics analyses demonstrated that the CSPB protein does not share structurally or immunologically relevant amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes. Digestive fate experiments conducted with the CSPB protein demonstrated that the full-length protein is rapidly digested in simulated gastric fluid, a characteristic shared among many proteins with a history of safe consumption. A small transiently stable CSPB protein fragment was very quickly degraded during short exposure to SIF. Rapid digestion of the full-length CSPB protein in SGF and SIF together with rapid degradation of the small transiently stable fragment in SIF indicates that it is highly unlikely that the CSPB protein and its fragment will reach absorptive cells of the intestinal mucosa. Finally, the CSPB protein represents no more than 0.000007% of the total protein in MON 87460 grain.

The safety of the *E. coli* NPTII protein was previously evaluated and it was concluded that NPTII poses no allergenic risk when ingested (FDA, 1998). The bioinformatic analysis utilizing an updated allergen database confirmed that the NPTII protein does not share structurally or immunologically relevant amino acid sequence similarities with known allergens. Furthermore, the NPTII protein was not detected in the grain of MON 87460. Taking into consideration the LOD of the ELISA method, it was conservatively calculated that, if present, NPTII represents less than 0.0000003% of the total protein in the grain of MON 87460.

SECTION 4. Assessment of the Potential for Toxicity of the CSPB and NPTII Proteins

4.1. Approach to the assessment of toxicity

Assessments of a protein's potential toxicity are based on the premise that a protein is not likely to have a toxic effect if:

1. The protein has a demonstrated history of safe use;
2. The protein has no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals; and
3. The protein does not exert any acute toxic effects to mammals.

A comprehensive analysis of these factors confirmed that CSPB and NPTII are not likely to exhibit toxic effects. The following subsections describe the history of safe use and potential mammalian toxicity of the CSPB and NPTII proteins produced in MON 87460. Information regarding the methods used to evaluate the structural similarity to known toxins and acute toxicity is also provided below. Data from this section are used to inform results of the exposure assessment presented in Section 5.

4.2. Assessment of the potential for toxicity of CSPB

CSPB possesses a strong safety profile. Its donor organism, *B. subtilis*, is ubiquitous in the environment, is used to manufacture food and is an ingredient in certain probiotic formulations. CSPB shares homology with a variety of proteins that are present in commonly consumed foods and have a history of safe use. CSPB is not homologous to known toxic or bioactive proteins. Finally CSPB did not exhibit any signs of toxicity when administered to mice via oral gavage. This weight of evidence supports the conclusion that CSPB is unlikely to exhibit toxic effects when consumed in food and feeds prepared from MON 87460.

4.2.1. Safety of the donor organism: *Bacillus subtilis*

The safety of *B. subtilis* and products derived from *B. subtilis* for use in food has been confirmed consistently by numerous reviews. In 1999, FDA designated enzyme preparations from this organism as GRAS (generally recognized as safe, FDA 1999). EFSA's Scientific Committee proposed *B. subtilis* for a qualified presumption of safety in December 2007 based on the extensive body of knowledge available about this species (EFSA, 2007). The U.S. Environmental Protection Agency (EPA) also exempted *B. subtilis* from further review under the Toxic Substances Control Act (TSCA) (EPA, 1997). A detailed safety profile of *B. subtilis* was reviewed as a part of the safety assessment of several enzymes used in food preparation including α-acetolactate, decarboxylase, α-amylase, maltogenic amylase, and pullulanase (de Boer and Diderichsen, 1991; Pedersen et al., 2002; Olempska-Beer et al., 2006). In addition, *B. subtilis* was tested for cytotoxicity in Chinese hamster ovary K1 (CHO-K1) cells, for production of hemolytic and nonhemolytic enterotoxins (Pedersen et al., 2002), acute toxicity in BALB/c mice, and chronic toxicity in mice, rabbits, and pigs (Sorokulova et al., 2007). No toxic effects were t were attributed to *B. subtilis* in these studies and no similarities to pathogenic bacteria were found, which led to the conclusion that *B. subtilis*

is non-pathogenic and safe for human consumption (Pedersen et al, 2002; Sorokulova et al., 2007).

As further confirmation of the safety of *B. subtilis*, spores and cultures of this organism and other *Bacillus* species have been sold worldwide as probiotics, including in the U.S., Mexico, Europe and South Asia (Sanders et al., 2003). Probiotics are dietary supplements containing potentially beneficial bacteria or yeasts. FAO/WHO (2002) defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Examples of commercial probiotic products intended for human or animal use and containing *Bacillus* spores include Bidisubtilis (Bidiphar, Vietnam), BioGrow (Provita Eurotech Ltd., UK), BioPlus 2B™ (Christian Hansen Hoersholm, Denmark), Biosporin (Biofarm, Ukraine), Biostart™ (Microbial Solutions, South Africa and Advanced Microbial Systems, USA), Lactipan Plus (Istituto Biochimico Italiano SpA, Italy), Liqualife™ (Cargill, USA), Medilac (Hanmi Pharmaceutical Co., China), Nature's First Food™ (Nature's First Law, USA) (reviewed in Hong et al., 2005). Human probiotic products are used for oral bacteriotherapy of gastrointestinal disorders since the ingestion of large amounts of *B. subtilis* is thought to restore the normal microbial flora following extensive antibiotic use or illness (Mazza, 1994).

4.2.2. Similarity of CSPB to other proteins with a history of safe use and consumption

The CSPB protein from *B. subtilis* is homologous to several bacterial proteins that are ubiquitous in the human diet and directly consumed in many common foods. CSPB is a member of the cold shock protein (CSP) family (Nakaminami et al., 2006; Karlson and Imai, 2003). This family includes bacterial and plant proteins possessing a cold shock domain (CSD) that has high amino acid sequence similarity to the sequence of the CSPB protein (Graumann et al., 1997; Karlson, 2003; Nakaminami, 2006). The cold shock domain database currently contains 547 entries representing cold shock proteins found in bacterial and plant species (http://www.chemie.uni-marburg.de/~csdbase/). A search of publicly available databases using the CSPB protein sequence has revealed that the CSPB protein present in MON 87460 shares amino acid identity to other naturally occurring CSD-containing proteins found in food and food products (Table VI-7). The amino acid identity ranges from 35% to 98.5% across different plant and bacterial species. CSPB from *B. subtilis* is homologous to the CSP proteins found in *E. coli*, *Lactobacillus*, *Lactococcous*, and *Bifidobacterium* species (Table VI-7), the most common types of bacteria used in the dairy industry to prepare cheese, sour cream, buttermilk, yogurt and probiotic products containing live bacterial cultures (Morea et al., 2001; Ogier et al., 2002; http://www.nationaldairycouncil.org). These bacteria are commonly present in gastrointestinal flora. In addition, *Bacillus*, *Lactobacillus* and *Lactoccoccus* species containing CSPs are involved in many fermentation processes of milk, meats, cereals and vegetables. Food fermentation is a widely practiced and ancient technology to preserve food or to make it more digestible (Caplice and Fitzgerald, 1999). Examples of

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fermented foods that are popular in different countries and prepared with the help of bacteria include European sausages prepared by fermentation of the raw meat with *Lactobacilli* cultures (*Lactobacillus sakei* and/or *Lactobacillus curvatus*) (Leroy et al., 2006), pickled vegetables, kefir, yogurt (Lopitz-Otsoa et al., 2006), products of soy fermentation including soy sauce (Tanasupawat et al., 2002) and okpehe (Oguntoyinbo et al., 2007), a traditional African fermented soup condiment produced by fermentation of cooked *Prosopis africana* seeds. Other examples include Dawadawa (made from soy or Locust beans), a popular condiment used in Africa and Asia to flavor soups and stews (Terlabie et al., 2006), thua nao (made from soy), popular in Asian food as a condiment for enhancing flavor in soups and curries (Inatsu et al., 2006) and natto, a commonly consumed food in Japan, made by fermenting cooked soybeans with *B. subtilis ssp. natto* (Ashikaga et al., 2000).

Cold shock domains (CSDs) with high similarity to the CSPB protein expressed in MON 87460 are also present in many crops and, therefore, are ubiquitous in the human diet and directly consumed in many common foods. Proteins that are homologous to *B. subtilis* CSPB were identified in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Table VI-7). The wheat cold shock protein, WCSP1 (Karlson et al., 2002), has been shown to be functionally similar to CSPB, as demonstrated by complementation of the *E. coli* cold-sensitive phenotype with WCSP1 and *in vitro* functional melting assays (Karlson et al., 2002; Nakaminami et al., 2006). It has been suggested that WCSP1 protein functions *in planta* as an RNA chaperone by unfolding the secondary structure of the nucleic acids and is involved in the regulation of translation at low temperatures (Nakaminami et al., 2006). Recently two CSD-containing proteins were described in rice (Chaikam and Karlson, 2008). Rice CSPs contain two distinct domains: an N-terminal CSD region and a glycine-rich domain. These CSD-containing proteins possess similar *in vitro* and *in vivo* functions as those of WCSP1 and bacterial CSPs (Chaikam and Karlson, 2008). Furthermore, the cold shock domains of CSD-containing proteins from wheat and rice CSPs share 40-50% identity to the entire sequence of CSPB from *B. subtilis*. Recently, Karlson and Imai (2003), through bioinformatic analyses of a GenBank expressed sequence tag (EST) database, have shown that proteins containing highly conserved CSDs are likely present in such food crops as corn, barley and soy. Digestion of plant CSD-containing proteins would be expected to release these highly conserved CSD portions or fragments thereof into the human digestive tract. It is recognized that human consumption of plant proteins with significant homology to CSPB occurs on a daily basis. The CSPB protein in MON 87460 is similar to several bacterial CSPs and CSD-containing proteins present in food crops that are ubiquitous in the human diet and directly consumed in common foods establishing a history of safe exposure for this protein.

Table VI-7. Amino Acid Sequence Identity between MON 87460-Produced CSPB Protein and Other Cold Shock Domain Containing Proteins Present in Foods

#	Proteins	Sequence Identity (%)																	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	MON 87460 CSPB	100	97	44	61	64	58	66	66	64	40	69	79	49	61	59	37	43	47
2	*B. subtilis* CSPB		100	45	61	64	58	66	66	64	40	69	79	49	61	59	38	42	46
3	*Bifidobacterium longum* CSPA			100	52	49	46	38	38	54	39	54	50	36	44	52	45	49	45
4	*E. coli* CSPA				100	68	77	59	59	39	65	68	57	44	58	57	44	54	53
5	*E. coli* CSPE					100	66	47	47	37	60	62	64	47	58	61	41	45	53
6	*E. coli* CSPB						100	47	47	34	60	62	55	46	58	58	43	48	50
7	*Lactobacillus acidophilus* CPSL							100	100	47	69	75	63	44	59	41	32	31	41
8	*Lactobacillus casei* CSPL								100	47	69	75	63	44	59	41	32	31	41
9	*Lactobacillus delbrueckii* CSPA									100	42	38	63	55	69	57	48	39	51
10	*Lactobacillus delbrueckii* CSPB										100	69	39	26	34	33	29	22	56
11	*Lactobacillus plantarum* CSPC											100	71	53	73	57	41	42	55
12	*Lactobacillus sakei* CSPA												100	51	65	42	32	33	36
13	*Lactococcus lactis* CSPA													100	62	52	42	44	42
14	*Lactococcus lactis* CSPB														100	60	33	32	53
15	*Corynebacterium glutamicum* CSP															100	38	54	60
16	*Agrobacterium tumefaciens* YSP																100	37	28
17	*Oryza sativa P0582D05*																	100	79
18	*Triticum aestivum* WCSP1																		100

Sequences of CSPs were extracted from publicly available database of cold shock proteins, CDBAse (http://www.chemie.uni-marburg.de/~csdbase/; Weber et al., 2002). Sequences were aligned and sequence identity was calculated using MegAlign module of Lasergene software suit for sequence analysis (version 7.1.0(44)) (DNASTAR Inc., Madison, WI, USA).

4.2.3. Estimated consumption of cold shock proteins

Proteins that are highly similar to MON 87460 CSPB are consumed in a wide variety of foods and are utilized in numerous food processes that have been in use for long periods of time. *Lactobacillus sp*. in dairy foods provide an example to estimate the consumption of CSP proteins. *Lactobacillus sp*. are the most common type of bacteria used in the dairy industry for preparation of products containing live bacterial cultures, including buttermilk, sour cream, cheddar cheese, mozzarella cheese and yogurt (http://www.nationaldairycouncil.org). *Lactobacillus* species contain CSPs that share 40-79% sequence identity with the CSPB in MON 87460 (Table IV-9). These bacteria resist gastric acid, bile salts and pancreatic enzymes, and, thus, readily colonize the intestinal tract (Rolfe, 2000). In the U.S. during 2006, *per capita* daily availabilities of buttermilk, sour cream, cheddar cheese and mozzarella cheese were 2.1 g, 5.2 g, 12.8 g and 12.9 g, respectively (http://www.ers.usda.gov). Also in 2006, *per capita* yogurt availability within the U.S. was 15.3 g per day. In other western countries such as the United Kingdom (UK), *per capita* daily yogurt consumption is similar (19 - 24 g; Henderson and Swan, 2002).

Yogurt is unique among dairy products in that standards exist for the number of colony-forming units (CFUs) that must present at the time of manufacture. Codex standards specify that each gram of yogurt contain a minimum of 10^7 *Lactobacilli* CFUs, a number reflected in a 2003 FDA advance notice of public rulemaking (Codex, 2003; FDA, 2003). Applying this level of *Lactobacilli* CFUs to an estimated *per capita* daily yogurt consumption of 19 g per day equates to approximately 190 million CFU per person per day. Expression of CSPs in *Lactobacillus* varies depending on temperature (Derzelle et al., 2000), making it difficult to estimate an average consumption rate based on bacterial exposure. It is known, however, that levels of bacterial CSPs can increase several fold in response to cold shock (Etchegaray and Inouye, 1999; Phadtare 2004). CSPs can be present in *E. coli*, an organism with a cold shock response that is similar to that observed in *Lactobacillus*, at levels of 10^6 copies per cell (Derzelle et al., 2000; Phadtare, 2004; Sauvageot et al., 2006; Thieringer et al., 1998). Assuming 10^6 copies of a 7 kDa *Lactobacillus* CSP is present in each cell, *per capita* daily CSP consumption through yogurt alone would be approximately 3 µg. Considering the prevalence of other dairy sources of bacterial CSPs such as buttermilk, sour cream and cheese and assuming they contain similar CSP levels as yogurt, it is likely that average consumption rates of just a few varieties of dairy foods a day would provide amounts of bacterial CSPs that far exceed the amount of CSPB a person would consume from MON 87460 even if it were the sole source of corn in the diet.

Within the U.S., the chronic daily adult dietary consumption of CSPB from MON 87460 is 0.033 µg/kg body weight/person, using the same assumptions for CSPB levels as in the acute dietary risk assessment in Section 5. Notably, this consumption level assumes that all corn consumed is MON 87460. In a 60 kg adult, that equates to 2 µg of CSPB consumed daily *per capita* from MON 87460. Given that MON 87460 is a product targeted for geographies that experience frequent drought stress, such as the western U.S. dryland, it is appropriate to assume a smaller portion of the diet will be MON 87460. The western U.S. dryland is approximately 15% of U.S. corn acres. Therefore a more reasonable estimate of CSPB consumption from MON 87460 is 0.3 µg *per capita*, per

day. This consumption level equates to 10% of that represented by yogurt alone. When other dairy-based sources of CSPs are considered, the likely contribution of CSPB from MON 87460 becomes even smaller.

Data on the consumption of natto, a fermented soy food is made with *B. subtilis ssp. natto*, further confirm that CSPB consumption from MON 87460 will represent a small fraction of human CSP consumption levels. Natto is consumed primarily in Japan and other Asian countries. The average daily *per capita* consumption rate of natto in Japan is 6.9 g (MHLW, 2002). To quantify CSPB consumption from natto, the level of CSPB protein was evaluated in 12 sources of natto purchased in the U.S. using an ELISA with a CSPB-specific antibody. All samples tested contained measurable quantities of CSPB protein with an average level of 12.5 ± 4.7 µg/g on a dry weight basis with a range of 5.6 - 19.6 µg/g. The samples had an average 43.4% fresh weight to dry weight conversion. Assuming a 6.9 g per capita daily natto consumption rate, a 43.4% fresh weight to dry weight conversion and a CSPB level of 12.5 µg/g, the average daily consumption of CSPB from natto is approximately 37.4 µg CSPB per person. As stated previously, the chronic daily U.S. dietary consumption of CSPB from MON 87460 is approximately 0.3 µg/person, more than a 100-fold lower level of consumption. Average daily CSPB consumption from natto alone is therefore significantly higher than even conservative estimates of CSPB consumption from MON 87460.

MON 87460 represents a minimal contribution to human CSP consumption and poses no safety risk. Estimates of CSP consumption in a single dairy product (yogurt) and natto show that the consumption rate of CSPB and homologous proteins is significantly higher than what would be consumed in MON 87460, establishing a history of safe use for CSPB. Proteins homologous to CSPB are present in many widely consumed foods and human consumption of CSPB itself is also well documented. Bacterial and plant sources of CSPs and other CSD-containing proteins are common in human diets throughout the world. An estimate of human CSP consumption from yogurt is ten times greater than estimates of CSPB consumption from MON 87460. Calculations of *B. subtilis* CSPB consumption from natto provide a further demonstration that CSPB from MON 87460 represents a fraction of ongoing human exposures. Plant sources such as wheat and rice represent still more dietary sources of CSD-containing proteins.

4.2.4. Similarity of CSPB to known toxins or other biologically active proteins

Bioinformatic analyses of amino acid sequences provide an additional assessment of the potential for protein toxicity. The goal of the bioinformatic analysis is to ensure that the introduced protein does not share homology to known toxins or antinutritional proteins associated with adverse health effects. Results for CSPB further confirm that this protein is not likely to exhibit toxic effects.

Potential structural similarities shared between the CSPB protein and sequences in a protein database were evaluated using the FASTA sequence alignment tool. The FASTA program directly compares amino acid sequences (i.e., primary, linear protein structure) and the alignment data may be used to infer shared higher order structural similarities between two sequences (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire sequence are often homologous.

Homologous proteins usually have common secondary structures, common three-dimensional configuration, and, consequently, may share similar functions.

FASTA bioinformatic alignment searches using the CSPB and NPTII protein sequences were performed with the TOXIN6 database to identify possible homology with proteins that may be harmful to human and animal health. The TOXIN6 database is a subset of 7,176 sequences derived from the protein database (PROTEIN) consisting of publicly available protein sequences from GenBank (GenBank protein database, release 163.0, December 15, 2007). The 7,176 sequence subset was selected using a keyword search and filtered to remove non-toxin proteins. Initially, all sequence descriptions contained in header lines and the associated protein sequence derived from the PROTEIN database were keyword screened using all possible combinations of upper and lower case characters spelling the words "toxic" and "toxin". The resulting 9,082 sequences and their respective descriptions were then filtered to exclude several terms used in combination with "toxic" or "toxin"; these exclusion terms were "synthetic", "anti", "putative", "like", "insect", "Cry", "Thuringiensis" and "toxin-reductase" are used to remove non-toxin protein sequences.

An *E-score* acceptance criterion of $<1x10^{-5}$ for any alignments was used to identify proteins from the TOXIN6 database with potential for significant shared structural similarity and function with CSPB proteins. The results of the search produced no alignments below an *E-score* of 1.0 and therefore, there were no alignments with shared significant similarity with known toxins.

The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the CSPB and any known toxic or other biologically active proteins that would be harmful to human or animal health.

4.2.5. Acute oral toxicity studies with CSPB

Most known protein toxins act through acute mechanisms to exert toxicity (Sjoblad et al., 1992; Pariza and Johnson, 2001, Hammond and Fuchs, 1998). The primary exceptions to this rule consist of certain anti-nutritional proteins such as lectins and protease inhibitors, which typically require a short-term (2-4 week) feeding study to manifest toxicity (Liener, 1994). The amino acid sequence of the CSPB protein produced in MON 87460 is not similar to any of these anti-nutritional proteins or to any other known mammalian protein toxin. Therefore, for the protein safety evaluation an acute administration of a single dose of the protein is considered to be an appropriate test to establish a No Observable Adverse Effect Level (NOAEL) (Pariza and Johnson, 2001). The NOAEL is the tested dose of the protein that causes no adverse effects in test animals and is used to estimate a safe level of exposure for humans to the food containing the introduced protein. The proteins used in these studies were produced by *E. coli* but shown to be physicochemically and functionally equivalent to the CSPB protein produced in MON 87460.

The CSPB protein was administered at a single dose of 4.7 mg/kg to 10 male and 10 female CD-1 mice. Additional groups of 10 male and 10 female mice were administered a comparable dose of bovine serum albumin (BSA) to serve as a protein control. Following dosing, all mice were given detailed clinical observations once daily (twice on day of dosing) for signs of mortality or toxicity. Food consumption was measured on

days 0, 7 and 14. Body weights were measured prior to dosing and on study days 0, 7 and 14. All animals were sacrificed on day 14 and subjected to a gross necropsy. There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the NOAEL for CSPB was considered to be 4.7 mg/kg, the highest dose tested. This dose was four orders of magnitude higher than conservative estimates for human exposure to CSPB from consumption of MON 87460 (see Section 5).

4.3. Assessment of the potential for toxicity of NPTII

NPTII has an established record of safety. Its source organism, *E. coli*, is ubiquitous in the environment and is present in the digestive tracts of vertebrate species. Enzyme preparations derived from *E. coli* have GRAS status (Flamm, 1991). NPTII has GRAS status when used in biotechnology-derived crops (Bradford et al., 2005). Additionally, no structurally relevant similarity exists between the NPTII protein and any known toxic or other biologically active proteins that would be harmful to human or animal health. Finally, an acute toxicity study with adult mice found no adverse effects when NPTII was administered at dose far exceeding those that would be experienced consuming grain produced by MON 87460.

4.3.1. Safety of NPTII donor organism: *E. coli*

The *nptII* gene was isolated from *E. coli* K-12 transposon *Tn5*. *E. coli* bacterium is ubiquitous in the environment and found in the digestive tracts of vertebrate species, including humans (Jefferson et al., 1986). The safety of *E. coli* has been previously assessed by the FDA as part of the safety evaluation of the chymosin enzyme preparation derived from *E. coli* K-12 (Flamm, 1991). As a result of the safety evaluation the donor organism was determined to be safe and the FDA affirmed the chymosin preparation as GRAS. *E. coli* is one of the most studied laboratory organisms with a long history of safe use in the laboratory environment. *E. coli* represents one of the best understood living organisms whose full genome has been sequenced (Blattner et al., 1997). It is classified in all major national and international safety guidelines as a biologically safe organism for the propagation of a broad range of gene cloning and expression vectors and has been used as such for protein production in many commercial applications (Bogosian and Kane, 1991). To date no virulence genes have been found so far in *E. coli* K-12 derivatives in contrast to the pathogenic *E. coli* strains (Mühldorfer and Hacker, 1994).

4.3.2. Similarity of NPTII to proteins with a history of safe use and consumption

The enzyme NPTII is the most commonly used antibiotic resistance marker, which inactivates neomycin and related antibiotics. NPTII is ubiquitous in *E. coli,* and, therefore, is normally present within the human gastrointestinal tract (Jefferson et al., 1986; Fuchs et al., 1993b). NPTII has been used as a selectable marker in a variety of crops including tomatoes, cotton, oilseed rape, and corn, and its safety has been thoroughly evaluated by the FDA (FDA, 1994; 1998; 2003). The FDA considers NPTII to be GRAS for use in biotechnology-derived crops (Bradford et al., 2005) and has also approved its use as a food additive (FDA, 1994). EPA has also established an exemption from the requirement of a tolerance for NPTII for use as a selectable marker in raw agricultural commodities (40 CFR Part 174.521). Numerous studies have suggested that the presence of this antibiotic-resistance gene in any crop or crop products will have no

impact on food safety (reviewed in Miki and McHugh, 2004). Studies using purified NPTII protein revealed that NPTII degrades rapidly in simulated gastric and intestinal fluids suggesting that the protein is unlikely to cause an allergic response (Fuchs et al., 1993a). An assessment of the ecological impact of the use of the NPTII protein in crops has been discussed by Nap et al. (1992). It was suggested that the amount of free kanamycin accumulating in soils, through the action of microorganisms or animal feces, is restricted by absorption to soil components so that no direct selection pressure for kanamycin resistant plants can occur (Nap et al., 1992). Also, enhancement of physiological fitness resulting from pleiotropic effects of *nptII* gene expression has not been documented (Nap et al., 1992). Thus, based on all the available evidence, it can be concluded that the NPTII protein is safe for use as a selectable marker in genetically modified plants (Flavell et al., 1992; Nap et al., 1992; Fuchs et al., 1993a and 1993b; WHO, 1993; FDA, 1994; Huppatz and Fitzgerald, 2000; Miki and McHugh, 2004).

4.3.3. Similarity of NPTII to known toxins or other biologically active proteins

Potential structural similarities shared between the NPTII protein and sequences in a protein database were evaluated using the FASTA sequence alignment tool. This comparison confirmed that no structurally relevant similarity exists between the NPTII protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.

The FASTA program directly compares amino acid sequences (i.e., primary, linear protein structure) and the alignment data may be used to infer shared higher order structural similarities between two sequences (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout the entire sequence are often homologous. Homologous proteins usually have common secondary structures, common three-dimensional configuration, and, consequently, may share similar functions.

FASTA bioinformatic alignment searches using the CSPB and NPTII protein sequences were performed with the TOXIN6 database to identify possible homology with proteins that may be harmful to human and animal health. The TOXIN6 database is a subset of 7,176 sequences derived from the protein database (PROTEIN) consisting of publicly available protein sequences from GenBank (GenBank protein database, release 163.0, December 15, 2007). The 7,176 sequence subset was selected using a keyword search and filtered to remove non-toxin proteins. Initially, all sequence descriptions contained in header lines and the associated protein sequence derived from the PROTEIN database were keyword screened using all possible combinations of upper and lower case characters spelling the words “toxic” and “toxin”. The resulting 9,082 sequences and their respective descriptions were then filtered to exclude several terms used in combination with “toxic” or ”toxin”; these exclusion terms were “synthetic”, “anti”, “putative”, “like”, “insect”, “Cry”, “Thuringiensis” and “toxin-reductase” and are used to remove non-toxin protein sequences.

An *E-score* acceptance criterion of $<1x10^{-5}$ for any alignments was used to identify proteins from the TOXIN6 database with potential for significant shared structural similarity and function with NPTII protein. The results of the search produced no

alignments below an *E-score* of 1.0 and therefore, there were no alignments with shared significant similarity with known toxins.

The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the NPTII protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.

4.3.4. Acute oral toxicity study with NPTII protein

The NPTII protein was administered by gavage to three groups of 10 male and 10 female CD-1 mice (Fuchs et al., 1993a). The total doses administered were 100, 1000, and 5000 mg/kg body weight but because of the limited solubility of the protein in the dosing vehicle (0.1M carbonate buffer), the dosage was subdivided into two separate doses about four hours apart. Additional groups of 10 male and 10 female mice were administered dosing vehicle as a control. Following dosing, all mice were observed twice daily for signs of mortality and moribundity and once daily for signs of toxicity. Body weights were measured prior to randomization and on study day 7. Food consumption was measured daily from study days 1 to 7. A gross necropsy was performed on all animals at the end of the study (day 7). There were no treatment-related effects on survival, clinical observations, body weight gain, food consumption or gross pathology. Therefore, the NOAEL for the NPTII protein was considered to be 5000 mg/kg, the highest dose tested.

4.4. Conclusions

A multipart toxicity assessment of CSPB and NPTII confirmed that these proteins are safe for human and animal consumption. The source organisms are ubiquitous in the environment and have well-established safety profiles. CSPB is homologous to a variety of proteins that are present in food. NPTII is GRAS for use in biotechnology-derived crops. The proteins have histories of consumption, further confirming that both CSPB and NTPII pose no threat to human and animal health. Finally, the proteins lack structural similarity to known toxins or biologically active proteins known to have adverse effects to mammals and did not cause acutely toxic effects in a mammalian assay.

SECTION 5. Dietary Exposure Assessment

Dietary exposure assessments translate protein expression levels into consumption estimates for humans and animals. For humans, these consumption estimates can be expressed as the amount of protein consumed per kg body weight per day and this value may be compared with results from animal feeding studies to establish a reasonable certainty that adverse effects are unlikely. For animals, exposure estimates place consumption values into the context of their total diet as a percentage of total protein consumed. The results of human and animal dietary exposure assessments for CSPB and NPTII expressed by MON 87460 establish that exposures will be extremely low.

5.1. Human dietary exposure assessment

An acute dietary safety assessment was conducted to estimate human dietary exposure to CSPB and NPTII from consumption of food derived from MON 87460. The assessment considers all sources of corn in the U.S. diet, assumes they are comprised entirely of MON 87460, and is based on 95th percentile consumption values in order to provide a conservative, high end estimate.

5.1.1. Human corn consumption

MON 87460 is intended for use in field corn and may also be used in sweet corn and popcorn. Thus all three types of corn were used to estimate potential exposure to CSPB and NPTII proteins from MON 87460. Acute exposure estimates were conducted using the Dietary Exposure Evaluation Model (DEEM-FCID version 2.03, Exponent, Inc.). DEEM-FCID utilizes U.S. food consumption data from the 1994-1996 and 1998 USDA Continuing Surveys of Food Intakes by Individuals (CSFII). Estimated human exposures to CSPB and NPTII from MON 87460 in the U.S. were considered using a conservative scenario of the 95th percentile estimate of acute corn consumption estimated on an "eater-only" basis.

DEEM-FCID separates field corn into six fractions: flour, meal, bran, starch, oil and syrup. However, corn oil and corn syrup were excluded from the assessment because they are essentially devoid of protein and would thus not contain significant amounts of either CSPB or NPTII. Corn starch was included in the assessment but, because of the very low protein content, any contribution from corn starch is expected to be minimal. Field corn is a blended commodity that is used primarily as animal feed and is processed before being consumed by humans. Popcorn and some forms of sweet corn (all except corn-on-the-cob) are also blended commodities. Thus, except for corn-on-the-cob, most MON 87460 grain entering the human food supply would likely be blended with other grain before being processed and/or consumed. However, the exposure calculations herein make the conservative assumption that grain from MON 87460 is not blended with other grain prior to consumption; i.e., for the purposes of this assessment, 100% of the corn-derived food products consumed were assumed to be derived from MON 87460. This is a very conservative assumption because MON 87460 will likely represent only a portion of the total corn grain consumed.

5.1.2. Human intake of the CSPB and NPTII proteins

For the purposes of this assessment, the concentration of the CSPB protein in flour, meal, bran, starch and popcorn was assumed to be equal to the mean expression level in whole

MON 87460 grain grown in the U.S., which was 0.063 µg/gram (ppm) fwt (Table VI-3). For sweet corn, a partially blended commodity, the concentration of the CSPB protein was assumed to be equal to the maximal expression level in MON 87460 grain grown in the U.S., which was 0.089 µg/gram (ppm) fwt (Table VI-3). This accounts for the cases in which MON 87460 sweet corn would not be blended (e.g., consumed on the cob). NPTII protein levels in grain from MON 87460 were below the assay limit of quantification of 0.0047 µg/gram fwt (Table VI-4). However, for the purposes of this assessment, the conservative assumption was made that NPTII protein in all of the above commodities was expressed at a level equal to the limit of quantification. These protein expression estimates are conservative because they assume that there is no loss of the CSPB and NPTII proteins during storage, processing and/or cooking of the grain or food. Based on these assumptions, the 95th percentiles for acute dietary intake of CSPB from MON 87460 are estimated to be 176 x 10^{-6} and 413 x 10^{-6} mg/kg for the general population and children 1-6 years of age, respectively. For NPTII, the 95th percentile estimates for acute dietary intake are 11.0 x 10^{-6} and 24.0 x 10^{-6} mg/kg for the general population and children 1-6 years of age, respectively (Table VI-8).

5.1.3. CSPB and NPTII margins of exposure

Margins of exposure (MOE) place anticipated human exposures in the context of the NOAELs obtained through animal feeding studies. An MOE is the ratio of the lowest NOAEL from an appropriate animal toxicity study to the estimated human exposure. No adverse health effects were observed when mice were administered a total of 4.7 mg/kg of CSPB or 5000 mg/kg of NPTII protein in one day, the highest doses tested. Therefore, based on an apparent absence of hazard associated with exposure to these proteins, a dietary risk assessment for these proteins would normally not be considered necessary. Nevertheless, MOEs were calculated for both proteins in order to provide further assurances of safety. The highest doses from both mouse gavage studies were used as the NOAEL.

Potential health risks from acute dietary intake of these proteins following consumption of food derived from MON 87460 were evaluated by calculating MOEs based on the acute mouse NOAELs for CSPB and NPTII and the 95th percentile "eater-only" estimates of acute dietary exposure from DEEM-FCID. The MOEs for acute dietary intake of CSPB were estimated to be 26,700 and 11,400 for the general population and children 1-6 years of age, respectively. For NPTII, the MOEs for acute dietary intake were estimated to be 454,000,000 and 208,000,000 for the general population and children 1-6 years of age, respectively (Table VI-8). These very large MOEs indicate that there are no meaningful risks to human health from dietary exposure to either the CSPB or NPTII proteins derived from MON 87460.

Table VI-8. Acute (95th Percentile, "eater-only") Dietary Intake and Margins of Exposure for the CSPB and NPTII Proteins from Consumption of MON 87460-derived Food Products in the U.S.[1]

Population	Protein Intake [2] (mg/kg/day x 10^{-6})		Margins of Exposure[3]	
	CSPB	NPTII	CSPB	NPTII
General Population	176	11.0	26,700	454,000,000
Children 1-6 yrs	413	24.0	11,400	208,000,000

[1]Includes sweet corn, popcorn, and field corn (flour, meal, bran and starch). Assumes 100% corn consumed is derived from MON 87460. Estimated using DEEM-FCID version 2.03, Exponent, Inc.

[2]Assumes that all corn flour, meal, bran, starch and popcorn consumed contained 0.063 µg/g FW of CSPB and 0.0047 µg /g FW of NPTII, and that all sweet corn consumed contained 0.089 µg /g FW and 0.0047 µg/g FW of CSPB and NPTII, respectively.

[3]Calculated by dividing NOAELs from acute mouse gavage studies (4.7 and 5000 mg/kg for CSPB and NPTII, respectively) by protein intake. Rounded to three significant figures.

5.2. Animal dietary exposure assessment

Animal feeding represents the largest use of corn in the U.S. In 2007, approximately 47% of harvested grain (or 152 million metric tons) was used as animal feed (USDA-ERS, 2008). In addition, corn silage (as forage) harvested from 5.9 million acres (approx. 7% of total acres planted) was fed to livestock (USDA-ERS, 2008). Corn is the primary grain fed to poultry, pigs, beef cattle, and lactating dairy cattle in the U.S. The dietary safety assessment of MON 87460 as a feed was based on an evaluation of the consumption of grain (broiler chicken and pig) and grain and forage (cow). The intake calculations make the conservative assumption that there is no loss of CSPB and NPTII proteins during the processing of corn grain or forage into animal feed. It also assumes that 100% of the corn grain or forage ending up in animal feed is derived from MON 87460 which could be the case if the farmer raised the corn that was fed to his livestock. However, larger livestock operations purchase commodity corn that is a blend of many different varieties.

5.2.1. Animal corn consumption

The amount of corn-derived feed consumed in the U.S. by pig, broiler chicken and cow was determined through literature references (NRC, 1994, 1998; Ouellet et al., 2003). The daily consumption of corn grain is ~40 g/kg body weight (bw)/day (assuming 60% dietary inclusion rate) for the young pig and ~24.6 g/kg bw/day for the finishing pig (assuming 80% dietary inclusion rate) (NRC, 1998). The four week old broiler consumes ~60 g/kg bw/day of corn grain when the inclusion rate of corn is 65% of the diet (NRC, 1994). The lactating dairy cow producing 33 kg of milk/day consumes about 7.8 g/kg bw/day of corn grain and about 18.8 g/kg bw/day of silage. Silage typically contains about 45% grain and 55% forage on a dry matter basis (Ouellet et al., 2003).

5.2.2. Animal dietary intake of the CSPB and NPTII proteins

For the purpose of this dietary intake calculation, the highest concentrations of the CSPB and NPTII proteins reported for MON 87460 grain and forage on a dry weight basis were used. This provides a conservative high end exposure scenario. The mean and high end range values of the CSPB and NPTII protein levels in grain used in this assessment were from corn hybrids containing MON 87460 grown in the U.S. The mean and high end range values of the CSPB and NPTII protein levels in forage used in this assessment were from data from three pooled sites in Chile (Part VI, Section 2.2).

The mean level of CSPB protein in MON 87460 grain is 0.072 µg/g DW (range 0.045 – 0.10 µg/g dw) and forage is 0.15 µg/g DW (range 0.09 – 0.22 µg/g dw). Corn silage contains about 45% grain and 55% forage on a dry matter basis (http://www.ag.ndsu.edu/pubs/ansci/dairy/as1253w.htm), so corn silage would contain approximately 0.115 µg/g DW of CSPB (0.072 µg/g dw in corn grain × 45% + 0.15 µg/g dw corn in forage × 55%) when using the mean level of CSPB for the grain and forage or 0.166 µg/g dw CSPB (0.10 µg/g dw in corn grain × 45% + 0.22 µg/g DW in forage × 55%) when using the high end of the range.

The mean level of NPTII protein in MON 87460 grain was below the level of quantification (0.0047 µg/g FW) and forage is 0.1 µg/g DW (range 0.1 – 0.2 µg/g DW). For this scenario the level of quantification was used for NPTII concentrations in grain. It was assumed that the corn grain was 85% dry matter so the level of quantification on a dry weight basis is 0.0055 µg/g DW (0.0047 µg/g FW/0.85). Corn silage contains about 45% grain and 55% forage on a dry matter basis (http://www.ag.ndsu.edu/pubs/ansci/dairy/as1253w.htm), so corn silage would contain approximately 0.057 µg/g dw of NPTII (0.0055 µg/g dw in corn grain × 45% + 0.1 µg/g dw corn in forage × 55%) when using the mean level of NPTII for the grain and forage or 0.115 µg NPTII /g DW (0.0055 µg/g dw in corn grain × 45% + 0.2 µg/g dw in forage × 55%) when using the high end of the range.

The estimated mean and maximum daily intake of the CSPB and NPTII proteins by poultry and livestock are shown in Table VI-9.

The broiler chicken, young pig, finishing pig, and lactating dairy would typically consume 18 g dietary protein/kg bw (NRC1994), 14 g dietary protein/kg bw (NRC 1998), 4 g dietary protein/kg bw (NRC 1998), and 6 g dietary protein/kg bw (NRC 2001), respectively. The highest percentage of CSPB protein (g/kg bw) per total protein consumed was in the dairy cow, 0.000067% of the total dietary protein intake (0.000004 g CSPB/kg bw divided by 6 g dietary protein which is the total dietary protein intake for the cow). The highest percentage of NPTII protein (g/kg bw) per total protein consumed was in the dairy cow, 0.000043% of the total dietary protein intake (0.0000026 g NPTII/kg bw divided by 6 g dietary intake which is the total dietary protein intake for the cow). The chicken and pig percentages of the CSPB and NPTII proteins consumed as part of the daily protein intake is much less than for the dairy cow.

Under the most conservative consumption scenarios, poultry, swine and lactating dairy cattle would consume less than 1 ppm of their total protein intake as CSPB and NPTII proteins from MON 87460.

Table VI-9. Mean and Maximum Daily Intakes of the CSPB and NPTII Proteins in Poultry and Livestock

Species	Total Corn Consumption (g /kg of body weight/day DW)	Trait Protein Intake (g/kg of body weight/day DW)			
		CSPB		NPTII	
		Mean	Highest Level	Mean	Highest Level
Chicken broiler[1]	60	0.0000043	0.0000060	0.0000003	0.0000003
Young pig[1]	40	0.0000029	0.0000040	0.0000002	0.0000002
Finishing pig[1]	24.6	0.0000018	0.0000025	0.0000001	0.0000001
Lactating dairy cow[2]	26.6	0.0000028	0.0000040	0.0000012	0.0000026

[1] Corn grain consumed × concentration of CSPB or NPTII protein in the grain

[2] Corn grain consumed × concentration of CSPB or NPTII protein in the grain + corn silage consumed × concentration of CSPB or NPTII protein in the corn silage

5.3. Conclusions

Human and animal dietary exposure assessments provide further confirmation that consuming food and feed derived from MON 87460 poses no meaningful health risks. Using upper 95th percentile consumption values, human MOEs for CSPB are 11.400 for children and 26,700 for adults. Under those same consumption scenarios, human MOEs for NPTII are 208,000,000 for children and 454,000,000 for adults. Animal exposures will also be low with chickens, swine and dairy cows consuming only nanogram quantities of each protein per kilogram of body weight. The human and animal dietary exposure assessments further support a conclusion that food and feed derived from MON 87460 are as safe as those derived from conventional corn.

SECTION 6. Other Data and Information about the Proteins Introduced into MON 87460

6.1. Heat stability

During some types of corn processing, the grain may be heated. CSPB from MON 87460 was evaluated under conditions that simulate this heating to understand the impacts of heat on immunodetectability. Depending on the type of processing, corn grain can be heated between 100 °C and 316 °C, and the treatment can last from a few seconds to a few hours (Rooney and Serna-Saldivar, 1994). To evaluate how heat treatment affects the immunodetectability of the CSPB protein, a temperature of 204°C and time interval of 15 min were chosen for the study as a representative heat treatment utilized during corn processing (Rooney and Serna-Saldivar, 1994). Grain of MON 87460 contains low levels of CSPB (Table VI-3 and VI-5) and it is not possible to detect the protein in the grain extract using western blot analysis. Therefore, the immunodetectability of the CSPB protein in corn grain extracts was evaluated using a validated ELISA assay following the extraction of the grain with a tris-borate buffer (TB).

The study demonstrated that the amount of the immunodetectable CSPB protein present in TB buffer extract of MON 87460 after heating was below the lower limit of quantitation (LOQ), or had decreased by at least 91% relative to the original value. Heating ground corn grain, in a manner similar to conditions employed for commercial corn processing, results in the loss of immunodetectable CSPB protein.

The level of the CSPB protein detected in the grain of MON 87460 prior to the heat treatment was 52.7 ± 1.9 ppb. After the heat treatment, the amount of immunodetectable CSPB protein had decreased below the limit of quantitation (LOQ) of the assay (5 ppb) (Table VI-10). The reduction in immunodetectable CSPB protein was conservatively calculated based on the value for LOQ:

$$\text{Minimum reduction in detected CSPB (\%)} = \left|\frac{(\text{Unheated CSPB (ppb)}) - (\text{LOQ (ppb)})}{(\text{Unheated CSPB (ppb)})}\right| \times 100\%$$

$$\text{Minimum reduction in detected CSPB (\%)} = \left|\frac{(52.7 \text{ ppb}) - (5 \text{ ppb})}{(52.7 \text{ ppb})}\right| \times 100\% = 91\%$$

Therefore, considering the LOQ, there was a 91% reduction in the quantity of immunodetectable CSPB when the grain was heated. As expected, the CSPB protein was not detected in the control substance, either before or after heating.

The results of this study demonstrate that the immunodetectable level of the CSPB protein in grain of MON 87460 was significantly impacted by heat treatment, falling below the LOQ of the ELISA used for quantitation. This implies that heating under conditions which mimic commercial processing of corn, result in a dramatic decrease in the amount of immunodetectable CSPB protein. This decrease is likely caused by protein degradation but may also be the result of decreased solubility or the loss of the epitopes recognizable by antibodies.

Table VI-10. Summary of the CSPB Protein Detected in Extracts of Heated and Unheated MON 87460 and Conventional Control

Sample	Treatment	Water loss (%)[1]	CSPB in grain (ppb)[3]	Standard Deviation[6]
Control corn	Unheated	N/A[2]	<LOD[4]	N/A
	Heated	4.0	<LOD	N/A
MON 87460	Unheated	N/A	52.7	1.9
	Heated	7.3	<LOQ[5]	0.1

[1] Water loss (%) = (unheated weight–heated weight)/unheated weight x 100%.
[2] N/A: not applicable
[3] Value refers mean calculated based on n=3.
[4] LOD: the limit of detection
[5] LOQ: the lower limit of quantitation (5 ppb).
[6] Standard deviation was calculated using Microsoft Excel 2002 (10.6834.6830) SP3

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Part VII. FOOD/FEED SAFETY AND NUTRITIONAL ASSESSMENT OF MON 87460

SECTION 1. Corn as the Comparable Food and Feed

Corn is widely used for a variety of food and feed purposes, and it is intended that MON 87460 will be utilized in the same manner and for the same uses as conventional corn. Corn grain and its processed products are consumed in a multitude of human food and animal feed products. Corn forage (as silage) is extensively consumed as an animal feed by ruminants.

SECTION 2. Historical Uses of Corn

Corn's versatility as a source of food, feed, and fuel stems from its starch content and ease of processing. Corn is processed into valuable food and industrial products, such as corn meal by dry milling, refined starch by wet milling, and ethanol by fermentation. Corn use globally is dominated by the use of field corn varieties/hybrids for animal feed although ethanol production from corn has been increasing for the past several years (Brookes, 2001; NCGA, 2008; Perry, 1988; Watson, 1988). In recent years the global demand for corn has increased, leading to higher prices for raw grain and its derivatives (NCGA, 2008).

Food uses include sweet corn, popcorn, and processed field corn, which are all varieties/hybrids of *Zea mays* subsp. *mays*. Of the corn used for food and industrial uses, the majority is processed by wet milling to produce starch and sweetener products (e.g., high fructose corn syrup) for use in foodstuffs. Non-food products such as industrial starches, corn gluten feed and corn gluten meal are also manufactured through the wet mill process (May, 1987; Watson, 1988). The primary products derived from the dry milling process are corn grits, corn meal, corn flour, and ethanol.

Because of its high starch content, corn is used as a valuable energy source in animal feed for domestic livestock, such as cattle, pigs and poultry. This starch content is also amenable to fermentation, providing ethanol for use as fuel. Whole corn is usually ground and mixed with a high-protein feed compound and with vitamin and mineral supplements to balance the ration according to the nutritional requirements of the animals being fed (Leath and Hill, 1987). Corn is also used for processing and the production of derivatives, which have a wide range of food, feed and industrial applications. Some of the processed fractions are used for animal feed, such as corn gluten, a resource that is rich in corn protein. Corn is also used for the production of feed additives.

OECD-FAO's joint 2008-2017 Agricultural Outlook forecasted that corn prices will remain 40 – 60% higher in the next decade than they have been for the last decade. The report also concluded that increased yields on existing agricultural land will be more important to improving commodity supplies than bringing new land into cultivation. In developing countries, economic growth, changing diets, and growing populations are driving added demand. In developed countries, fuel uses are the largest source of new demand. These factors along with diminished stocks and climate change will lead to variability in agricultural product supply and possibly result in price spikes (OECD/FAO, 2008).

2.1. Corn as a food source

Corn is the leading cereal in the U.S. Most of the human consumption is in the form of corn-based ingredients produced by the wet mill process including high fructose corn syrup, starch, sweetners, cereals, oil and alcohol. Other food-based ingredients are derived from the dry mill process, and include, corn meal, flour, grits and oil. Corn grain is also used for the production of tortillas and other ethnic Mexican prepared foods by the alkaline cooking process. Fresh-cooked corn provides macronutrients, vitamins and minerals in the human diet. Corn is a significant source of the nutritionally essential sulfur-containing amino acids, methionine and cystine. Corn contains colored pigments called carotenoids, which are primarily responsible for the yellow color of corn grain. The carotenes are precursors for the production of vitamin A and also function as antioxidants. Corn grain is a significant source of Vitamin E (tocopherol), which also serves as an antioxidant (White and Weber, 2003).

Corn is an excellent raw material for the manufacture of starch, not only because of price and availability, but also because the starch is easily recovered in high yield and purity. Approximately 6.5 billion pounds of starch were produced in the U.S. and sold into food and industrial markets in 2004 (CRA, 2007). Starch can be converted to a variety of sweetener and fermentation products including high fructose corn syrup and ethanol. Starch is used as a food ingredient in: dairy and ice cream; batters and breading; baked goods; soups, sauces and gravies; salad dressings; meat, poultry, and fish analogues; confections; and, in drinks. Corn oil, commercially processed from the germ, is another important food ingredient derived from corn grain. In 2004, approximately 1.2 billion pounds of corn oil were produced in the U.S. (CRA, 2007).

2.2. Corn as a feed source

Animal feeding represents the largest use of corn in the U.S. In 2007, approximately 46% of harvested grain was used as animal feed (NCGA, 2008). In addition, corn silage (as forage) harvested from 5.9 million acres (approx. 7% of total acres planted) was fed to livestock (USDA-NASS, 2008). Corn gluten meal, corn gluten feed, and distillers dried grains, derived as co-products by wet and dry milling, are also important components of livestock feed.

The corn kernel contains about 83% carbohydrate in the form of starch, pentosans, dextrins, sugars, cellulose, and hemicellulose. Starch is the biggest component in the carbohydrate fraction and provides most of the energy. The fiber portion includes cellulose and hemicellulose, which are generally available to ruminants but not to nonruminants. Corn grain contains approximately 4% (w/w) oil (White and Weber, 2003), which has a high content of 18:2 linoleic acid, one of the essential polyunsaturated fatty acids needed by swine and poultry. Although corn grain has a relatively low protein content (10% DW; http://www.cropcomposition.org) compared to other cereal grains, it is a major source of essential amino acids due to the high percentage incorporated in animal diets. Corn grain is a good source of methionine, but a poor source for lysine and tryptophan. Methionine and lysine are the two most limiting amino acids for poultry, swine and other livestock fed corn-based diets (NRC, 1994, 1998, and 2001).

Calcium and phosphorus are important minerals in animal nutrition. Corn grain has low levels of calcium, and thus, is not a big contributor to calcium in the animal diet. On the

other hand, corn grain is a source of phosphorus in the animal diet (Ensminger et al., 1990). Nutritionists incorporate supplemental sources of calcium, phosphorus, sodium, magnesium, iron, zinc, copper, manganese, iodine, and selenium as needed to balance animal diets. Corn grain is a source of a number of vitamins in animal feed, which include: vitamins A, B1 (thiamin), B2 (riboflavin), B6 (pyridioxine), C (ascorbic acid), E, folate, niacin and pantothenic acid. While the content of niacin in corn grain is relatively high, it exists in a bound form (niacytin) that is not biologically available to monogastric animals. Nutritionists supplement animal diets with vitamins, since their levels in corn grain are insufficient to meet dietary needs.

Corn silage is a major forage ingredient for feedlot and dairy cattle due its importance as a palatable energy source (Newcomb, 1995). Corn gluten feed and meal are byproducts of the wet milling process and are incorporated into animal diets. Gluten meal contains high levels of protein (~60%) and is an important source of carotenoids. It is commonly used in feed for cattle, fish, poultry, pets, and other animals but primarily in poultry diets. Corn gluten feed (wet or dry) is an excellent feed that is a significant source of protein (~20%), low in starch, high in digestible fiber, and low in oil and is used mainly in dairy and beef cattle diets. In addition, with the increasing use of U.S. corn in dry mill plants to produce ethanol, the distillers dried grains co-product will be in greater supply and is expected to replace small amounts of corn grain in livestock and poultry diets.

SECTION 3. Comparison of the Composition and Characteristics of MON 87460 to Conventional Corn

Compositional comparisons between biotechnology-derived crops and conventional varieties represent an integral part of a nutritional and safety assessment. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus documents for corn composition (OECD, 2002; 2006). These principles are accepted globally and have been employed previously in assessments of corn products derived through biotechnology.

Compositional equivalence between biotechnology-derived crops and conventional varieties provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). The OECD consensus documents emphasize quantitative measurements of essential nutrients, and known anti-nutrients and toxicants. This is predicated on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and anti-nutritional concerns. Crop components to be analyzed in comparative assessments include proximates (moisture, fat, protein, ash), carbohydrates by calculation, fiber, amino acids, fatty acids, vitamins and minerals. Anti-nutrients to be assessed include phytic acid, trypsin inhibitor, and raffinose. The secondary plant metabolites, *p*-coumaric acid, ferulic acid, and furfural, are measured as indicators of the effect of trait modification on metabolism. The components analyzed represent over 90% of the non-starch biomass of corn grain. Proximates, fiber, and selected minerals are assessed in corn forage.

Levels of the components in grain and forage of the biotechnology-derived crop are compared to i) corresponding levels in a non-modified comparator, typically the near isogenic parental line grown under identical conditions, and ii) natural ranges generated from an in-study evaluation of commercial varieties or from data published in the scientific literature.

These guidelines framed the strategy for the compositional assessment of MON 87460. The compositional assessment was conducted on samples harvested from two different growing seasons, the first in 2006 in the U.S. and the second during 2006/2007 in Chile. Production in the first season was conducted under normal agronomic practices at all sites. A strip-plot design adopted in the second season allowed production under two separate irrigation regimens at all sites. The irrigation regimens included a well-watered and a water-limited treatment. Differential water treatments provide an assessment of whether compositional equivalence is maintained between MON 87460 and the conventional control under conditions where MON 87460 is likely to be cultivated. Appendix J presents data for individual sites. Finally, Section 4 presents data on a targeted list of secondary metabolites that are associated with plant stress responses. Data from these comprehensive compositional analyses confirm that MON 87460 is a safe and wholesome product that is compositionally equivalent to conventional corn.

3.1. Assessment of significant nutrients, antinutrients, and key secondary metabolites in corn forage and grain – U.S. 2006

In 2006, forage and grain tissues of MON 87460 and control corn were harvested from plants grown under commercially acceptable agronomic practices at each of six field sites in the U.S. within corn production regions. Two sites were in Iowa (IAE, IAW), one each was in Illinois (IL), Indiana (IN), Kansas (KS) and Nebraska (NE). Each site received water as is typical of the growing area. Four sites were rainfed (IAE, IAW, IL, IN) and two (KS, NE) received supplemental irrigation. These conditions provide a comparison of MON 87460 and the control under conditions common to corn production. Table VII-1 presents temperature data and applied water from the production period.

Three different conventional commercial corn hybrids were also grown at each of the six sites. This allowed harvest of forage and grain from a total of 18 commercial references to provide information on natural variation in the levels of analyzed nutrients and anti-nutrients. Compositional analysis included the significant nutrients, antinutrients, and key secondary metabolites, consistent with OECD guidelines. Appendix I presents compositional analysis methods.

Starting seed was planted in a randomized complete block design with three replicates per block. Tissue was collected from MON 87460 and the control from all three blocks; tissue from the three different commercial references grown at each site was collected from a single block. Forage was collected at the early dent (R5) plant growth stage, and grain was collected at physiological maturity.

The compositional data set was examined for evidence of statistically significant differences between MON 87460 and the control. Seven sets of statistical analyses were made, six based on the data from each of the replicated field sites and the seventh based on data from a combination of all six field sites. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods.

Commercial references were included to provide data for the development of a 99% tolerance interval for each component analyzed. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial corn. The tolerance interval illustrates the compositional variability that currently occurs in corn grown commercially. It allows statistically significant differences between MON 87460 and the

control to be placed in biological perspective. This comparative evaluation also considers natural ranges in corn component levels published in the literature or in the International Life Sciences Institute (ILSI) Crop Composition Database (http://www.cropcomposition.org).

Forage and grain samples were harvested from all plots and analyzed for nutritional and anti-nutrient components. Compositional analyses of the forage samples included measurement of proximates (moisture, fat, protein, ash), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. Compositional analyses of the grain samples included measurement of proximates (moisture, fat, protein, ash), carbohydrates by calculation, ADF, NDF, total dietary fiber (TDF), total amino acid composition, fatty acid composition (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (vitamin B1 [thiamine], vitamin B2 [riboflavin], vitamin B6 [pyridoxine], vitamin E, niacin, folic acid), furfural, raffinose, phytic acid, *p*-coumaric acid, and ferulic acid. Methods for analysis were based on internationally-recognized procedures and literature publications.

In total, 77 different analytical components were measured (9 in forage, 68 in grain). Of these evaluated components, 15 had more than 50% of the observations below the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from statistical analysis. These included 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, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural. These components are known to be present at low levels in corn grain (OECD, 2002). Therefore, 62 components (9 in forage and 53 in grain) were statistically assessed using a mixed model analysis of variance method.

The compositional data set was examined for evidence of statistically significant differences between MON 87460 and the control. Statistical evaluation of the composition data involved a comparison of the forage and grain from the test substance to those of the control. There were a total of 434 comparisons made (seven sets of comparisons × 53 components from grain and seven sets of comparisons × nine components from forage).

Mean values, ranges, and statistical analyses for the combined-site data are presented in Table VII-2 for forage and Tables VII-3 to VII-8 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table VII-9. Literature and ILSI Crop Composition Database ranges for corn components are provided in Table VII-27.

The statistical analysis showed that, for 407 (93.7%) of the 434 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). Of the 27 statistically significant differences (three from the combined-site analysis and 24 from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

3.1.1. Levels of nutrients in corn forage and grain

Corn forage and grain contain a variety of key nutrients that provide much of this crop's value as a food and feed. The OECD consensus document on compositional considerations

for corn describes the nutrients present in corn grain or processed corn products and includes proximates, fiber, minerals, total amino acids, fatty acids (FA) and vitamins (OECD, 2002). A comprehensive comparison of MON 87460 and the control confirms that the two materials are compositionally equivalent with respect to nutrients.

In the combined-site analysis of forage, no significant differences were found between MON 87460 and the control. In the combined-site analysis of grain, 50 of the 53 comparisons were not significantly different ($p>0.05$). The three differences were detected in the values for ash, 18:0 stearic acid and 20:1 eicosenoic acid. However, values for 18:0 stearic acid were not significantly different ($p>0.05$) at any of the individual sites. The magnitude of the combined-site difference in the 18:0 stearic acid value was small (0.069% of total FA) and the mean component values for the test and control substances were within the 99% tolerance interval established from the commercial reference varieties grown at the same sites. Only one out of the six individual site comparisons for ash and 20:1 eicosenoic acid values showed a significant difference ($p<0.05$). The magnitude of the differences in ash (0.082 % DW) and 20:1 eicosenoic acid (0.0078% of total FA) was extremely small, and the mean values for these two components were within the 99% tolerance interval established from the commercial references grown at the same sites. Furthermore, this lack of reproducibility across multiple sites established that the differences observed in the combined-site analysis in values for these two components were not biologically significant. These findings confirmed that these minor differences reflected the natural variability of conventional corn.

For forage, 51 of the 54 individual site comparisons were not significantly different ($p>0.05$). The three differences were in the values for carbohydrates by calculation, moisture, and protein, with each component difference being observed at only a single site. This lack of reproducibility across multiple sites indicated that there were no meaningful trends in differences in the values for these three components and that this limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for moisture (two sites), and cystine, histidine, lysine, methionine, valine, 18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, 22:0 behenic acid, ADF, TDF, thiamine, folic acid, and riboflavin (each at a single site only). The lack of reproducibility in differences across multiple sites and the fact that the mean values for these components at these sites were within the 99% tolerance interval established from the commercial references confirmed that the limited number of site differences in values for these components were of no biological significance.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. The limited number of differences observed in this study reflected the natural variation of conventional corn and supported the compositional equivalence of MON 87460 to conventional corn.

3.1.2. Assessment of levels of key anti-nutrients and secondary metabolites in corn forage and grain

The OECD consensus document on compositional considerations for corn describes the anti-nutrients and secondary metabolites present in corn grain or processed corn products (OECD, 2002). The anti-nutrients assessed included phytic acid and raffinose. Phytic acid is widely

distributed in plants and can limit the uptake of minerals such as calcium in higher animals (Lott et al., 2000; Novak and Haslberger, 2000). Raffinose is a nondigestible oligosaccharide that is considered to be an antinutrient due to gas production and the resulting flatulence caused by its consumption (Voragen, 1998). The secondary metabolites included ferulic acid, *p*-coumaric acid, and furfural. Ferulic acid and *p*-coumaric acid are derived from the aromatic amino acids, phenylalanine and tyrosine (Douglas, 1999), and serve as precursors for a large group of phenylpropanoid compounds. The non-starch polysaccharide pentosans are a major source of furfural (Adams et al., 1997).

No combined-site differences ($p>0.05$) between values for grain anti-nutrient components and secondary metabolites in MON 87460 and the control were recorded. Individual site differences ($p<0.05$) were observed in values for raffinose, phytic acid, and ferulic acid. For each component, these differences were observed at a single site only. As only one out of six individual site comparisons was recorded for each of these components, these differences represented no meaningful trend and were considered to be of no biological significance. The limited number of differences recorded in this study reflected the natural variation of conventional corn.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the control. Thus, a comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 to conventional corn.

3.1.3. Conclusions for U.S. 2006

Statistical analysis of the compositional data showed that there were no significant differences ($p>0.05$) for 407 (93.7%) of the 434 comparisons made between the mean component values of MON 87460 and the control. Of the 27 (three from the combined-site analysis and 24 from the individual site analyses) statistically significant differences, all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

Furthermore, the limited number of component differences was characterized by extremely small differences in magnitude, and a lack of reproducibility across the six individual sites. These factors established that the limited number of differences observed in this study reflected no meaningful trends and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460, and consequently the foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn with a history of safe consumption.

Table VII-1. Monthly Temperature and Monthly Accumulated Water Data for the 2006 U.S. Field Production

Site[1]	Measurement	May	June	July	August	September	October
IAE	Accumulated water (in.)	2.5	2.3	2.3	6.2	2.4	2.1
	Avg Max temp (°F)	72	81	86	86	76	59
	Avg Min temp (°F)	51	60	65	62	49	37
	Range[2] (°F)	30 - 98	45 - 97	54 - 95	55 - 96	32 - 94	19 - 94
IAW	Accumulated water (in.)	1.8	0.4	2.8	4.9	8.7	1.5
	Avg Max temp (°F)	72	84	88	83	71	60
	Avg Min temp (°F)	50	59	63	66	48	38
	Range[2] (°F)	34 - 91	49 - 93	52 - 99	58 - 93	34 - 86	23 - 90
IL	Accumulated water (in.)	2.1	1.5	3.7	3.1	2.8	2.7
	Avg Max temp (°F)	72	81	88	82	75	60
	Avg Min temp (°F)	51	57	64	62	48	36
	Range[2] (°F)	36 - 94	46 - 92	46 - 97	51 - 96	34 - 88	19 - 92
IN	Accumulated water (in.)	4.8	5.5	3.4	5.6	2.4	5.9
	Avg Max temp (°F)	71	80	86	83	75	63
	Avg Min temp (°F)	51	58	65	63	53	41
	Range[2] (°F)	40 - 92	52 - 89	50 - 96	55 - 93	37 - 87	25 - 90
KS	Accumulated water (in.)	5.3	8.7	8	10.9	2	1.9
	Avg Max temp (°F)	81	92	96	92	80	68
	Avg Min temp (°F)	53	62	66	67	51	42
	Range[2] (°F)	37 - 100	52 - 102	54 - 109	52 - 107	36 - 92	23 - 96
NE	Accumulated water (in.)	1.9	3.8	5.6	9.5	4.8	0.8
	Avg Max temp (°F)	79	88	91	86	76	63
	Avg Min temp (°F)	51	61	65	63	50	37
	Range[2] (°F)	37 - 96	52 - 101	55 - 103	52 - 100	36 - 93	18 - 96

[1] Site codes are as follows: IAE = Benton County, IA; IAW = Greene County, IA; IL = Stark County, IL; IN = Parke County, IN; KS = Pawnee County, KS; NE = York County, NE.

[2] The range is the absolute maximum and minimum temperature in each month.

Table VII-2. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	24.10 (0.96) [17.78 - 34.43]	24.64 (0.96) [19.11 - 29.21]	-0.54 (0.92) [-6.50 - 8.60]	-2.49, 1.41	0.567	(19.44 - 30.49) [13.04, 35.77]
Neutral Detergent Fiber (% DW)	38.69 (1.99) [31.10 - 49.44]	38.75 (1.99) [27.73 - 48.35]	-0.056 (1.08) [-11.60 - 6.51]	-2.33, 2.22	0.959	(32.12 - 49.62) [24.23, 56.48]
Mineral						
Calcium (% DW)	0.21 (0.018) [0.14 - 0.30]	0.22 (0.018) [0.13 - 0.33]	-0.018 (0.012) [-0.075 - 0.089]	-0.048, 0.013	0.189	(0.12 - 0.25) [0.044, 0.35]
Phosphorus (% DW)	0.18 (0.0089) [0.14 - 0.22]	0.19 (0.0089) [0.14 - 0.23]	-0.0095 (0.0055) [-0.069 - 0.022]	-0.021, 0.0021	0.101	(0.090 - 0.26) [0.074, 0.32]
Proximate						
Ash (% DW)	3.76 (0.35) [2.17 - 5.34]	4.21 (0.35) [2.94 - 8.01]	-0.44 (0.37) [-3.73 - 1.22]	-1.39, 0.50	0.281	(2.67 - 4.43) [1.52, 5.75]
Carbohydrates (% DW)	86.45 (0.54) [83.78 - 88.75]	85.77 (0.54) [81.88 - 89.26]	0.68 (0.49) [-2.08 - 2.89]	-0.57, 1.93	0.220	(84.97 - 88.89) [82.09, 90.80]
Moisture (% FW)	70.94 (1.25) [64.70 - 77.90]	71.46 (1.25) [66.50 - 75.70]	-0.52 (0.37) [-2.50 - 2.40]	-1.30, 0.25	0.174	(64.20 - 75.50) [59.32, 81.14]
Protein (% DW)	7.56 (0.25) [6.65 - 8.57]	7.85 (0.25) [6.45 - 10.24]	-0.30 (0.20) [-2.63 - 1.14]	-0.71, 0.12	0.146	(5.80 - 8.63) [4.92, 10.30]
Total Fat (% DW)	2.23 (0.20) [1.07 - 3.24]	2.17 (0.20) [1.28 - 2.88]	0.059 (0.13) [-0.88 - 0.90]	-0.22, 0.34	0.659	(1.60 - 3.62) [0, 4.67]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-3. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E. [Range]	Control Mean ± S.E.[1] [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Ash (% DW)	1.54 (0.039) [1.33 - 1.83]	1.46 (0.039) [1.32 - 1.79]	0.082 (0.038) [-0.22 - 0.38]	0.0033, 0.16	0.041	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	84.22 (0.56) [81.40 - 87.04]	84.10 (0.56) [81.31 - 86.05]	0.13 (0.20) [-1.57 - 1.98]	-0.30, 0.56	0.539	(82.11 - 87.06) [80.32, 89.92]
Moisture (% FW)	9.94 (0.18) [9.12 - 11.00]	10.09 (0.18) [9.17 - 11.20]	-0.15 (0.15) [-1.36 - 0.83]	-0.54, 0.25	0.377	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	10.50 (0.54) [8.19 - 13.21]	10.74 (0.54) [8.77 - 13.33]	-0.24 (0.18) [-2.05 - 1.35]	-0.61, 0.13	0.195	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.74 (0.051) [3.44 - 4.06]	3.71 (0.051) [3.57 - 3.96]	0.029 (0.067) [-0.52 - 0.32]	-0.14, 0.20	0.678	(2.95 - 4.40) [2.08, 5.12]
Fiber						
Acid Detergent Fiber (% DW)	3.03 (0.25) [1.57 - 4.94]	3.02 (0.25) [1.94 - 4.08]	0.0095 (0.36) [-2.51 - 3.00]	-0.79, 0.81	0.979	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	8.97 (0.32) [6.45 - 11.63]	8.95 (0.32) [7.82 - 12.22]	0.019 (0.46) [-4.07 - 3.32]	-1.00, 1.03	0.967	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	12.59 (0.34) [10.42 - 14.57]	12.15 (0.34) [10.76 - 14.87]	0.44 (0.35) [-3.32 - 3.67]	-0.28, 1.16	0.216	(10.65 - 16.26) [8.11, 17.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-4. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Calcium (% DW)	0.0054 (0.00019) [0.0047 - 0.0061]	0.0054 (0.00019) [0.0048 - 0.0063]	-0.00006 (0.00007) [-0.00059 - 0.00056]	-0.00020, 0.00009	0.431	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	1.89 (0.14) [1.47 - 4.61]	1.86 (0.14) [1.54 - 3.43]	0.022 (0.16) [-1.31 - 2.11]	-0.32, 0.37	0.892	(1.14 - 2.56) [0.39, 3.21]
Iron (mg/kg DW)	18.24 (0.62) [15.02 - 24.88]	18.30 (0.62) [14.17 - 20.58]	-0.067 (0.50) [-2.34 - 7.02]	-1.34, 1.21	0.898	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.11 (0.0042) [0.095 - 0.13]	0.12 (0.0042) [0.095 - 0.13]	-0.0013 (0.0016) [-0.010 - 0.013]	-0.0047, 0.0020	0.418	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	6.79 (0.43) [5.02 - 8.64]	6.89 (0.43) [5.50 - 8.34]	-0.097 (0.12) [-0.97 - 0.68]	-0.41, 0.22	0.462	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.31 (0.011) [0.27 - 0.35]	0.32 (0.011) [0.27 - 0.37]	-0.0085 (0.0047) [-0.030 - 0.034]	-0.018, 0.0014	0.089	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.38 (0.0030) [0.36 - 0.39]	0.38 (0.0030) [0.35 - 0.39]	0.0019 (0.0037) [-0.025 - 0.038]	-0.0060, 0.0097	0.624	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	20.86 (0.95) [18.24 - 24.75]	21.24 (0.95) [17.41 - 25.20]	-0.38 (0.32) [-3.02 - 1.85]	-1.03, 0.27	0.238	(16.78 - 28.17) [11.61, 32.63]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-5. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Alanine (% DW)	0.80 (0.047) [0.60 - 1.04]	0.82 (0.047) [0.64 - 1.04]	-0.011 (0.013) [-0.10 - 0.10]	-0.037, 0.016	0.410	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.45 (0.019) [0.33 - 0.54]	0.44 (0.019) [0.38 - 0.52]	0.0067 (0.011) [-0.071 - 0.087]	-0.022, 0.035	0.577	(0.34 - 0.51) [0.24, 0.60]
Aspartic acid (% DW)	0.65 (0.028) [0.52 - 0.79]	0.66 (0.028) [0.54 - 0.78]	-0.0062 (0.0075) [-0.065 - 0.060]	-0.022, 0.0096	0.419	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.23 (0.0085) [0.19 - 0.27]	0.23 (0.0085) [0.20 - 0.26]	-0.0040 (0.0018) [-0.016 - 0.012]	-0.0087, 0.00069	0.079	(0.19 - 0.24) [0.15, 0.27]
Glutamic acid (% DW)	2.07 (0.12) [1.52 - 2.66]	2.09 (0.12) [1.64 - 2.67]	-0.025 (0.034) [-0.26 - 0.28]	-0.097, 0.046	0.462	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.39 (0.013) [0.33 - 0.45]	0.39 (0.013) [0.34 - 0.43]	0.0019 (0.0041) [-0.024 - 0.035]	-0.0085, 0.012	0.656	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.32 (0.012) [0.25 - 0.38]	0.32 (0.012) [0.27 - 0.37]	-0.00085 (0.0049) [-0.029 - 0.040]	-0.013, 0.012	0.868	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.38 (0.021) [0.28 - 0.50]	0.38 (0.021) [0.31 - 0.48]	-0.0022 (0.0088) [-0.042 - 0.070]	-0.025, 0.020	0.810	(0.30 - 0.41) [0.22, 0.49]

Table VII-5 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Leucine (% DW)	1.41 (0.088) [1.01 - 1.85]	1.43 (0.088) [1.11 - 1.87]	-0.020 (0.026) [-0.20 - 0.22]	-0.075, 0.035	0.453	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.30 (0.0076) [0.26 - 0.34]	0.30 (0.0076) [0.26 - 0.33]	0.0024 (0.0040) [-0.023 - 0.027]	-0.0080, 0.013	0.579	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.22 (0.013) [0.16 - 0.28]	0.22 (0.013) [0.17 - 0.26]	-0.00089 (0.0030) [-0.019 - 0.019]	-0.0086, 0.0068	0.777	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.56 (0.031) [0.41 - 0.72]	0.56 (0.031) [0.45 - 0.72]	-0.0059 (0.0090) [-0.067 - 0.074]	-0.025, 0.013	0.518	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	1.01 (0.047) [0.78 - 1.23]	1.02 (0.047) [0.83 - 1.21]	-0.0048 (0.017) [-0.082 - 0.17]	-0.050, 0.040	0.793	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.53 (0.028) [0.40 - 0.64]	0.53 (0.028) [0.43 - 0.67]	-0.0070 (0.0086) [-0.089 - 0.046]	-0.025, 0.011	0.430	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.37 (0.016) [0.30 - 0.45]	0.37 (0.016) [0.31 - 0.45]	-0.00059 (0.0050) [-0.036 - 0.037]	-0.011, 0.010	0.908	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.066 (0.0027) [0.054 - 0.088]	0.068 (0.0027) [0.055 - 0.085]	-0.0015 (0.0017) [-0.014 - 0.016]	-0.0050, 0.0021	0.394	(0.047 - 0.070) [0.037, 0.081]

Table VII-5 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Tyrosine (% DW)	0.32 (0.024) [0.16 - 0.43]	0.30 (0.024) [0.15 - 0.43]	0.014 (0.022) [-0.12 - 0.21]	-0.044, 0.071	0.565	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.52 (0.024) [0.40 - 0.64]	0.52 (0.024) [0.43 - 0.62]	-0.0019 (0.011) [-0.053 - 0.079]	-0.029, 0.026	0.866	(0.42 - 0.54) [0.33, 0.62]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-6. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
16:0 Palmitic (% Total FA)	12.12 (0.20) [11.60 - 15.21]	11.94 (0.20) [11.45 - 12.38]	0.18 (0.19) [-0.28 - 2.84]	-0.31, 0.66	0.394	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.17 (0.0073) [0.15 - 0.20]	0.17 (0.0073) [0.15 - 0.23]	-0.0020 (0.0036) [-0.042 - 0.015]	-0.0095, 0.0055	0.576	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.05 (0.033) [1.88 - 2.34]	1.98 (0.033) [1.80 - 2.10]	0.069 (0.022) [-0.041 - 0.33]	0.013, 0.13	0.024	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	20.26 (0.18) [19.32 - 21.08]	20.49 (0.18) [19.50 - 21.77]	-0.23 (0.12) [-1.13 - 0.85]	-0.48, 0.015	0.064	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	63.34 (0.35) [59.90 - 65.07]	63.34 (0.35) [61.88 - 64.70]	-0.0079 (0.26) [-3.07 - 1.05]	-0.67, 0.65	0.976	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.28 (0.012) [1.17 - 1.46]	1.27 (0.012) [1.22 - 1.33]	0.0066 (0.015) [-0.046 - 0.20]	-0.029, 0.042	0.673	(0.89 - 1.56) [0.39, 1.85]
20:0 Arachidic (% Total FA)	0.41 (0.0078) [0.39 - 0.44]	0.41 (0.0078) [0.37 - 0.45]	0.0037 (0.0032) [-0.017 - 0.024]	-0.0031, 0.010	0.263	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.18 (0.0024) [0.17 - 0.19]	0.19 (0.0024) [0.17 - 0.22]	-0.0078 (0.0027) [-0.035 - 0.0086]	-0.013, -0.0022	0.007	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.20 (0.012) [0.14 - 0.27]	0.20 (0.012) [0.14 - 0.27]	-0.0044 (0.013) [-0.099 - 0.078]	-0.037, 0.028	0.742	(0.069 - 0.28) [0, 0.37]

[1]FA = fatty acid S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-7. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Folic Acid (mg/kg DW)	0.30 (0.012)	0.30 (0.012)	0.0058 (0.0059)	-0.0094, 0.021	0.371	(0.19 - 0.31)
	[0.25 - 0.36]	[0.24 - 0.35]	[-0.033 - 0.041]			[0.13, 0.38]
Niacin (mg/kg DW)	18.59 (0.77)	18.52 (0.77)	0.069 (0.53)	-1.29, 1.42	0.901	(15.07 - 32.38)
	[15.53 - 22.23]	[15.26 - 21.85]	[-3.73 - 4.43]			[4.67, 36.68]
Thiamine HCl Vitamin B1 (mg/kg DW)	3.31 (0.16)	3.21 (0.16)	0.094 (0.066)	-0.077, 0.26	0.216	(2.43 - 4.17)
	[2.67 - 3.89]	[2.33 - 3.89]	[-0.44 - 0.54]			[1.84, 4.94]
Riboflavin/Vitamin B2 (mg/kg DW)	1.54 (0.084)	1.44 (0.084)	0.10 (0.097)	-0.15, 0.36	0.331	(0.95 - 2.42)
	[0.95 - 2.04]	[0.94 - 1.94]	[-0.88 - 0.60]			[0.047, 2.91]
Pyridoxine HCl/ Vitamin B6 (mg/kg DW)	6.10 (0.25)	6.24 (0.25)	-0.13 (0.17)	-0.48, 0.21	0.436	(4.93 - 7.53)
	[5.03 - 7.49]	[5.21 - 7.41]	[-1.55 - 1.66]			[3.12, 8.09]
Vitamin E (mg/kg DW)	14.73 (0.80)	14.69 (0.80)	0.045 (0.53)	-1.31, 1.40	0.935	(5.96 - 17.70)
	[11.09 - 20.02]	[9.47 - 18.44]	[-3.95 - 4.77]			[0, 26.07]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-8. Comparison of the Antinutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites (U.S. 2006)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.83 (0.038) [0.60 - 1.00]	0.84 (0.038) [0.69 - 1.09]	-0.0087 (0.028) [-0.15 - 0.19]	-0.080, 0.063	0.767	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.19 (0.0081) [0.15 - 0.22]	0.18 (0.0081) [0.15 - 0.22]	0.014 (0.0082) [-0.036 - 0.050]	-0.0074, 0.035	0.155	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1772.22 (47.57) [1561.63 - 1966.67]	1693.18 (47.57) [1245.83 - 1997.77]	79.04 (62.05) [-210.94 - 533.93]	-80.47, 238.55	0.258	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	115.95 (4.15) [99.45 - 136.67]	126.55 (4.15) [94.77 - 156.25]	-10.61 (4.60) [-38.32 - 27.59]	-22.44, 1.22	0.069	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero**.**

Table VII-9. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control (U.S. 2006)

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[2]
Forage						
IL						
Carbohydrates (% DW)	87.31	85.75	1.81	0.007	[86.23 - 88.01]	[82.09, 90.80]
IN						
Moisture (% FW)	66.23	67.80	-2.31	0.008	[64.70 - 67.40]	[59.32, 81.14]
Protein (% DW)	6.75	7.23	-6.60	0.031	[6.68 - 6.90]	[4.92, 10.30]
Grain						
Combination of all sites						
Ash (% DW)	1.54	1.46	5.60	0.041	[1.33 - 1.83]	[0.55, 2.30]
18:0 Stearic (% Total FA)	2.05	1.98	3.50	0.024	[1.88 - 2.34]	[1.00, 2.51]
20:1 Eicosenoic (% Total FA)	0.18	0.19	-4.05	0.007	[0.17 - 0.19]	[0.15, 0.33]
IAE						
Moisture (% FW)	9.78	9.22	6.08	0.049	[9.57 - 10.00]	[7.58, 12.13]
Histidine (% DW)	0.31	0.32	-2.68	0.032	[0.30 - 0.32]	[0.20, 0.36]
Methionine (% DW)	0.21	0.20	2.45	0.035	[0.20 - 0.21]	[0.14, 0.28]
Valine (% DW)	0.51	0.53	-3.71	0.028	[0.48 - 0.52]	[0.33, 0.62]
18:3 Linolenic (% Total FA)	1.22	1.25	-2.45	0.040	[1.17 - 1.25]	[0.39, 1.85]
Total Dietary Fiber (% DW)	12.23	11.60	5.40	0.029	[11.94 - 12.52]	[8.11, 17.95]
Raffinose (% DW)	0.20	0.15	27.28	0.009	[0.19 - 0.20]	[0.039, 0.26]
IAW						
22:0 Behenic (% Total FA)	0.24	0.21	12.99	0.015	[0.20 - 0.25]	[0, 0.37]
Thiamine HCl (mg/kg DW)	2.85	2.48	14.81	0.011	[2.77 – 2.9]	[1.84, 4.94]
Ferulic Acid (µg/g DW)	1753.10	1847.90	-5.13	0.003	[1661.13 - 1914.78]	[395.96, 3485.38]

Table VII-9 (cont.). Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control (U.S. 2006)

Tissue/Site/ Components (Units)[a]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[b]
IL (cont.)						
Moisture (% FW)	9.94	10.43	-4.73	0.013	[9.71 - 10.30]	[7.58, 12.13]
Folic Acid (mg/kg DW)	0.28	0.27	4.19	0.033	[0.27 - 0.29]	[0.13, 0.38]
IN						
Cystine (% DW)	0.19	0.20	-5.55	0.039	[0.19 - 0.20]	[0.15, 0.27]
18:1 Oleic (% Total FA)	20.05	20.63	-2.83	0.025	[19.96 - 20.13]	[11.92, 39.78]
18:2 Linoleic (% Total FA)	63.81	63.03	1.24	0.026	[63.67 - 63.95]	[45.91, 72.47]
Acid Detergent Fiber (% DW)	2.55	3.63	-29.68	0.036	[2.52 - 2.60]	[0.62, 5.72]
Riboflavin/Vitamin B2 (mg/kg DW)	1.52	1.09	39.64	0.039	[1.45 - 1.57]	[0.047, 2.91]
Phytic Acid (% DW)	0.63	0.77	-17.45	0.007	[0.60 - 0.66]	[0.50, 1.11]
KS						
Lysine (% DW)	0.33	0.31	4.51	0.045	[0.31 - 0.34]	[0.22, 0.36]
20:1 Eicosenoic (% Total FA)	0.18	0.19	-7.81	0.043	[0.17 - 0.18]	[0.15, 0.33]
NE						
Ash (% DW)	1.57	1.38	14.01	0.007	[1.49 - 1.62]	[0.55, 2.30]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

3.2. Assessment of significant nutrients, antinutrients, and key secondary metabolites in corn forage and grain – Chile 2006/2007

Forage and grain samples of MON 87460 and its conventional control were harvested from plants grown at each of four field sites in Chile in 2006 - 2007. These field sites were located in commercial corn production regions of Chile. The sites were Calera de Tango (CT), Colina (CL), Lumbreras (LUM) and Quillota (QUI). These sites are well-suited for corn production, but as they typically do not receive any rainfall during the growing season, all water was applied to each site occurred through controlled irrigation. At each site, a strip-plot design was used allowing comparisons of the test and control substances under two separate irrigation regimens, well-watered and water-limited. The well-watered treatment was managed to provide optimal grain yield. The water-limited treatment was managed to impose a drought stress by withholding irrigation during approximately the V10 – R2 growth stages, which represents the growth stages when corn grain yield potential is most susceptible to drought stress (Campos et al., 2006). In addition to MON 87460 and its conventional control, four different conventional commercial corn hybrids were also grown at each site. This allowed harvest of forage and grain from a total of 16 commercial references from each water treatment to provide information on natural variation in the levels of analyzed nutrient and anti-nutrients under well-watered and water-limited conditions. Compositional analysis included components consistent with OECD guidelines.

The experiment was arranged in a strip-plot design with three replicates per site, with irrigation treatment (well-watered or water-limited) as the whole plot and substance type as the sub-plot. The whole plot factor was arranged as a randomized complete block design. The strip-plot factor consisted of the test, control, and reference substances.

Tissue was collected from MON 87460 and the control from all three blocks for each treatment; tissue from the four different commercial references grown at each site was collected from a single block for each treatment. Forage was collected at the early dent (R5) plant growth stage; grain was collected at physiological maturity.

Within each treatment, the composition of forage and grain of MON 87460 was compared to that of the conventional control across sites (combined-site analysis) and within site (individual site analysis). For a site to be included in the combined-site analysis, commercial reference varieties had to exhibit phenotypic responses indicative of a treatment effect. Specifically, reference varieties in the water-limited plots had to exhibit a minimum 15% reduction in yield compared to reference materials in the well-watered plots. Moderate water deficits result in approximately a 15% yield loss annually for corn grown in both temperate and tropical regions (Barker et al., 2005). Assessments for plant height, ear height and days to 50% silking were also made as reduced height and a delay in silking are indicators of moisture deficit in corn (Campos et al., 2006). CT, CL and LUM met these criteria (Appendix F). QUI did not meet these criteria and therefore, it was not possible to include this site in the combined-site analysis. Data from QUI are presented separately in Appendix K. Table VII.10 presents temperature data and applied water from the production period.

The compositional data set was examined for evidence of statistically significant differences between MON 87460 and the control. Statistical comparisons between the test and control substances were performed within each irrigation treatment. A range of component values and a statistical population were determined for the commercial reference varieties within

each irrigation treatment. Thus, four sets of statistical analyses were made for each treatment, three based on the data from each of the replicated field sites and the fourth based on data from the combined sites. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods.

Commercial references were included to provide data for the development of a 99% tolerance interval for each component analyzed. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial corn. The tolerance interval illustrates the compositional variability that occurs in corn currently grown commercially. It allows statistically significant differences between MON 87460 and the control to be placed in biological perspective. This comparative evaluation can also consider natural ranges in corn component levels published in the literature or in the International Life Sciences Institute (ILSI) Crop Composition Database (http://www.cropcomposition.org).

3.2.1. Levels of nutrients, anti-nutrients and key secondary metabolites (well-watered)

The well-watered plots provide a compositional comparison between MON 87460 and a conventional control grown under conditions that are optimal for corn growth and development. Results confirm that MON 87460 and the conventional control are compositionally equivalent when produced under well-watered conditions.

Forage and grain samples were harvested from all well-watered plots and analyzed for nutritional and anti-nutrient components as described in Section 3.1. In total, 77 different analytical components were measured (9 in forage, 68 in grain). Of these evaluated components, 16 had more than 50% of the observations below the assay limit of quantitation (LOQ). Components with more than 50% of observations below the assay LOQ were excluded from statistical analysis. These included 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 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural. These components are known to be present at low levels in corn grain (OECD, 2002). Therefore, 61 components (9 in forage, 52 in grain) were statistically assessed using a mixed model analysis of variance method.

The compositional data set was examined for evidence of statistically significant differences between MON 87460 and the control. Statistical evaluation of the composition data involved a comparison of the forage and grain from the test substance to those of the control. There were a total of 244 comparisons made (four sets of comparisons × 52 components from grain and four sets of comparisons × nine components from forage).

Mean values, ranges, and statistical analyses for the combined-site data are presented in Table VII-11 for forage and Tables VII-12 to VII-17 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table VII-19. Literature and ILSI Crop Composition Database ranges for corn components are provided in Table VII-27.

The statistical analysis showed that, for 230 (94.3%) of the 244 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). For the 14 statistically significant differences (two from the combined-site analysis and 12 from the individual site analyses) all mean component values of the test and control substances were within the 99% tolerance interval established from the

commercial references. Therefore, these differences were within the natural variability of corn for these components.

3.2.2. Levels of nutrients in forage and grain (well-watered)

A description of nutrients present in corn grain is provided in the OECD consensus document on compositional considerations for corn (OECD, 2002). A comparative assessment of levels of proximates, fiber, minerals, total amino acids, fatty acids, and vitamins follows.

In the combined-site analysis of forage, no significant differences were found between MON 87460 and the control. In the combined-site analysis of grain, 50 of the 52 comparisons were not significantly different ($p>0.05$). Differences included values for total fat and magnesium. Individual site comparisons between values for total fat and magnesium in MON 87640 and the control grain showed a significant difference ($p<0.05$) only at a single site. This lack of reproducibility across multiple sites established that the differences observed in the combined-site analysis in values for these two components were of no biological significance. The magnitude of the differences in total fat (0.17% DW) and magnesium (0.01% of DW) were extremely small, and the mean values for these two components were within the 99% tolerance interval established from the commercial references grown at the same sites. These findings confirmed that these minor differences reflected the natural variability of conventional corn.

For forage, 23 of the 27 individual site comparisons were not significantly different ($p>0.05$). Differences included values for carbohydrates by calculation, moisture, ADF, and calcium, with each component difference being observed at only a single site. This lack of reproducibility across all sites established that there were no meaningful trends in values for these components and that this limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for serine, threonine, 18:0 stearic acid, 18:2 linoleic acid, 18:3 linolenic acid, and vitamin E. For each component, these differences were observed at a single site only. This lack of reproducibility in differences across multiple sites and the fact that the mean values for these components at these sites were within the 99% tolerance interval established from the commercial references confirmed that the limited number of site differences in values for these components were of no biological significance.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. The limited number of differences recorded in this study reflected the natural variation of corn and supported the compositional equivalence of MON 87460 and conventional corn.

3.2.3. Assessment of levels of anti-nutrients and key secondary metabolites (well-watered)

A description of the anti-nutrients and secondary metabolites present in corn grain is provided in the OECD consensus document on compositional considerations for corn (OECD, 2002). The anti-nutrients and key secondary metabolites analyzed in this study are the same as those listed in Section 3.1.2.

The statistical analysis highlighted no differences within or across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the control. Thus, a

comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 and conventional corn.

3.2.4. Conclusion (well-watered)

Statistical analysis of the composition data showed that, for 230 (94.3%) of the 244 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). Of the 14 statistically significant differences (two from the combined-site analysis and 12 from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

Furthermore, the limited numbers of component differences were characterized by extremely small differences in magnitude, and a lack of reproducibility across all individual sites. These factors established that the differences observed in this study reflected no meaningful trends and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460 grown under well-watered conditions, and consequently the foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn grown under the same conditions. MON 87460 grown under well-watered conditions is as safe as conventional corn with a history of safe consumption.

3.2.5. Levels of nutrients, anti-nutrients and key secondary metabolites (water-limited)

The water-limited treatment allowed a compositional comparison between MON 87460 and the conventional control grown under conditions intended to impose drought stress by withholding irrigation during approximately V10 - R2, the growth stages when yield potential is most susceptible to drought stress. Irrigation management in this treatment was intended to provide well-watered conditions outside of the V10 - R2 growth stages.

Forage and grain samples were harvested from all water-limited plots and analyzed for the same nutritional and anti-nutrient components assessed in the evaluation of samples from the well-watered plots. Mean values, ranges, and statistical analyses for the combined-site data are presented in Table VII-19 for forage and Tables VII-20 to VII-25 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table VII-26. Literature and ILSI Crop Composition Database ranges for corn components are provided in Table VII-27.

The statistical analysis showed that, for 233 (95.5%) of the 244 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). Of the eleven statistically significant differences (two from the combined-site analysis and nine from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

3.2.6. Levels of nutrients in forage and grain (water-limited)

In the combined-site analysis of forage, eight of the nine comparisons between MON 87460 and the control were not significantly different ($p>0.05$). The single difference was in total

fat values. However, the mean values for total fat in the test and control substances were within the 99% tolerance interval established from the commercial reference varieties grown at the same sites, indicating that the difference was of no biological significance. Values for total fat were not observed to be significantly different ($p>0.05$) at any of the individual sites. In the combined-site analysis of grain, 51 of the 52 comparisons were not significantly different ($p>0.05$). The single difference was in 20:1 eicosenoic acid values. However, the mean values for 20:1 eicosenoic acid in the test and control substances were within the 99% tolerance interval established from the commercial references grown at the same sites, indicating that the difference was of no biological significance. Values for 20:1 eicosenoic acid were observed to be significantly different ($p>0.05$) at only one of the individual sites.

For forage, 26 of the 27 individual site comparisons were not significantly different ($p>0.05$). Individual site differences included only a single value for moisture. This lack of reproducibility across multiple sites established that there are no meaningful trends in differences in values for this component and that the limited number of differences constituted no biological significance. For grain nutrients, individual site differences in components not recorded in the combined-site analysis included values for iron, phosphorus, 18:1 oleic acid, 22:0 behenic acid, folic acid, vitamin E, and phytic acid. For each component, these differences were observed at a single site only. The fact that the mean values for these components at multiple sites were within the 99% tolerance interval established from the commercial references and the lack of reproducibility in differences across these sites confirmed that the limited number of site differences in values for these components were of no biological significance.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of nutrient components from MON 87460 and the control. Those limited number of differences observed in this study reflected the natural variation of conventional corn and supported the compositional equivalence of MON 87460 and conventional corn.

3.2.7. Assessment of levels of anti-nutrients and key secondary metabolites (water-limited)

No combined-site differences between values for grain anti-nutrient components (phytic acid and raffinose) and secondary metabolites (*p*-coumaric acid and ferulic acid) in MON 87460 and the control were recorded. Individual site differences were observed for a single value for phytic acid. As only one out of six individual site comparisons was recorded for this component, this difference represented no meaningful trend and was of no biological significance. The limited number of differences reflected the natural variation of conventional corn.

In summary, statistical analysis highlighted no consistent differences across sites in the levels of anti-nutrient components and secondary metabolites in MON 87460 and the conventional control. Thus, a comprehensive evaluation of anti-nutrient components and key secondary metabolites supported the compositional equivalence of MON 87460 and conventional corn.

3.2.8. Conclusion (water-limited)

Statistical analysis of the composition data showed that, for 233 (96.3%) of the 244 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). Of the 11 statistically significant differences

(one from the combined-site analysis and ten from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

Furthermore, the limited number of component differences was characterized by extremely small differences in magnitude, and a lack of reproducibility across all individual sites. These factors established that the limited number of differences observed in this study reflected no meaningful trends and were of no biological significance.

Therefore, the corn grain and forage derived from MON 87460 grown under water-limited conditions, and the intended foods and feeds derived from MON 87460, can be considered compositionally equivalent to those derived from conventional corn grown under the same conditions. MON 87460 grown under limited water availability is as safe as conventional corn with a history of safe consumption.

3.2.9. Overall conclusions from compositional analysis of MON 87460 from U.S. 2006 and Chile 2006/2007 field productions

The compositional analyses of MON 87460 were based on forage and grain harvested from two different growing seasons, the first during 2006 in the U.S. conducted under normal agronomic practice, and the second during 2006/2007 in Chile under well-watered and water-limited conditions. Thus, the multi-year study allowed a determination of whether food and feed derived from MON 87460 exhibits compositional equivalence to conventional corn under a broad range of environmental conditions.

Components evaluated in samples harvested from both productions included 1) moisture, protein, carbohydrates by calculation fat, fiber, and ash in a proximate analysis, 2) essential macro- and micro-nutrients in a nutritional analysis, and 3) known endogenous toxicants and anti-nutrients.

Overall, a comprehensive evaluation of key nutrient, anti-nutrients and secondary metabolites from MON 87460 and the control showed no biologically significant differences. The statistical differences were small in magnitude and not reproducible across multiple sites. All mean component values of the test and control substances were within the 99% tolerance interval established from commercial references. Therefore, the corn grain and forage derived from MON 87460 and the foods and feeds derived from such, can be considered compositionally equivalent to those derived from conventional corn.

Table VII-10. Monthly Temperature and Monthly Accumulated Water Data for the 2006/2007 Chile Field Production

Site[1]	Measurement	December	January	February	March	April	May
CL	Accumulated water (in.), well-watered	0.9	10.3	8.5	9.4	2.8	0.0
	Accumulated water (in.), water-limited[2, 3]	0.9	10.3	4.7	5.6	2.8	0.0
	Avg Max temp (°F)	NA[5]	88	85	82	74	67
	Avg Min temp (°F)	NA[5]	53	49	47	41	33
	Range[4] (°F)	NA[5]	46 - 97	42 - 94	41 - 94	33 - 89	26 - 78
CT	Accumulated water (in.), well-watered	2.8	9.4	8.5	10.3	2.8	0.0
	Accumulated water (in.), water-limited[2, 3]	3.8	9.4	2.8	6.6	2.8	0.0
	Avg Max temp (°F)	NA[5]	84	79	79	71	66
	Avg Min temp (°F)	NA[5]	52	50	50	41	37
	Range[4] (°F)	NA[5]	46 - 91	42 - 90	42 - 88	31 - 87	29 – 77
LUM	Accumulated water (in.), well-watered	2.8	9.4	8.5	10.3	1.9	0.0
	Accumulated water (in.), water-limited[2, 3]	2.8	9.4	2.8	6.6	2.8	0.0
	Avg Max temp (°F)	NA[5]	81	78	79	73	67
	Avg Min temp (°F)	NA[5]	52	50	49	42	37
	Range[4] (°F)	NA[5]	47 - 89	42 - 89	42 - 94	32 - 87	28 - 77

[1] Site codes are as follows: CL = Colina; CT = Calera de Tango; LUM = Lumbreras.
[2]Water limitation began at the V10 growth stage which occurred at approximately February 7.
[3]Water limitation ended at the R2 growth stage which occurred at approximately March 13.
[4]The range is the absolute maximum and minimum temperature in each month.
[5]Temperature data are available from January 6 through May 25; planting occurred in late December and early January. Rainfall did not occur during the production period.

Table VII-11. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	28.37 (1.45) [17.95 - 34.70]	30.43 (1.45) [24.98 - 35.12]	-2.07 (1.79) [-10.93 - 5.34]	-5.85, 1.72	0.264	(25.07 - 37.22) [16.01, 45.98]
Neutral Detergent Fiber (% DW)	42.02 (1.52) [36.08 - 50.00]	44.51 (1.52) [39.08 - 47.24]	-2.49 (1.81) [-8.10 - 8.70]	-6.10, 1.11	0.171	(37.84 - 49.16) [27.28, 58.88]
Mineral						
Calcium (% DW)	0.26 (0.020) [0.24 - 0.28]	0.27 (0.020) [0.22 - 0.39]	-0.0091 (0.019) [-0.11 - 0.051]	-0.048, 0.029	0.628	(0.17 - 0.36) [0.043, 0.46]
Phosphorus (% DW)	0.16 (0.0077) [0.12 - 0.19]	0.16 (0.0077) [0.13 - 0.20]	0.0011 (0.0061) [-0.030 - 0.033]	-0.011, 0.013	0.852	(0.13 - 0.18) [0.086, 0.22]
Proximate						
Ash (% DW)	4.71 (0.22) [4.25 - 5.35]	4.89 (0.22) [3.88 - 6.05]	-0.18 (0.20) [-1.33 - 0.73]	-0.59, 0.22	0.366	(4.12 - 6.12) [2.42, 8.00]
Carbohydrates (% DW)	87.61 (0.42) [86.51 - 89.58]	87.11 (0.42) [85.87 - 88.50]	0.50 (0.40) [-0.52 - 2.34]	-0.29, 1.29	0.208	(85.54 - 89.52) [82.51, 92.09]
Moisture (% FW)	74.02 (0.73) [70.90 - 75.90]	75.19 (0.73) [74.20 - 78.00]	-1.17 (0.70) [-4.40 - 1.70]	-2.65, 0.31	0.113	(71.40 - 76.80) [69.22, 81.25]
Protein (% DW)	6.53 (0.40) [5.29 - 7.10]	6.71 (0.40) [6.01 - 7.44]	-0.18 (0.22) [-1.20 - 1.09]	-0.62, 0.25	0.407	(5.56 - 7.39) [4.12, 8.77]
Total Fat (% DW)	1.16 (0.16) [0.57 - 1.96]	1.30 (0.16) [0.51 - 2.33]	-0.14 (0.23) [-0.71 - 0.68]	-0.60, 0.33	0.557	(0.20 - 2.26) [0, 3.59]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-12. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Ash (% DW)	1.44 (0.038) [1.35 - 1.53]	1.42 (0.038) [1.26 - 1.60]	0.015 (0.048) [-0.22 - 0.20]	-0.083, 0.11	0.751	(1.14 - 1.47) [0.90, 1.76]
Carbohydrates (% DW)	85.17 (0.27) [82.98 - 87.63]	85.53 (0.27) [84.91 - 86.31]	-0.37 (0.36) [-2.41 - 2.56]	-1.08, 0.35	0.310	(83.60 - 86.65) [81.08, 89.71]
Moisture (% FW)	12.09 (0.15) [11.80 - 12.50]	12.09 (0.15) [11.30 - 12.80]	0 (0.21) [-0.70 - 0.70]	-0.44, 0.44	1.000	(11.00 - 12.20) [10.10, 13.35]
Protein (% DW)	9.50 (0.23) [7.57 - 11.32]	9.32 (0.23) [8.55 - 9.77]	0.18 (0.29) [-1.93 - 1.61]	-0.40, 0.76	0.533	(8.69 - 11.33) [5.83, 13.57]
Total Fat (% DW)	3.89 (0.082) [3.45 - 4.23]	3.72 (0.082) [3.60 - 3.90]	0.17 (0.071) [-0.42 - 0.61]	0.018, 0.32	0.029	(3.16 - 4.07) [2.47, 4.68]
Fiber						
Acid Detergent Fiber (% DW)	2.57 (0.21) [2.08 - 3.18]	2.47 (0.21) [1.41 - 4.41]	0.11 (0.27) [-1.43 - 0.96]	-0.44, 0.65	0.696	(1.95 - 3.76) [0.29, 5.01]
Neutral Detergent Fiber (% DW)	8.66 (0.34) [8.19 - 9.45]	8.60 (0.34) [7.74 - 9.70]	0.063 (0.44) [-1.41 - 1.62]	-0.86, 0.99	0.887	(7.15 - 9.41) [5.23, 10.90]
Total Dietary Fiber (% DW)	12.70 (0.44) [11.59 - 16.00]	12.53 (0.44) [11.20 - 13.98]	0.17 (0.50) [-1.15 - 2.97]	-0.90, 1.24	0.737	(10.24 - 13.51) [6.72, 16.07]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-13. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Calcium (% DW)	0.0047 (0.00045) [0.0035 - 0.0058]	0.0045 (0.00045) [0.0036 - 0.0059]	0.00014 (0.00029) [-0.00060 - 0.0012]	-0.00044, 0.00072	0.630	(0.0032 - 0.0057) [0.00076, 0.0080]
Copper (mg/kg DW)	1.86 (0.24) [1.58 - 2.17]	1.87 (0.24) [1.47 - 2.81]	-0.011 (0.27) [-0.89 - 0.37]	-0.60, 0.58	0.966	(1.29 - 4.16) [0, 5.74]
Iron (mg/kg DW)	16.60 (0.68) [14.53 - 20.30]	16.95 (0.68) [15.22 - 19.95]	-0.36 (0.61) [-2.07 - 1.58]	-1.58, 0.86	0.561	(14.37 - 19.48) [10.40, 25.42]
Magnesium (% DW)	0.12 (0.0034) [0.10 - 0.14]	0.11 (0.0034) [0.10 - 0.11]	0.0094 (0.0037) [-0.0076 - 0.028]	0.0021, 0.017	0.012	(0.095 - 0.13) [0.064, 0.16]
Manganese (mg/kg DW)	6.27 (0.40) [5.25 - 7.08]	6.03 (0.40) [4.64 - 7.58]	0.24 (0.29) [-1.51 - 1.61]	-0.59, 0.87	0.434	(4.55 - 9.02) [0.69, 10.70]
Phosphorus (% DW)	0.32 (0.0099) [0.29 - 0.34]	0.30 (0.0099) [0.26 - 0.33]	0.019 (0.0098) [-0.034 - 0.086]	-0.00060, 0.038	0.057	(0.27 - 0.36) [0.21, 0.40]
Potassium (% DW)	0.40 (0.011) [0.37 - 0.41]	0.40 (0.011) [0.36 - 0.45]	-0.0039 (0.0084) [-0.078 - 0.047]	-0.021, 0.013	0.643	(0.32 - 0.42) [0.25, 0.47]
Zinc (mg/kg DW)	22.00 (1.00) [19.20 - 25.09]	21.02 (1.00) [18.36 - 25.34]	0.99 (0.80) [-3.91 - 5.47]	-0.60, 2.57	0.219	(18.12 - 29.69) [7.39, 38.63]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-14. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Alanine (% DW)	0.71 (0.018) [0.63 - 0.86]	0.70 (0.018) [0.63 - 0.76]	0.017 (0.025) [-0.077 - 0.14]	-0.032, 0.066	0.500	(0.66 - 0.89) [0.44, 1.06]
Arginine (% DW)	0.39 (0.012) [0.32 - 0.47]	0.38 (0.012) [0.32 - 0.43]	0.0079 (0.017) [-0.030 - 0.14]	-0.026, 0.042	0.639	(0.34 - 0.46) [0.23, 0.55]
Aspartic Acid (% DW)	0.61 (0.013) [0.54 - 0.70]	0.60 (0.013) [0.56 - 0.63]	0.0088 (0.017) [-0.057 - 0.073]	-0.024, 0.042	0.598	(0.58 - 0.77) [0.39, 0.88]
Cystine (% DW)	0.22 (0.0043) [0.20 - 0.23]	0.21 (0.0043) [0.20 - 0.22]	0.0030 (0.0047) [-0.0082 - 0.017]	-0.0064, 0.012	0.527	(0.20 - 0.24) [0.16, 0.27]
Glutamic Acid (% DW)	1.84 (0.047) [1.63 - 2.21]	1.80 (0.047) [1.62 - 1.97]	0.043 (0.064) [-0.22 - 0.35]	-0.085, 0.17	0.503	(1.64 - 2.26) [1.09, 2.72]
Glycine (% DW)	0.35 (0.0063) [0.32 - 0.39]	0.34 (0.0063) [0.31 - 0.35]	0.0063 (0.0077) [-0.029 - 0.041]	-0.0090, 0.022	0.414	(0.31 - 0.38) [0.26, 0.42]
Histidine (% DW)	0.29 (0.0056) [0.26 - 0.34]	0.29 (0.0056) [0.26 - 0.30]	0.0034 (0.0074) [-0.031 - 0.037]	-0.011, 0.018	0.645	(0.24 - 0.30) [0.20, 0.34]
Isoleucine (% DW)	0.33 (0.0092) [0.29 - 0.42]	0.33 (0.0092) [0.30 - 0.36]	0.0014 (0.013) [-0.044 - 0.074]	-0.024, 0.026	0.908	(0.30 - 0.41) [0.19, 0.49]

Table VII-14 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Leucine (% DW)	1.23 (0.034) [1.08 - 1.52]	1.19 (0.034) [1.08 - 1.30]	0.036 (0.046) [-0.13 - 0.28]	-0.057, 0.13	0.441	(1.06 - 1.53) [0.66, 1.87]
Lysine (% DW)	0.28 (0.0045) [0.26 - 0.31]	0.28 (0.0045) [0.26 - 0.29]	0.00075 (0.0055) [-0.027 - 0.018]	-0.010, 0.012	0.892	(0.25 - 0.31) [0.19, 0.35]
Methionine (% DW)	0.18 (0.0073) [0.16 - 0.21]	0.18 (0.0073) [0.16 - 0.19]	0.0032 (0.0065) [-0.021 - 0.027]	-0.0098, 0.016	0.625	(0.18 - 0.23) [0.14, 0.26]
Phenylalanine (% DW)	0.49 (0.012) [0.43 - 0.60]	0.48 (0.012) [0.43 - 0.52]	0.011 (0.017) [-0.056 - 0.10]	-0.023, 0.044	0.535	(0.44 - 0.60) [0.28, 0.72]
Proline (% DW)	0.88 (0.027) [0.77 - 1.07]	0.87 (0.027) [0.82 - 0.95]	0.012 (0.030) [-0.092 - 0.16]	-0.049, 0.072	0.705	(0.72 - 0.99) [0.48, 1.18]
Serine (% DW)	0.47 (0.012) [0.43 - 0.54]	0.45 (0.012) [0.40 - 0.50]	0.019 (0.016) [-0.041 - 0.071]	-0.013, 0.051	0.224	(0.43 - 0.55) [0.32, 0.65]
Threonine (% DW)	0.32 (0.0069) [0.28 - 0.37]	0.32 (0.0069) [0.29 - 0.33]	0.0043 (0.0090) [-0.033 - 0.040]	-0.014, 0.022	0.634	(0.30 - 0.37) [0.23, 0.42]
Tryptophan (% DW)	0.051 (0.0020) [0.039 - 0.063]	0.051 (0.0020) [0.046 - 0.054]	0.00095 (0.0025) [-0.013 - 0.012]	-0.0039, 0.0058	0.701	(0.040 - 0.059) [0.022, 0.078]

Table VII-14 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Tyrosine (% DW)	0.23 (0.023) [0.12 - 0.35]	0.25 (0.023) [0.13 - 0.32]	-0.022 (0.032) [-0.11 - 0.079]	-0.089, 0.044	0.496	(0.14 - 0.32) [0, 0.53]
Valine (% DW)	0.46 (0.011) [0.42 - 0.56]	0.46 (0.011) [0.41 - 0.49]	0.0017 (0.014) [-0.054 - 0.077]	-0.027, 0.031	0.909	(0.41 - 0.54) [0.29, 0.62]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-15. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
16:0 Palmitic (% Total FA)	10.93 (0.13) [10.63 - 11.60]	11.15 (0.13) [10.96 - 11.32]	-0.22 (0.16) [-0.69 - 0.33]	-0.54, 0.10	0.173	(9.53 - 12.33) [7.43, 14.09]
18:0 Stearic (% Total FA)	1.80 (0.045) [1.74 - 1.97]	1.78 (0.045) [1.66 - 1.91]	0.020 (0.047) [-0.11 - 0.22]	-0.078, 0.12	0.675	(1.28 - 2.13) [0.60, 2.58]
18:1 Oleic (% Total FA)	20.93 (0.37) [20.29 - 21.28]	21.01 (0.37) [19.78 - 21.93]	-0.079 (0.29) [-1.20 - 0.51]	-0.69, 0.53	0.786	(22.13 - 31.09) [12.40, 36.28]
18:2 Linoleic (% Total FA)	64.51 (0.45) [63.92 - 65.27]	64.21 (0.45) [63.22 - 65.48]	0.30 (0.42) [-0.81 - 1.59]	-0.59, 1.19	0.485	(55.17 - 64.97) [49.61, 73.18]
18:3 Linolenic (% Total FA)	1.18 (0.016) [1.15 - 1.23]	1.21 (0.016) [1.19 - 1.25]	-0.027 (0.014) [-0.049 - -0.013]	-0.056, 0.00085	0.057	(1.00 - 1.32) [0.72, 1.66]
20:0 Arachidic (% Total FA)	0.31 (0.011) [0.29 - 0.34]	0.32 (0.011) [0.29 - 0.34]	-0.0054 (0.0058) [-0.026 - 0.0090]	-0.018, 0.0067	0.367	(0.29 - 0.42) [0.19, 0.52]
20:1 Eicosenoic (% Total FA)	0.18 (0.0039) [0.17 - 0.20]	0.19 (0.0039) [0.17 - 0.20]	-0.0041 (0.0039) [-0.016 - 0.0089]	-0.012, 0.0038	0.301	(0.20 - 0.31) [0.10, 0.36]
22:0 Behenic (% Total FA)	0.15 (0.020) [0.062 - 0.26]	0.13 (0.020) [0.063 - 0.17]	0.015 (0.026) [-0.075 - 0.097]	-0.039, 0.070	0.559	(0.061 - 0.33) [0, 0.48]

[1]DW = dry weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-16. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Folic Acid (mg/kg DW)	0.26 (0.018) [0.23 - 0.29]	0.27 (0.018) [0.22 - 0.33]	-0.0094 (0.016) [-0.085 - 0.029]	-0.044, 0.025	0.569	(0.26 - 0.41) [0.11, 0.55]
Niacin (mg/kg DW)	19.17 (1.77) [16.42 - 21.50]	19.23 (1.77) [17.42 - 21.17]	-0.054 (1.81) [-1.62 - 2.69]	-3.66, 3.56	0.976	(14.92 - 26.80) [5.96, 38.50]
Thiamine HCl/Vitamin B1 (mg/kg DW)	2.86 (0.084) [2.61 - 3.19]	2.86 (0.084) [2.74 - 3.06]	0.00065 (0.088) [-0.22 - 0.44]	-0.19, 0.19	0.994	(2.94 - 4.78) [1.01, 6.00]
Riboflavin/Vitamin B2 (mg/kg DW)	2.01 (0.14) [1.61 - 2.54]	1.97 (0.14) [1.46 - 2.63]	0.040 (0.14) [-1.02 - 0.61]	-0.24, 0.32	0.781	(1.62 - 2.62) [0.87, 3.38]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	6.32 (0.27) [5.49 - 7.39]	6.83 (0.27) [6.17 - 7.37]	-0.51 (0.33) [-1.32 - 0.21]	-1.21, 0.19	0.143	(4.01 - 6.70) [1.86, 8.29]
Vitamin E (mg/kg DW)	11.90 (0.40) [10.64 - 13.57]	10.99 (0.40) [9.30 - 12.78]	0.91 (0.54) [-0.81 - 2.32]	-0.23, 2.05	0.110	(2.83 - 11.69) [0, 19.32]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-17. Comparison of the Antinutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, well-watered)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Antinutrient						
Phytic Acid (% DW)	0.76 (0.035) [0.58 - 0.93]	0.76 (0.035) [0.63 - 0.90]	0.0069 (0.043) [-0.21 - 0.31]	-0.083, 0.096	0.873	(0.58 - 0.97) [0.28, 1.15]
Raffinose (% DW)	0.11 (0.013) [0.075 - 0.12]	0.11 (0.013) [0.077 - 0.14]	-0.0015 (0.0050) [-0.022 - 0.013]	-0.012, 0.0089	0.767	(0.028 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1849.20 (114.60) [1265.68 - 2240.00]	1753.19 (114.60) [820.14 - 2128.15]	96.01 (160.59) [-660.93 - 1232.32]	-224.84, 416.86	0.552	(1504.52 - 2224.72) [1019.70, 2703.40]
p-Coumaric Acid (µg/g DW)	137.39 (15.51) [68.64 - 188.64]	149.37 (15.51) [64.03 - 204.06]	-11.98 (18.53) [-88.07 - 87.50]	-50.95, 26.99	0.526	(84.79 - 239.33) [0, 378.84]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.

[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-18. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control (Chile 2006/2007, well-watered)

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[2]
Forage						
CT						
Moisture (% FW)	72.53	76.10	-4.69	<0.001	70.90 - 75.00	[69.22, 81.25]
Carbohydrates (% DW)	87.22	86.14	1.26	0.048	86.98 - 87.46	[82.51, 92.09]
Acid Detergent Fiber (% DW)	22.84	29.47	-22.51	0.002	17.95 - 31.28	[16.01, 45.98]
Calcium (% DW)	0.26	0.32	-18.99	0.047	0.25 - 0.28	[0.043, 0.46]
Grain						
Combination of all sites						
Total Fat (% DW)	3.89	3.72	4.52	0.029	3.45 - 4.23	[2.47, 4.68]
Magnesium (% DW)	0.12	0.11	8.64	0.012	0.10 - 0.14	[0.064, 0.16]
CL						
Vitamin E (mg/kg DW)	12.46	10.50	18.66	0.002	12.37 - 12.51	[0, 19.32]
CT						
18:2 Linoleic (% Total FA)	64.64	63.82	1.28	0.048	64.11 - 65.10	[49.61, 73.18]
LUM						
Total Fat (% DW)	3.96	3.61	9.54	0.010	3.80 - 4.23	[2.47, 4.68]
Magnesium (% DW)	0.13	0.11	15.54	0.022	0.11 - 0.14	[0.064, 0.16]
Serine (% DW)	0.50	0.44	13.33	0.024	0.47 - 0.54	[0.32, 0.65]
Threonine (% DW)	0.35	0.32	10.08	0.047	0.33 - 0.37	[0.23, 0.42]
18:0 Stearic (% Total FA)	1.83	1.71	6.83	0.024	1.74 - 1.97	[0.60, 2.58]
18:3 Linolenic (% Total FA)	1.20	1.23	-2.41	0.042	1.17 - 1.23	[0.72, 1.66]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-19. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	27.15 (1.45) [23.03 - 32.00]	26.73 (1.45) [23.10 - 30.79]	0.42 (1.79) [-6.30 - 8.90]	-3.36, 4.21	0.816	(20.73 - 33.39) [11.54, 42.87]
Neutral Detergent Fiber (% DW)	39.06 (1.52) [33.29 - 44.10]	40.10 (1.52) [31.81 - 50.61]	-1.04 (1.81) [-9.65 - 8.94]	-4.65, 2.56	0.565	(36.08 - 49.33) [25.58, 58.01]
Mineral						
Calcium (% DW)	0.32 (0.020) [0.20 - 0.44]	0.34 (0.020) [0.26 - 0.41]	-0.023 (0.019) [-0.14 - 0.11]	0.002, 0.015	0.219	(0.21 - 0.37) [0.085, 0.50]
Phosphorus (% DW)	0.16 (0.0077) [0.14 - 0.18]	0.17 (0.0077) [0.15 - 0.21]	-0.0078 (0.0061) [-0.033 - 0.014]	-0.020, 0.0044	0.204	(0.13 - 0.19) [0.077, 0.23]
Proximate						
Ash (% DW)	5.29 (0.22) [4.51 - 6.29]	5.49 (0.22) [4.59 - 6.90]	-0.20 (0.20) [-1.02 - 0.94]	-0.60, 0.21	0.332	(4.80 - 6.62) [3.59, 7.93]
Carbohydrates (% DW)	85.92 (0.42) [84.14 - 88.81]	86.02 (0.42) [84.51 - 87.52]	-0.10 (0.40) [-1.84 - 1.29]	-0.89, 0.69	0.798	(84.11 - 87.54) [81.74, 90.41]
Moisture (% FW)	74.98 (0.73) [72.00 - 77.40]	75.42 (0.75) [73.00 - 77.60]	-0.44 (0.70) [-3.20 - 4.10]	-1.92, 1.04	0.552	(73.40 - 77.50) [70.85, 80.94]
Protein (% DW)	7.47 (0.40) [5.49 - 8.76]	7.67 (0.40) [6.52 - 9.14]	-0.20 (0.22) [-1.02 - 0.36]	-0.63, 0.24	0.373	(5.56 - 8.59) [2.94, 11.20]
Total Fat (% DW)	1.32 (0.16) [0.50 - 1.92]	0.84 (0.16) [0.20 - 1.66]	0.47 (0.23) [-0.42 - 0.92]	0.010, 0.94	0.045	(0.20 - 1.76) [0, 3.25]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-20. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximates						
Ash (% DW)	1.47 (0.038) [1.24 - 1.75]	1.50 (0.038) [1.39 - 1.63]	-0.032 (0.048) [-0.31 - 0.35]	-0.13, 0.066	0.505	(1.27 - 1.63) [1.06, 1.93]
Carbohydrates (% DW)	84.21 (0.27) [82.64 - 85.64]	84.10 (0.27) [82.95 - 85.98]	0.11 (0.36) [-2.06 - 1.84]	-0.60, 0.82	0.754	(82.10 - 85.17) [80.40, 87.76]
Moisture (% FW)	12.10 (0.15) [11.60 - 12.50]	11.98 (0.15) [11.30 - 12.50]	0.12 (0.21) [-0.70 - 1.20]	-0.31, 0.56	0.563	(11.70 - 13.20) [10.50, 14.11]
Protein (% DW)	10.30 (0.23) [9.41 - 11.45]	10.44 (0.23) [9.17 - 11.50]	-0.13 (0.29) [-1.61 - 1.41]	-0.71, 0.45	0.645	(9.99 - 12.19) [8.12, 13.56]
Total Fat (% DW)	4.02 (0.082) [3.71 - 4.28]	3.96 (0.082) [3.47 - 4.23]	0.054 (0.071) [-0.19 - 0.54]	-0.096, 0.20	0.459	(3.18 - 4.22) [2.07, 5.10]
Fiber						
Acid Detergent Fiber (% DW)	2.59 (0.21) [1.85 - 3.58]	2.33 (0.21) [1.83 - 3.05]	0.26 (0.27) [-0.53 - 0.99]	-0.28, 0.80	0.342	(1.83 - 3.39) [0.88, 4.63]
Neutral Detergent Fiber (% DW)	8.87 (0.34) [7.33 - 11.31]	8.22 (0.34) [7.91 - 8.66]	0.64 (0.44) [-1.25 - 3.07]	-0.28, 1.57	0.161	(6.08 - 10.36) [2.87, 13.22]
Total Dietary Fiber (% DW)	12.48 (0.44) [10.78 - 14.43]	12.15 (0.44) [11.06 - 13.70]	0.33 (0.50) [-1.99 - 2.19]	-0.73, 1.40	0.515	(10.57 - 14.56) [6.50, 17.54]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-21. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Calcium (% DW)	0.0052 (0.00045) [0.0046 - 0.0065]	0.0050 (0.00045) [0.0041 - 0.0063]	0.00017 (0.00029) [-0.0014 - 0.0015]	-0.00041, 0.00075	0.563	(0.0035 - 0.0070) [0, 0.010]
Copper (mg/kg DW)	2.19 (0.24) [1.88 - 2.49]	2.16 (0.24) [1.87 - 2.30]	0.031 (0.27) [-0.40 - 0.62]	-0.56, 0.62	0.910	(1.39 - 2.76) [0.22, 3.82]
Iron (mg/kg DW)	17.67 (0.68) [16.38 - 19.27]	18.60 (0.68) [16.12 - 22.21]	-0.93 (0.61) [-3.53 - 1.02]	-2.14, 0.29	0.131	(15.90 - 24.66) [7.05, 30.38]
Magnesium (% DW)	0.13 (0.0034) [0.11 - 0.14]	0.13 (0.0034) [0.10 - 0.14]	0.00044 (0.0037) [-0.022 - 0.024]	-0.0069, 0.0077	0.905	(0.11 - 0.14) [0.083, 0.16]
Manganese (mg/kg DW)	6.71 (0.40) [5.28 - 8.66]	6.54 (0.40) [5.25 - 7.77]	0.18 (0.30) [-1.13 - 2.28]	-0.45, 0.80	0.565	(4.78 - 9.35) [0.72, 11.82]
Phosphorus (% DW)	0.32 (0.0099) [0.25 - 0.36]	0.33 (0.0099) [0.27 - 0.38]	-0.0095 (0.0098) [-0.074 - 0.075]	-0.029, 0.010	0.334	(0.30 - 0.38) [0.25, 0.42]
Potassium (% DW)	0.40 (0.011) [0.37 - 0.43]	0.40 (0.011) [0.37 - 0.43]	-0.0024 (0.0082) [-0.038 - 0.038]	-0.019, 0.015	0.777	(0.36 - 0.43) [0.29, 0.49]
Zinc (mg/kg DW)	23.30 (1.00) [18.36 - 26.77]	24.37 (1.00) [21.29 - 27.79]	-1.07 (0.80) [-3.62 - 2.49]	-2.66, 0.52	0.183	(18.25 - 30.44) [6.01, 42.60]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-22. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Alanine (% DW)	0.78 (0.018) [0.67 - 0.85]	0.79 (0.018) [0.68 - 0.89]	-0.010 (0.025) [-0.12 - 0.12]	-0.059, 0.039	0.682	(0.77 - 0.96) [0.59, 1.09]
Arginine (% DW)	0.43 (0.012) [0.41 - 0.44]	0.42 (0.012) [0.34 - 0.47]	0.013 (0.017) [-0.058 - 0.10]	-0.022, 0.047	0.457	(0.41 - 0.50) [0.32, 0.56]
Aspartic Acid (% DW)	0.65 (0.013) [0.59 - 0.71]	0.65 (0.013) [0.59 - 0.73]	-0.0072 (0.017) [-0.090 - 0.098]	-0.040, 0.026	0.665	(0.63 - 0.76) [0.52, 0.88]
Cystine (% DW)	0.23 (0.0043) [0.22 - 0.25]	0.23 (0.0043) [0.20 - 0.24]	-0.00090 (0.0047) [-0.021 - 0.027]	-0.010, 0.0085	0.848	(0.20 - 0.26) [0.15, 0.30]
Glutamic Acid (% DW)	2.01 (0.047) [1.74 - 2.21]	2.03 (0.047) [1.71 - 2.29]	-0.019 (0.064) [-0.31 - 0.32]	-0.15, 0.11	0.772	(1.94 - 2.44) [1.51, 2.80]
Glycine (% DW)	0.36 (0.0063) [0.34 - 0.39]	0.36 (0.0063) [0.33 - 0.39]	0.0018 (0.0077) [-0.033 - 0.037]	-0.014, 0.017	0.818	(0.35 - 0.42) [0.30, 0.45]
Histidine (% DW)	0.31 (0.0056) [0.28 - 0.32]	0.31 (0.0056) [0.27 - 0.34]	-0.0022 (0.0074) [-0.031 - 0.034]	-0.017, 0.013	0.768	(0.27 - 0.33) [0.23, 0.36]
Isoleucine (% DW)	0.37 (0.0092) [0.32 - 0.38]	0.37 (0.0092) [0.32 - 0.41]	-0.0065 (0.013) [-0.052 - 0.048]	-0.031, 0.018	0.605	(0.34 - 0.44) [0.27, 0.50]

Table VII-22 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Leucine (% DW)	1.36 (0.034) [1.16 - 1.47]	1.37 (0.034) [1.13 - 1.56]	-0.011 (0.046) [-0.22 - 0.24]	-0.10, 0.081	0.806	(1.29 - 1.65) [0.98, 1.91]
Lysine (% DW)	0.29 (0.0045) [0.27 - 0.31]	0.29 (0.0045) [0.28 - 0.31]	-0.0044 (0.0055) [-0.023 - 0.029]	-0.016, 0.0067	0.428	(0.28 - 0.31) [0.25, 0.34]
Methionine (% DW)	0.20 (0.0073) [0.18 - 0.22]	0.20 (0.0073) [0.16 - 0.22]	0.0010 (0.0065) [-0.023 - 0.038]	-0.012, 0.014	0.873	(0.19 - 0.30) [0.095, 0.35]
Phenylalanine (% DW)	0.53 (0.012) [0.46 - 0.58]	0.54 (0.012) [0.45 - 0.61]	-0.0044 (0.017) [-0.079 - 0.086]	-0.038, 0.029	0.797	(0.51 - 0.63) [0.41, 0.72]
Proline (% DW)	0.96 (0.027) [0.85 - 1.04]	0.97 (0.027) [0.84 - 1.11]	-0.011 (0.030) [-0.15 - 0.16]	-0.072, 0.050	0.722	(0.78 - 1.03) [0.64, 1.23]
Serine (% DW)	0.51 (0.012) [0.45 - 0.58]	0.51 (0.012) [0.43 - 0.59]	-0.0023 (0.016) [-0.087 - 0.11]	-0.034, 0.030	0.886	(0.48 - 0.60) [0.36, 0.71]
Threonine (% DW)	0.35 (0.0069) [0.32 - 0.39]	0.35 (0.0069) [0.31 - 0.39]	0.0015 (0.0090) [-0.042 - 0.067]	-0.016, 0.019	0.868	(0.33 - 0.39) [0.28, 0.44]
Tryptophan (% DW)	0.053 (0.0020) [0.046 - 0.059]	0.052 (0.0020) [0.042 - 0.063]	0.0012 (0.0025) [-0.0044 - 0.0095]	-0.0037, 0.0060	0.639	(0.043 - 0.063) [0.031, 0.082]

Table VII-22 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Tyrosine (% DW)	0.29 (0.023) [0.18 - 0.33]	0.24 (0.023) [0.12 - 0.35]	0.050 (0.032) [-0.11 - 0.16]	-0.017, 0.12	0.133	(0.25 - 0.41) [0.12, 0.52]
Valine (% DW)	0.50 (0.011) [0.44 - 0.51]	0.51 (0.011) [0.45 - 0.55]	-0.0078 (0.014) [-0.068 - 0.050]	-0.037, 0.021	0.590	(0.47 - 0.58) [0.39, 0.64]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-23. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
16:0 Palmitic (% Total FA)	11.06 (0.13) [10.54 - 11.33]	11.18 (0.13) [10.75 - 11.45]	-0.12 (0.16) [-0.39 - 0.32]	-0.45, 0.20	0.447	(9.84 - 12.33) [7.71, 14.14]
18:0 Stearic (% Total FA)	1.86 (0.045) [1.73 - 1.95]	1.86 (0.045) [1.68 - 2.08]	-0.0062 (0.047) [-0.15 - 0.25]	-0.10, 0.092	0.896	(1.30 - 2.10) [0.71, 2.57]
18:1 Oleic (% Total FA)	20.99 (0.37) [20.20 - 21.60]	20.83 (0.37) [19.59 - 21.98]	0.16 (0.29) [-1.03 - 1.33]	-0.45, 0.77	0.589	(20.78 - 29.13) [12.15, 35.55]
18:2 Linoleic (% Total FA)	64.29 (0.45) [63.27 - 65.10]	64.30 (0.45) [62.75 - 65.65]	-0.0089 (0.42) [-1.32 - 0.94]	-0.90, 0.88	0.983	(56.51 - 64.46) [50.63, 72.71]
18:3 Linolenic (% Total FA)	1.19 (0.016) [1.13 - 1.25]	1.21 (0.016) [1.12 - 1.26]	-0.013 (0.014) [-0.079 - 0.049]	-0.041, 0.015	0.354	(1.03 - 1.38) [0.67, 1.76]
20:0 Arachidic (% Total FA)	0.31 (0.011) [0.30 - 0.34]	0.32 (0.011) [0.30 - 0.33]	-0.0034 (0.0058) [-0.025 - 0.014]	-0.016, 0.0087	0.561	(0.30 - 0.41) [0.18, 0.52]
20:1 Eicosenoic (% Total FA)	0.18 (0.0039) [0.16 - 0.19]	0.18 (0.0039) [0.17 - 0.20]	-0.0082 (0.0039) [-0.025 - 0.014]	-0.016, -0.00030	0.042	(0.18 - 0.27) [0.11, 0.34]
22.0 Behenic (% Total FA)	0.12 (0.020) [0.058 - 0.20]	0.12 (0.020) [0.059 - 0.15]	0.0022 (0.026) [-0.092 - 0.13]	-0.052, 0.056	0.933	(0.062 - 0.18) [0, 0.32]

[1]FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-24. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Folic Acid (mg/kg DW)	0.29 (0.018) [0.25 - 0.37]	0.28 (0.018) [0.23 - 0.35]	0.011 (0.016) [-0.062 - 0.13]	-0.023, 0.046	0.497	(0.26 - 0.42) [0.098, 0.58]
Niacin (mg/kg DW)	18.54 (1.77) [16.23 - 25.00]	21.73 (1.77) [16.36 - 42.06]	-3.18 (1.81) [-24.26 - 2.78]	-6.79, 0.43	0.083	(13.64 - 27.42) [2.23, 41.53]
Thiamine HCl/Vitamin B1 (mg/kg DW)	3.10 (0.084) [2.84 - 3.42]	2.98 (0.084) [2.71 - 3.19]	0.12 (0.088) [-0.11 - 0.45]	-0.070, 0.30	0.203	(2.87 - 4.33) [1.55, 5.85]
Riboflavin/Vitamin B2 (mg/kg DW)	2.12 (0.14) [1.43 - 2.89]	2.29 (0.14) [1.64 - 2.81]	-0.16 (0.14) [-1.30 - 0.50]	-0.45, 0.12	0.255	(1.81 - 2.78) [0.88, 3.61]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	6.17 (0.27) [5.43 - 6.57]	6.15 (0.27) [4.97 - 8.27]	0.013 (0.33) [-1.96 - 1.20]	-0.69, 0.71	0.969	(5.30 - 8.22) [2.06, 9.98]
Vitamin E (mg/kg DW)	13.01 (0.40) [12.16 - 14.24]	12.16 (0.40) [10.15 - 13.64]	0.84 (0.54) [-0.45 - 2.42]	-0.30, 1.99	0.135	(2.84 - 15.53) [0, 22.61]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-25. Comparison of the Antinutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control for Combined Sites (Chile 2006/2007, water-limited)

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Antinutrient						
Phytic Acid (% DW)	0.79 (0.035) [0.63 - 0.89]	0.77 (0.035) [0.60 - 0.89]	0.022 (0.043) [-0.16 - 0.27]	-0.067, 0.11	0.612	(0.67 - 0.94) [0.40, 1.12]
Raffinose (% DW)	0.11 (0.013) [0.087 - 0.14]	0.12 (0.013) [0.097 - 0.15]	-0.0087 (0.0050) [-0.018 - 0.0025]	-0.019, 0.0017	0.095	(0.061 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1923.79 (114.60) [1208.67 - 2352.27]	1852.11 (114.60) [1088.34 - 2301.59]	71.68 (160.59) [-852.84 - 788.80]	-249.18, 392.53	0.656	(1011.40 - 2539.86) [0, 4071.51]
p-Coumaric Acid (µg/g DW)	137.29 (15.51) [85.52 - 168.18]	149.45 (15.51) [66.48 - 208.43]	-12.16 (18.53) [-122.91 - 65.49]	-51.13, 26.81	0.519	(84.15 - 259.68) [0, 378.67]

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-26. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control (Chile 2006/2007, water-limited)

Tissue/Site/ Components (Units)[a]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[b]
Forage						
Combination of all sites						
Total Fat (% DW)	1.32	0.84	56.03	0.045	0.50 - 1.92	[0, 3.25]
CT						
Moisture (% FW)	72.13	74.23	-2.83	0.005	72.00 - 72.30	[70.85, 80.94]
Grain						
Combination of all sites						
20:1 Eicosenoic (% Total FA)	0.18	0.18	-4.43	0.042	0.16 - 0.19	[0.11, 0.34]
CL						
22:0 Behenic (% Total FA)	0.17	0.11	57.68	0.029	0.13 - 0.20	[0, 0.32]
Vitamin E (mg/kg DW)	13.34	11.16	19.54	0.001	12.57 - 14.24	[0, 22.61]
Phytic Acid (% DW)	0.87	0.69	25.47	0.012	0.84 - 0.89	[0.40, 1.12]
CT						
Iron (mg/kg DW)	17.61	18.81	-6.34	0.046	17.06 -18.24	[7.05, 30.38]
Phosphorus (% DW)	0.32	0.35	-8.35	0.027	0.32 - 0.32	[0.25, 0.42]
20:1 Eicosenoic (% Total FA)	0.17	0.18	-9.16	0.016	0.16 - 0.17	[0.11, 0.34]
Folic Acid (mg/kg DW)	0.30	0.25	19.36	0.046	0.25 - 0.37	[0.098, 0.58]
LUM						
18:1 Oleic (% Total FA)	20.38	20.89	-2.46	0.030	20.20 - 20.48	[12.15, 35.55]

[a]DW= dry weight; FA=fatty acid.
[c]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-27. Literature and ILSI Database Ranges of Components of Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Forage		
Proximates (% DW)		
Ash	2.43-9.64[a]; 2-6.6[b]	1.527 – 9.638
Carbohydrates	83.2-91.6[b]; 76.5-87.3[a]	76.4 – 92.1
Fat, total	0.35-3.62[b]; 1.42-4.57[a]	0.296 – 4.570
Moisture (% fw)	56.5-80.4[a];55.3-75.3[b]	49.1 – 81.3
Protein	4.98-11.56[a]	3.14 – 11.57
Fiber (% DW)		
Acid detergent fiber (ADF)	18.3-41.0[b]; 17.5-38.3[a]	16.13 – 47.39
Neutral detergent fiber (NDF)	26.4-54.5[b]; 27.9-54.8[a]	20.29 – 63.71
Minerals (% DW)		
Calcium	0.0969-0.3184[b]	0.0714 – 0.5768
Phosphorous	0.1367-0.2914[b]	0.0936 – 0.3704
Grain		
Proximates (% DW)		
Ash	1.1-3.9[d]; 0.89-6.28[b]	0.616 – 6.282
Carbohydrates	77.4-87.2[b]; 82.2-88.1[a]	77.4 – 89.5
Fat, total	3.1-5.7[d]; 2.48-4.81[b]	1.742 – 5.823
Moisture (% FW)	7-23[d]; 8.18-26.2[b]	6.1 – 40.5
Protein	6-12[d]; 9.7-16.1[c]	6.15 – 17.26
Fiber (% dw)		
Acid detergent fiber (ADF)	3.3-4.3[d]; 2.46-11.34[a,b]	1.82 – 11.34
Neutral detergent fiber (NDF)	8.3-11.9[d]; 7.58-15.91[b]	5.59 – 22.64
Total dietary fiber (TDF)	10.99-11.41[h]	8.82 – 35.31
Minerals		
Calcium (% DW)	0.01-0.1[d]	0.00127 – 0.02084
Copper (mg/kg DW)	0.9-10[d]	0.73 – 18.50
Iron (mg/kg DW)	1-100[d]	10.42 – 49.07
Magnesium (% DW)	0.09-1[d]	0.0594 – 0.194
Manganese (mg/kg DW)	0.7-54[d]	1.69 – 14.30
Phosphorous (% DW)	0.26-0.75[d]	0.147 – 0.533
Potassium (% DW)	0.32-0.72[d]	0.181 – 0.603
Zinc (mg/kg DW)	12-30[d]	6.5 – 37.2

Table VII-27 (cont.). Literature and ILSI Database Ranges of Components of Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Grain		
Amino Acids (% DW)		
Alanine	N/A	0.439 – 1.393
Arginine	N/A	0.119 – 0.639
Aspartic acid	N/A	0.335 – 1.208
Cystine	N/A	0.125 – 0.514
Glutamic acid	N/A	0.965 – 3.536
Glycine	N/A	0.184 – 0.539
Histidine	N/A	0.137 – 0.434
Isoleucine	N/A	0.179 – 0.692
Leucine	N/A	0.642 – 2.492
Lysine	N/A	0.172 – 0.668
Methionine	N/A	0.124 – 0.468
Phenylalanine	N/A	0.244 – 0.930
Proline	N/A	0.462 – 1.632
Serine	N/A	0.235 – 0.769
Threonine	N/A	0.224 – 0.666
Tryptophan	N/A	0.0271 – 0.215
Tyrosine	N/A	0.103 – 0.642
Valine	N/A	0.266 – 0.855
Fatty Acids	(% total fat)	(% total fatty acid)
16:0 Palmitic	7-19[e]	7.94 – 20.71
16:1 Palmitoleic	1[e]	0.095 – 0.447
18:0 Stearic	1-3[e]	1.02 – 3.40
18:1 Oleic	20-46[e]	17.4 – 40.2
18:2 Linoleic	35-70[e]	36.2 – 66.5
18:3 Linolenic	0.8-2[e]	0.57 – 2.25
20:0 Arachidic	0.1-2[e]	0.279 – 0.965
20:1 Eicosenoic	-	0.170 – 1.917
22:0 Behenic	-	0.110 – 0.349
Vitamins (mg/kg DW)		
Folic acid	0.3[d]	0.147 – 1.464
Niacin	9.3-70[d]	10.37 – 46.94
Vitamin B_1	3-8.6[e]	1.26 – 40.00
Vitamin B_2	0.25-5.6[e]	0.50 – 2.36
Vitamin B_6	5.3[d]; 9.6[e]	3.68 – 11.32
Vitamin E	3-12.1[e]; 17-47[d]	1.5 – 68.7

Table VII-27 (cont.). Literature and Historical Ranges of Components of Corn Forage and Grain

Tissue/ Component[1]	Literature Range[2]	ILSI Range[3]
Grain		
Antinutrients (% DW)		
Phytic acid	0.48-1.12[a]	0.111 – 1.570
Raffinose	0.08-0.30[e]	0.020 – 0.320
Secondary Metabolites (µg/g dw)		
Ferulic acid	113-1194[f]; 3000[g]	291.9 – 3885.8
p-Coumaric acid	22-75[f]	53.4 – 576.2

[1]FW=fresh weight; DW=dry weight; Niacin =Vitamin B_3; Vitamin B_1 =Thiamine; Vitamin B_2 =Riboflavin; Vitamin B_6 =Pyridoxine ; N/A = not available as percent dry weight.
[2]Literature range references: [a]Ridley et al., 2002. [b]Sidhu et al., 2000. [c]Jugenheimer, 1976. [d]Watson, 1987. [e]Watson, 1982. [f]Classen *et al.*, 1990. [g]Dowd and Vega, 1996. [h]Choi et al., 1999.
[3]ILSI range is from ILSI CCD, 2006.

Conversions: % DW x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg DW; mg/100g dw x 10 = mg/kg DW

SECTION 4. Other Information Relevant to the Safety and Nutritional Assessment of MON 87460

4.1. Compositional analyses of additional secondary metabolites

As part of the comparative approach described previously, the OECD consensus documents also suggest additional components can be considered for characterization of food and feed derived from new products. Because MON 87460 is expected to be grown in regions subjected to frequent drought stress, additional secondary metabolites selected for further comparative evaluation included those known to be generally associated with stress responses in a range of plants and tissues, and thus possibly relevant in corn. There is no evidence in the literature for such components that are unique to corn.

Selected additional secondary metabolites included osmoprotectants, such as sugars and polyols (sucrose, glucose, fructose, sorbitol, mannitol, and glycerol), free proline, glycine betaine and choline (Yancey, 2004; Yancey, 2005), as well as metabolites that are generally associated with stress responses such as salicylic acid (Yuan and Lin, 2008), and abscisic acid (Wasilewska, et al., 2008). These 11 metabolites were measured in forage and grain of MON 87460 and the control. No safety issues are evident for these metabolites and most represent an extremely minor fraction of corn biomass.

Field design and sampling are described in Section 3.2. Samples from test, control, and reference substances from all four sites in Chile were subjected to additional compositional analysis. This section describes results from the three sites where two distinct treatments, well-watered and water-limited, were achieved. Data from the fourth site (QUI) are presented in Appendix K.

Statistical comparisons between the test and control substances were performed within each irrigation treatment. A range of component values and a statistical population were determined for the reference substances within each irrigation treatment. Thus, four sets of statistical analyses were made for each treatment, three based on the data from each of the replicated field sites and the fourth based on data from a combination of all three field sites. Statistically significant differences were determined at the 5% level of significance ($p<0.05$) using established statistical methods.

4.2. Levels of additional secondary metabolites (well-watered)

Two of the 11 metabolites (sorbitol, mannitol) had more than half of the observations below the assay limit of quantitation (LOQ). Metabolites with more than half of the observations below the assay LOQ were excluded from statistical analysis. Therefore, nine metabolites in both forage and grain were statistically assessed using a mixed model analysis of variance method.

There were a total of 72 comparisons made (four sets of comparisons × nine components from grain and four sets of comparisons × nine components from forage). Mean values, ranges, and statistical analyses for the combined-site data are presented in Table VII-28 for forage and Table VII-29 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table VII-32.

The statistical analysis showed that there were no significant differences ($p>0.05$) for 68 (94.4%) of the 72 comparisons. Of the four detected differences (one from the combined-site analysis and three from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references or differences between test and control were of exceedingly small magnitude. Therefore, these differences were within the natural variability of corn for these components.

4.3. Assessment of levels of additional secondary metabolites (well-watered)

In the combined-site analysis of forage, eight of the nine comparisons between MON 87460 and the control were not significantly different ($p>0.05$). The single difference was for abscisic acid. However, the magnitude of the combined-site difference in the abscisic acid values was exceedingly small (21.37 ppb FW). Individual site comparisons of abscisic acid revealed a significant difference ($p<0.05$) at only one of the three sites. In the combined-site analysis of grain, none of the nine comparisons were significantly different.

For forage, individual site differences in components not recorded in the combined-site analysis were values for choline and free proline observed at a single site only. In the individual site analysis of grain, no comparisons were significantly different.

4.4. Conclusion (well-watered)

In summary, statistical analysis highlighted no consistent differences across sites in the levels of the additional secondary metabolites from MON 87460 and the control. The limited number of differences observed in this study reflected the natural variation of corn and allowed the conclusion that levels of key osmoprotectants and metabolites generally associated with stress do not differ between MON 87460 and conventional corn. This supported the assessment of MON 87460 as compositionally equivalent to conventional corn.

4.5. Levels of additional secondary metabolites (water-limited)

Mean values, ranges, and statistical analyses for the combined-site data are presented in Table VII-30 for forage and Table VII-31 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table VII-33.

The statistical analysis showed that, for 61 (84.7%) of the 72 comparisons made between the mean component values of MON 87460 and the control, there were no significant differences ($p>0.05$). Of the 11 statistically significant differences (one from the combined-site analysis and ten from the individual site analyses), all mean component values of the test and control substances were within the 99% tolerance interval established from the commercial references. Therefore, these differences were within the natural variability of corn for these components.

4.6. Assessment of additional secondary metabolites (water-limited)

In the combined-site analysis of forage, no significant differences ($p<0.05$) were found between MON 87460 and the control. In the combined-site analysis of grain, eight of the nine comparisons were not significantly different. The single difference included values for sucrose. Individual site comparisons between values for sucrose in MON 87460 and the control grain showed a component difference at two of the three sites. The magnitude of the

differences were small (0.17 and 0.32% DW at the two sites) and the mean values were within the 99% tolerance interval established from the commercial references grown at the same sites. Therefore, these differences were within the natural variability of corn for these components.

For forage, 21 of the 27 individual site comparisons were not significantly different ($p>0.05$). Differences included values for abscisic acid, choline, glycine betaine and salicylic acid. The differences for abscisic acid and glycine betaine were observed at an individual site. The value for glycine betaine was lower for MON 87460, and the value for salicylic acid was higher for MON 87460 relative to the control. For choline, the magnitude of the differences was small and the mean values were within the 99% tolerance interval established from the commercial references grown at the same sites. For grain, individual site differences in components not detected in the combined-site analysis include values for abscisic acid and glycerol, both at a single site. The limited number and lack of reproducibility in differences across all sites confirmed that differences in values for these components were not biologically significant.

4.7. Conclusion (water-limited)

In summary, statistical analysis highlighted no consistent differences across sites in the levels of metabolite components from MON 87460 and the control. The limited number of differences observed in this study reflects the natural variation of corn and supports the conclusion that levels of key osmoprotectants and metabolites potentially associated with stress do not differ between MON 87460 and conventional corn. This supported the conclusion that MON 87460 is compositionally equivalent to conventional corn.

4.8. Overall conclusion on levels of additional secondary metabolites in MON 87460 grown under different irrigation treatments

A supplementary analysis of secondary metabolites was conducted for samples from the Chilean trial. The compositional assessment included a number of metabolites considered to be associated with stress tolerance. Statistical comparisons between the test and control substances were performed within each water treatment and showed very similar results. The few detected differences were either of exceedingly small magnitude or the mean component values of the test and control substances were within the 99% tolerance interval. Therefore, these differences were within the natural variability of corn for these components. The evaluation of these additional metabolites further supports the compositional equivalence of MON 87460 to conventional corn.

Table VII-28. Comparison of the Additional Secondary Metabolite Composition of Forage from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Well-Watered Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Free Proline (% DW)	0.023 (0.0025) [0.014 - 0.031]	0.019 (0.0025) [0.012 - 0.024]	0.0035 (0.0018) [-0.0077 - 0.014]	-0.00026, 0.0073	0.066	(0.0094 - 0.030) [0, 0.042]
Abscisic Acid (ppb FW)	37.03 (8.19) [11.90 - 122.00]	15.66 (7.97) [10.30 - 21.70]	21.38 (9.64) [-5.50 - 106.90]	1.01, 41.75	0.040	(12.70 - 23.80) [1.22, 33.02]
Choline (ppm FW)	137.00 (6.68) [114.00 - 159.00]	128.77 (6.68) [94.90 - 145.00]	8.23 (6.28) [-10.00 - 36.00]	-4.99, 21.46	0.207	(111.00 - 154.00) [76.96, 179.64]
Glycerol (% DW)	0.16 (0.0085) [0.11 - 0.21]	0.14 (0.0085) [0.12 - 0.18]	0.019 (0.0097) [-0.023 - 0.048]	-0.00090, 0.039	0.060	(0.097 - 0.18) [0.024, 0.25]
Glycine Betaine (ppm FW)	84.24 (9.15) [66.40 - 104.00]	76.27 (9.15) [55.60 - 91.00]	7.98 (11.23) [-17.40 - 24.90]	-15.64, 31.59	0.486	(4.46 - 147.00) [0, 271.19]
Salicylic Acid (ppm DW)	0.14 (0.049) [0.072 - 0.21]	0.19 (0.049) [0.060 - 0.30]	-0.046 (0.045) [-0.15 - 0.060]	-0.14, 0.051	0.327	(0.11 - 0.34) [0, 0.51]
Fructose (% DW)	8.56 (1.15) [6.74 - 10.29]	9.06 (1.15) [7.63 - 9.80]	-0.50 (0.89) [-1.86 - 0.77]	-2.38, 1.38	0.581	(4.32 - 10.04) [1.20, 14.57]
Glucose (% DW)	9.22 (1.07) [7.31 - 10.43]	9.68 (1.07) [7.63 - 11.04]	-0.45 (0.96) [-2.26 - 0.87]	-2.48, 1.58	0.642	(4.19 - 11.67) [1.01, 16.70]
Sucrose (% DW)	0.46 (1.07) [0.10 - 1.03]	0.86 (1.07) [0.094 - 2.35]	-0.40 (1.11) [-1.58 - 0.46]	-2.61, 1.82	0.722	(0.076 - 5.36) [0, 9.76]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-29. Comparison of the Additional Secondary Metabolite Composition of Grain from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Well-Watered Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Free Proline (% DW)	0.058 (0.011) [0.029 - 0.090]	0.055 (0.011) [0.030 - 0.083]	0.0026 (0.0035) [-0.0036 - 0.010]	-0.0048, 0.0099	0.476	(0.0093 - 0.076) [0, 0.12]
Abscisic Acid (ppb FW)	9.73 (2.00) [3.61 - 20.70]	11.49 (2.00) [7.24 - 21.90]	-1.76 (2.71) [-10.40 - 8.40]	-7.16, 3.63	0.517	(9.48 - 116.00) [0, 162.21]
Choline (ppm FW)	219.89 (13.09) [181.00 - 255.00]	235.78 (13.09) [203.00 - 265.00]	-15.89 (10.18) [-48.00 - 33.00]	-37.49, 5.71	0.138	(174.00 - 264.00) [129.07, 327.26]
Glycerol (% DW)	0.023 (0.0035) [0.020 - 0.029]	0.022 (0.0035) [0.017 - 0.030]	0.0013 (0.0024) [-0.0017 - 0.0051]	-0.0035, 0.0061	0.595	(0.015 - 0.037) [0, 0.048]
Glycine Betaine (ppm FW)	2.27 (0.33) [1.31 - 3.11]	2.41 (0.33) [1.32 - 4.19]	-0.14 (0.26) [-1.08 - 0.36]	-0.70, 0.41	0.588	(0.50 - 7.67) [0, 12.03]
Salicylic Acid (ppm FW)	0.088 (0.018) [0.065 - 0.12]	0.094 (0.018) [0.069 - 0.11]	-0.0060 (0.016) [-0.030 - 0.030]	-0.039, 0.027	0.705	(0.061 - 0.71) [0, 0.95]
Fructose (% DW)	0.44 (0.029) [0.34 - 0.50]	0.44 (0.029) [0.25 - 0.53]	-0.0074 (0.034) [-0.19 - 0.24]	-0.078, 0.063	0.830	(0.21 - 0.57) [0, 0.87]
Glucose (% DW)	0.46 (0.029) [0.35 - 0.55]	0.48 (0.029) [0.34 - 0.56]	-0.018 (0.035) [-0.20 - 0.069]	-0.091, 0.054	0.607	(0.23 - 0.54) [0.038, 0.81]
Sucrose (% DW)	1.77 (0.17) [1.47 - 2.49]	1.82 (0.17) [1.40 - 2.47]	-0.053 (0.076) [-0.33 - 0.085]	-0.21, 0.11	0.490	(1.47 - 2.86) [0.41, 3.46]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-30. Comparison of the Additional Secondary Metabolite Composition of Forage from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Free Proline (% DW)	0.018 (0.0025) [0.013 - 0.027]	0.018 (0.0025) [0.011 - 0.025]	-0.00028 (0.0018) [-0.0092 - 0.0069]	-0.0041, 0.0035	0.876	(0.011 - 0.025) [0, 0.036]
Abscisic Acid (ppb FW)	31.60 (7.97) [20.40 - 54.30]	29.42 (7.97) [15.80 - 56.60]	2.18 (9.46) [-31.10 - 31.80]	-17.91, 22.27	0.820	(16.00 - 58.50) [0, 94.59]
Choline (ppm FW)	155.11 (6.68) [134.00 - 181.00]	145.89 (6.68) [136.00 - 163.00]	9.22 (6.28) [-13.00 - 34.00]	-4.00, 22.45	0.159	(118.00 - 166.00) [66.54, 217.46]
Glycerol (% DW)	0.14 (0.0085) [0.11 - 0.17]	0.14 (0.0085) [0.12 - 0.17]	-0.0038 (0.0097) [-0.029 - 0.042]	-0.024, 0.016	0.697	(0.10 - 0.19) [0.025, 0.24]
Glycine Betaine (ppm FW)	116.80 (9.15) [73.20 - 138.00]	119.79 (9.15) [89.60 - 176.00]	-2.99 (11.23) [-64.00 - 32.50]	-26.61, 20.63	0.793	(7.19 - 189.00) [0, 357.15]
Salicylic Acid (ppm FW)	0.21 (0.049) [0.10 - 0.58]	0.24 (0.049) [0.12 - 0.49]	-0.024 (0.045) [-0.39 - 0.34]	-0.12, 0.073	0.607	(0.12 - 0.47) [0, 0.82]
Fructose (% DW)	11.12 (1.15) [7.77 - 20.17]	10.91 (1.15) [7.52 - 14.95]	0.21 (0.89) [-2.41 - 7.53]	-1.67, 2.09	0.813	(7.53 - 14.83) [0.69, 18.60]
Glucose (% DW)	12.31 (1.07) [8.60 - 20.87]	11.87 (1.07) [8.60 - 15.05]	0.43 (0.96) [-2.82 - 8.81]	-1.59, 2.46	0.655	(8.11 - 15.87) [1.24, 20.22]
Sucrose (% DW)	2.27 (1.07) [0.10 - 6.36]	1.88 (1.07) [0.11 - 4.07]	0.39 (1.11) [-3.62 - 4.83]	-1.82, 2.60	0.724	(0.12 - 4.68) [0, 8.87]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-31. Comparison of the Additional Secondary Metabolite Composition of Grain from MON 87460 and Conventional Control for Combined-Sites from the 2006/2007 Chile Production Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Free Proline (% DW)	0.051 (0.011) [0.029 - 0.076]	0.058 (0.011) [0.029 - 0.079]	-0.0063 (0.0035) [-0.027 - 0.016]	-0.014, 0.0011	0.089	(0.013 - 0.056) [0, 0.11]
Abscisic Acid (ppb FW)	11.43 (2.00) [8.78 - 17.70]	13.54 (2.00) [6.79 - 23.90]	-2.11 (2.71) [-15.12 - 7.01]	-7.50, 3.29	0.438	(7.37 - 120.00) [0, 176.41]
Choline (ppm FW)	238.11 (13.09) [191.00 - 308.00]	241.56 (13.09) [209.00 - 284.00]	-3.44 (10.18) [-45.00 - 76.00]	-25.04, 18.16	0.739	(202.00 - 306.00) [104.72, 381.48]
Glycerol (% DW)	0.030 (0.0035) [0.023 - 0.049]	0.029 (0.0035) [0.018 - 0.043]	0.00069 (0.0024) [-0.020 - 0.017]	-0.0041, 0.0055	0.776	(0.019 - 0.045) [0, 0.060]
Glycine Betaine (ppm FW)	2.21 (0.33) [1.52 - 3.24]	1.99 (0.33) [1.18 - 3.98]	0.21 (0.26) [-2.22 - 1.68]	-0.34, 0.77	0.421	(0.50 - 11.40) [0, 21.14]
Salicylic Acid (ppm FW)	0.11 (0.018) [0.073 - 0.19]	0.12 (0.018) [0.084 - 0.15]	-0.0026 (0.016) [-0.074 - 0.060]	-0.036, 0.031	0.871	(0.057 - 0.60) [0, 1.00]
Fructose (% DW)	0.47 (0.029) [0.37 - 0.60]	0.48 (0.029) [0.38 - 0.63]	-0.011 (0.034) [-0.26 - 0.19]	-0.082, 0.059	0.739	(0.29 - 0.74) [0, 1.12]
Glucose (% DW)	0.48 (0.029) [0.38 - 0.59]	0.50 (0.029) [0.39 - 0.64]	-0.015 (0.035) [-0.26 - 0.17]	-0.087, 0.058	0.671	(0.32 - 0.77) [0, 1.17]
Sucrose (% DW)	1.63 (0.17) [1.33 - 1.86]	1.86 (0.17) [1.37 - 2.27]	-0.23 (0.076) [-0.72 - 0.17]	-0.39, -0.069	0.008	(1.41 - 2.19) [0.61, 2.84]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-32. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control from the 2006/2007 Chile Production Conducted under Well-Watered Conditions

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[2]
Forage						
Combination of all sites						
Abscisic Acid (ppb FW)	37.03	15.66	136.54	0.040	(11.90 - 122.00)	[1.22, 33.02]
CL						
Abscisic Acid (ppb FW)	75.23	15.63	381.24	0.003	(18.50 - 122.00)	[1.22, 33.02]
CT						
Free Proline (% DW)	0.027	0.019	40.22	0.025	(0.023 - 0.031)	[0, 0.042]
Choline (ppm FW)	135.67	108.97	24.50	<0.001	[114.00 - 148.00]	[76.96, 179.64]

[1]DW= dry weight; FW=fresh weight.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table VII-33. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control from the 2006/2007 Chile Production Conducted under Water-Limited Conditions

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	99% Tolerance Interval[2]
Forage						
CL						
Glycine Betaine ppm FW)	102.40	147.00	-30.34	0.016	[73.20 - 122.00]	[0, 357.15]
Salicylic Acid (ppm FW)	0.11	0.33	-68.43	0.002	[0.10 - 0.11]	[0, 0.82]
CT						
Abscisic Acid (ppb FW)	42.27	23.57	79.35	0.043	[32.90 - 54.30]	[0, 94.59]
Choline (ppm FW)	157.00	144.00	9.03	0.024	[153.00 - 160.00]	[66.54, 217.46]
LUM						
Choline (ppm FW)	167.67	151.67	10.55	0.035	[153.00 - 181.00]	[66.54, 217.46]
Salicylic Acid (ppm FW)	0.41	0.26	57.57	0.016	[0.27 - 0.58]	[0, 0.82]
Grain						
Combination of all sites						
Sucrose (% DW)	1.63	1.86	-12.40	0.008	[1.33 - 1.86]	[0.61, 2.84]
CL						
Abscisic Acid (ppb FW)	10.03	16.59	-39.52	0.041	[8.78 - 11.90]	[0, 176.41]
Glycerol (% DW)	0.030	0.023	31.03	0.045	[0.025 - 0.034]	[0, 0.060]
CT						
Sucrose (% DW)	1.71	2.03	-15.85	0.010	[1.44 - 1.86]	[0.61, 2.84]
LUM						
Sucrose (% DW)	1.41	1.58	-10.55	0.040	[1.33 - 1.54]	[0.61, 2.84]

[1]DW= dry weight; FW=fresh weight.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

SECTION 5. Substantial Equivalence of MON 87460 to Conventional Corn

A detailed compositional assessment of key nutrients, anti-nutrients and other components in grain and forage confirms that MON 87460 is substantially equivalent to conventional corn. Data from a set of six sites in the U.S. that are representative of typical agronomic practices in the U.S. Corn Belt and a set of three sites in Chile that considered well-watered and water-limited conditions all support this conclusion. Supplemental data on secondary metabolites potentially associated with stress tolerance extend the comparison beyond the typical list of compositional analytes and further confirm that there are no biologically meaningful compositional differences in forage and grain from MON 87460 and conventional corn. This conclusion extends to the foods and feeds produced from MON 87460.

Collectively, these data and a history of safe use of the host organism, corn, as a common source of human food and animal feed, support a conclusion of "no concerns" for every criterion specified in the flowcharts outlined in the FDA's Food Policy document (FDA, 2002) (FigureVII-1). MON 87460 is not materially different in composition, safety or agronomic characteristics from conventional corn other than its ability to produce better yields than the conventional control under water-limited conditions. Sales and consumption of corn grain and processed products derived from MON 87460 would be fully consistent with the FDA's Food Policy, the Federal Food, Drug and Cosmetic Act, and current practices for the development and introduction of new corn varieties.

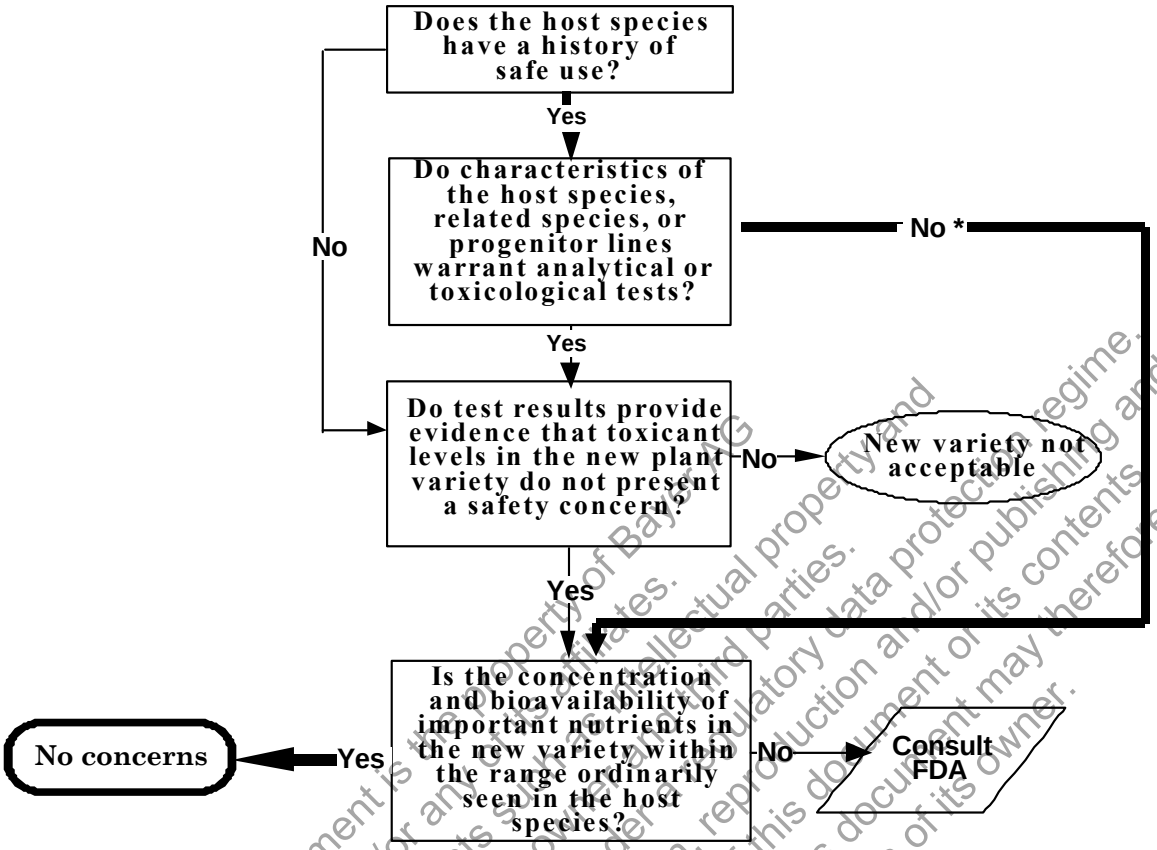

Figure VII-1. Safety Assessment of New Varieties: the Host Plant

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APPENDICES

APPENDIX A. Materials and Methods Used for Molecular Analyses of MON 87460

Materials

The DNA used in molecular analyses was isolated from MON 87460 seeds (seed lot number GLP-0604-17132-S). Additional DNA extracted from seeds of various generations of MON 87460 (see lot numbers GLP-0704-18549-S; GLP-0604-17132-S; GLP-0604-17132-S; GLP-0704-18550-S; GLP-0609-17631-S; GLP-0609-17631-S; GLP-0703-18435-S) was used in generation stability analyses. The control DNA was isolated from the seed of a conventional corn with the same genetic background (seed lot number GLP-0604-17133-S). The reference substances included the PV-ZMAP595 plasmid, probe templates generated from this plasmid, and the size estimation molecular weight standards. As a positive control on Southern blots, PV-ZMAP595 plasmid DNA was digested with combination of enzymes to produce the banding patterns that were most relevant to the assessment of the test substance digested with appropriate enzyme(s). The plasmid DNA was was digested first and then added to pre-digested conventional corn genomic DNA. The molecular weight standards include the 1 kb DNA Extension Ladder (Invitrogen) and λ DNA/*Hin*d III fragments (Invitrogen) for size estimations on Southern blots. The 100 bp and 500 bp DNA ladders (Invitrogen) were used for size estimations for PCR analyses.

Characterization of the Materials

The quality of the source materials from MON 87460 and conventional corn were verified by PCR analysis to confirm the presence or absence of MON 87460 except the materials used in the generational stability analyses where the identity of the materials was confirmed by the generation stability Southern blots themselves. The stability of the genomic DNA was confirmed in each Southern analysis by observation of the digested DNA sample on an ethidium bromide-stained agarose gel.

DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA samples from MON 87460 and conventional corn used in the insert and copy number, copy number of each element, backbone analysis, and PCR analyses were isolated from corn seeds that were processed into a fine powder using a Harbil® 5G high-speed paint shaker. DNA was extracted from the processed seeds using the method described by Rogers and Bendich (Rogers and Bendich 1985).

Genomic DNA was isolated using the following method. Place about 6 grams of processed seed tissue in a 50 mL centrifuge tube and add ~16 ml of cetyltriethylammonium bromide (CTAB) extraction buffer [1.5% CTAB, 75 mM tris(hydroxymethyl)aminomethane (Tris) pH 8.0, 100 mM ethylenediaminetetraacetic acid (EDTA), 1.05 M NaCl, 0.75% polyvinyl pyrrolidone (PVP) (40K)] and 8-10 µL of 10 mg/mL RNase. Incubate the samples at 65°C for 25-35 minutes and mix halfway through the incubation. Let the samples cool to room temperature, and add 16 mL of 24:1 chloroform: isoamyl alcohol, mix for 5 minutes and centrifuge for 5 minutes at 16,000 × *g* and 20-25°C to separate the aqueous and organic phases. Transfer the upper aqueous phase to a clean 50 mL centrifuge tube, add 1.6 mL of 10% (w/v) CTAB (10% CTAB, 0.71M NaCl) solution, mix by inversion, and add 16 mL of 24:1 chloroform: isoamyl

alcohol. Mix the tubes for 5 minutes before centrifuge for 5 minutes at 16,000 × *g* and 20-25°C to separate the aqueous and organic phases. Transfer the upper aqueous phase to a clean 50 mL centrifuge tube which contains 15 mL of CTAB precipitation buffer (1% CTAB, 50 mM Tris HCl, pH 8.0, 10 mM EDTA, pH 8.0). Mix the tubes gently by inversion, and let stand at RT for 50-70 minutes. Centrifuge for 9-11 minutes at 16,000 × *g* and 20-25°C to pellet the DNA. Discard the supernatant. Add 2 mL of high salt TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0, 1M NaCl) and incubate at 35-60°C with gentle shaking until the pellet goes into solution. Precipitate the DNA by adding 1/10 the volume of 3 M sodium acetate, pH 5.2, and two times the volume of 100% ethanol. Mix by inversion. Remove the DNA using a pipet tip, inoculation loop, or closed pasteur pipet. Place the DNA in a clean 1.5 mL microcentrifuge tube containing 0.5-1.0 mL of 70% (v/v) ethanol, microcentrifuge for 5 minutes at maximum speed to pellet the DNA, and discard the supernatant. Dry the DNA pellet by vacuum drying for ≤10 minutes or by air drying for ≤2 hours. Resuspend the DNA pellet in 500-1000 µL of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA, pH 8.0). To facilitate resuspension of the DNA, additional TE buffer may be added and/or the solution may be heated up to 70°C for 1-4 hours. Store the DNA in a 4°C refrigerator or -20°C or -80°C freezer.

Quantification of Genomic DNA

Quantification of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer with Roche molecular size marker IX as a DNA calibration standard.

Restriction Enzyme Digestion of Genomic DNA

Approximately 10 or 20 µg of genomic DNA extracted from the test and control substances were used for restriction enzyme digestions. When digesting genomic DNA with *Hin*d III (Roche) or *Eco*R V (Roche), 10X Buffer B (Roche) was used. When digesting genomic DNA with the enzyme combination *Eco*O109 I (New England BioLabs, Beverly, MA) and *Not* I (Roche), NEbuffer 4 (New England BioLabs) was used. Finally, 100× BSA (New England BioLabs) was added to the *Eco*O109 I/*Not* I digests to a final concentration of 1×. All digests were performed at 37°C in a total volume of approximately 500 µl using ~100 units of the appropriate restriction enzyme(s).

DNA Probe Preparation for Southern Blot Analyses

Probe template DNA containing sequences of plasmid PV-ZMAP595 was prepared by PCR amplification using a standard procedure based on Sambrook and Russell (Sambrook and Russell 2001). Approximately 25 ng of each probe template were radiolabeled with either ^{32}P-deoxycytidine triphosphate (dCTP) or ^{32}P-deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using the random priming method (RadPrime DNA Labeling System, Invitrogen) or PCR method. Probe locations relative to the genetic elements in plasmid PV-ZMAP595 are depicted in Figure IV-6 and Figure IV-7.

Southern Blot Analyses of Genomic DNA

Digested DNA was separated using 0.8% (w/v) agarose gel electrophoresis. Except for generational stability analyses, DNA samples were loaded on the gels for a long run and a short run in an effort to provide better resolution of larger DNA fragments while retaining smaller DNA fragments on the gel. After transferring the DNA to the membrane, Southern blots were hybridized at 55°C, 60°C, or 65°C. The table below lists the temperature and radiolabeling conditions of the probes used in this study. Multiple

exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

Probe	DNA Probe	Labeling Method	Probe labeled with dNTP (^{32}P)	Hybridization Temperature (°C)
1	T-DNA Probe 1	RadPrime	dCTP	65
2	T-DNA Probe 2	RadPrime	dCTP	65
3	T-DNA Probe 3	RadPrime	dCTP	65
4	Backbone Probe 1	RadPrime	dCTP	65
5	Backbone Probe 2	RadPrime	dCTP	65
6	Backbone Probe 3	RadPrime	dCTP	65
7	P-*Ract1* Probe	RadPrime	dCTP	65
8	I-*Ract1* Probe	PCR	dCTP	65
9	CS-*cspB* Probe	PCR	dATP	60
10	T-*tr7* Probe	PCR	dATP	55
11	*loxP* + P-*35S* Probe	RadPrime	dCTP	60
12	CS-*nptII* Probe	RadPrime	dCTP	65
13	T-*nos* + *loxP* + Left Border Probe	RadPrime	dATP	60

dNTP = deoxyribonucleotide triphosphate

DNA Sequence Analyses of the MON 87460 Insert

Overlapping PCR products were generated that span the insert in MON 87460. These products were sequenced to determine the nucleotide sequence of the insert in MON 87460 as well as the nucleotide sequence of the genomic DNA flanking the 5' and 3' ends of the insert.

The PCR analyses were conducted using approximately 75 ng of genomic DNA template or approximately 10 ng of plasmid DNA in a 50 µl reaction volume containing a final concentration of 2 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM each deoxyribonucleotide triphosphate (dNTP), and 1 unit of DNA polymerase mix. The DNA polymerase mix used to generate the products was Platinum *Taq* (Invitrogen) or Platinum High Fidelity *Taq* (Invitrogen).

Aliquots of each PCR reaction were separated on a 1.0 % (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. The PCR product was sequenced with the multiple primers used for PCR amplification. In addition, primers internal to the PCR primers were used to sequence other regions of the amplified product. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

PCR and DNA Sequence Analyses of the Parental Corn Genome

To demonstrate that the DNA sequences flanking the insert in MON 87460 are native to the corn genome, PCR analysis was performed on genomic DNA from both MON 87460 and conventional corn. The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87460. One primer designed from the genomic DNA sequence flanking the 5' end of the insert was paired with a second primer located in the genomic DNA sequence flanking the 3' end of the insert.

The PCR analyses were conducted using approximately 75 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgCl_2$, 0.2 µM of each primer, 0.1 mM each dNTP, and 1 unit of Platinum *Taq* DNA polymerase High Fidelty (Invitrogen).

Aliquots of each PCR reaction were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the product was of the expected size prior to sequencing. The PCR product was sequenced with the primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

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APPENDIX B. Supplemental Information on the Function of CSPB in MON 87460

Cold shock proteins confer environmental stress tolerance in bacteria

CSPB produced in MON 87460 belongs to the cold-shock protein (CSP) family, which has been extensively studied in bacteria. Early investigations of bacterial responses to cold-induced stress led to the discovery of CSPs, a group of small proteins that contain a highly conserved RNA-binding sequence identified as a cold shock domain (CSD).

In bacteria, a variety of environmental stresses are known to disrupt normal cell physiology, in part due to the production of RNA secondary structures which leads to a reduction in protein synthesis. Under environmental stress, CSD-containing proteins have been shown to bind to a broad array of RNA, including RNA secondary structures (Cristofari and Darlix, 2002), leading to maintenance of mRNA levels, sustainable translation, and improved cellular function. While some members of the bacterial CSP family accumulate strictly in response to temperature shifts (Etchegaray et al., 1996), others, including the *B. subtilis* CSPB protein, are also involved in maintaining normal cellular functions at both optimal temperatures (Graumann et al., 1997) and under nutrient limitation (Anderson et al., 2006).

In actively transcribing *B. subtilis* cells, CSPB is localized around the nucleoid, co-localizing with the ribosomes (Mascarenhas et al., 2001; Weber et al., 2001). In stationary-phase cells CSPB is distributed throughout the cell, indicating that specific localization of CSPB depends on cell development stage (Weber et al., 2001). Accumulation of the CSPB protein in *B. subtilis* cells occurs after transition from exponential growth to stationary phase (Graumann et al., 1997; Graumann and Marahiel, 1999), indicating that CSPB accumulation in cells can be triggered under several stress conditions that share a common signal such as inactivation of ribosomes (Schindler et al., 1999; Graumann et al., 1997). Stability of the protein both *in vivo* and *in vitro* depends on the protein's ability to form a complex with nucleic acids, most likely mRNAs (Schindler et al., 1999). In the absence of polynucleic acids, the CSPB protein has a very low thermodynamic stability and is susceptible to rapid proteolytic degradation (Schindler et al., 1999).

The CSPB protein produced in MON 87460 is identical to the native CSPB protein produced in *B. subtilis* with the exception of one amino acid change in the second position from leucine to valine (L2V) that was necessary for cloning purposes. Bacterial CSPs are composed of approximately 67-73 amino acid residues (Graumann et al., 1997) and although typically acidic in nature, contain several positively charged amino acid residues that may facilitate binding to the negatively charged backbones of polynucleotides.

The structure of CSPB protein has been previously described (PDB accession number 1NMF) (Schindelin et al., 1993; Schindelin et al., 1994). The CSPB protein in MON 87460 consists of 66 amino acids and has an isoelectric point of 4.31. The protein is composed of five antiparallel β-strands forming a five-strand β-barrel similar to the structure of CSPA protein from *E. coli* (PDB accession number 1MJC) (Schindelin at al, 1993; Newkirk at al, 1994). Experimental evidence proposes that CSPs bind at the single-stranded mRNA loop and then progressively cover this region forcing the stem to

open (Phadtare et al., 2002). It was suggested that CSPs bind to single stranded nucleic acids, RNA and ssDNA, but do not appear to bind to dsDNA (Max et al, 2006). The stable association of CSPs with nucleic acids has been confirmed by co-crystallization of the *B. subtilis* CSPB protein in a complex with single stranded polynucleotides (Bienert et al., 2004; Max et al., 2006). The crystal structure data revealed the stoichiometry and sequence determinants of the binding of single-stranded nucleic acids to a preformed site on CSPB. All CSPs possess binding sites for single stranded nucleic acids called RNA-binding ribonucleoprotein (RNP) motifs (Newkirk et al, 1994; Schröder et al., 1995). CSPB protein, like other CSPs, contains two conserved RNP motifs: RNP1 and RNP2. Within the CSPB RNP domains four aromatic amino acids, phenylalanines 15, 27, and 30 (F15, F27, and F30) and histidine 29 (H29) are required for the double-stranded polynucleotide "melting" capability (Figure 1).

These amino acids are conserved in CSPs and are thought to be essential for their function in bacteria (Phadtare et al., 2002). *In vitro* studies suggest that by binding to RNA secondary structures, CSPs reduce the free energy required for misfolded RNA to unfold and adopt the correct configuration (Herschlag et al., 1995). These findings together with the described mechanism of RNA unfolding led to the classification of CSPs as RNA chaperones.

Figure 1. Protein Sequence of the *Bacillus subtilis* CSPB Variant
The figure shows the relative position of the β–sheets and the four aromatic amino acids (in bold, red) required for double-stranded polynucleotide "melting" capability.

CSD-containing proteins also confer environmental stress tolerance in plants

Similar to bacteria, CSD-containing proteins in plants also bind RNA, unfold RNA secondary structures caused by environmental stress, and help maintain cellular functions under stress. These plant CSD-containing proteins share a high level of similarity to the bacterial CSPs and have been shown to share *in vitro* and *in vivo* functions with bacterial CSPs (Karlson and Imai, 2003; Kim et al, 2007; Nakaminami et al., 2005 and 2006; Chaikam and Karlson, 2008; Fusaro et al., 2007). Plant CSD-containing proteins have been reported to respond to abiotic stresses in *Arabidopsis* (Fusaro et al., 2007), wheat (Karlson et al., 2002), and rice (Chaikam and Karlson, 2008), and to play an important role in various aspects of plant development (Fusaro et al. 2007; Chaikam and Karlson, 2008). Direct relationships between the ability of CSD-containing proteins to bind RNA and/or ssDNA and stress tolerance have been established (Nakaminami et al., 2006; Castiglioni et al., 2008) and results of *in vitro* experiments show that plant CSD-containing proteins can bind RNA, synthetic mRNA, and ssDNA (Sasaki et al., 2007). The apparent absence of binding sequence specificity indicates that plant CSD-containing proteins could be involved in a more general response to stress by binding RNAs and, therefore, helping cells to maintain cellular functions following the stress. CSD-

containing proteins from rice and *Arabidopsis* have been shown to be highly expressed in apical meristems, ovules, embryos, and seeds (Fusaro et al. 2007; Chaikam and Karlson, 2008) and, therefore, could potentially affect growth rate, flowering time, and seed development. The CSD-containing proteins have been localized both in the cytoplasm and the nuclei (Sasaki et al., 2007; Fusaro et al. 2007) indicating that these proteins can potentially be involved in multiple steps of RNA metabolism including localization, translation and stability.

CSPB protein expressed in MON 87460 has a similar structure and function to other CSD-containing proteins

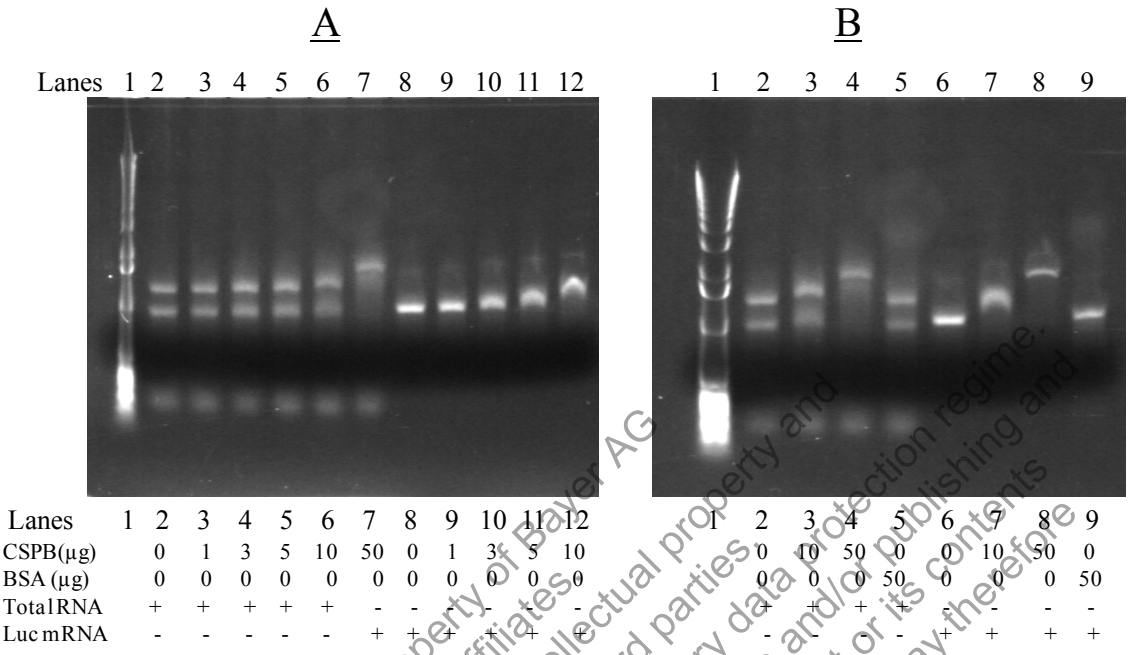
As with bacterial and other plant CSD-containing proteins, the CSPB protein produced in MON 87460, binds RNA, unfolds RNA secondary structures, and accumulates in actively growing tissues. Data from *in vitro* and *in vivo* experiments indicate that CSPB binds plant RNA, but not dsDNA. CSPB was also effective in unfolding secondary RNA structures *in vitro,* while variants of the CSPB protein (CSPB_F30R and CSPB_F30A) with impaired RNA binding functions were unable to bind and unfold RNA (Table 1). Gel shift experiments demonstrate that CSPB is capable of binding both ribosomal RNA (rRNA) and messenger RNA (mRNA) (Figure 2). To demonstrate the *in vivo* interaction between the CSPB protein and RNA in corn plants expressing CSPB, a CSPB/RNA complex was co-immunoprecipitated from leaf tissue, confirming that CSPB interacts with corn RNA *in vivo* (Figure 3).

Table 1. *In vitro* Melting Activity of CSPB and CSPB Variants

Protein	Specific Activity (pmoles opened DLP[1] /µg CSPB)
CSPB	0.905 ± 0.033
CSP_F30R	<LOD[2]
CSPB_F30A	<LOD

[1]DLP – Dual-Labeled Probe
[2]LOD – Limit of Detection

The melting activities of CSPB and the different variants were measured for all proteins using 3 µg of protein for each repeat. The activity represents the average of three repeats. In this assay, a hairpin-shaped (stem-loop) molecular beacon is labeled with a fluorophore at the 5'end and quencher at the 3'-terminus. Due to the close proximity of the fluorescent tag and quencher in the hairpin conformation, the fluorescence is efficiently quenched. When a CSPB protein "melts" the hairpin conformation, the fluorescent tag and quencher are spatially separated which permits fluorescence. CSPB is cold shock protein B. CSPB_F30A contains alanine instead of phenylalanine in position 30 and CSPB_F30R contains an arginine instead of phenylalanine in the same position. CSPB, CSPB_F30A and CSPB_F30R were produced in *E. coli*.



Lanes	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9
CSPB(µg)		0	1	3	5	10	50	0	1	3	5	10		0	10	50	0	0	10	50	0
BSA (µg)		0	0	0	0	0	0	0	0	0	0	0		0	0	0	50	0	0	0	50
Total RNA		+	+	+	+	+	-	-	-	-	-	-		+	+	+	+	-	-	-	-
Luc mRNA		-	-	-	-	-	+	+	+	+	+	+		-	-	-	-	+	+	+	+

Figure 2. Gel Shift Analysis of rRNA and mRNA

(A) Aliquots of total RNA (0.5 µg) purified from MON 87460 leaf tissue and *in vitro* transcribed Luciferase mRNA (Luc mRNA, 0.5 µg) were incubated with increasing amounts of CSPB and mixed with ethidium bromide. The shifting in the banding of the RNA was observed on 10% agarose gels under ultraviolet (UV) light.

(B) BSA was used as a control and mixed with total RNA (0.5 µg) purified from MON 87460 and Luciferase mRNA (Luc mRNA, 0.5 µg) and compared to shifts produced by CSPB. No shifts are observed for increasing amounts of BSA. Lane 1 in each gel contains a 1Kb molecular weight ladder.

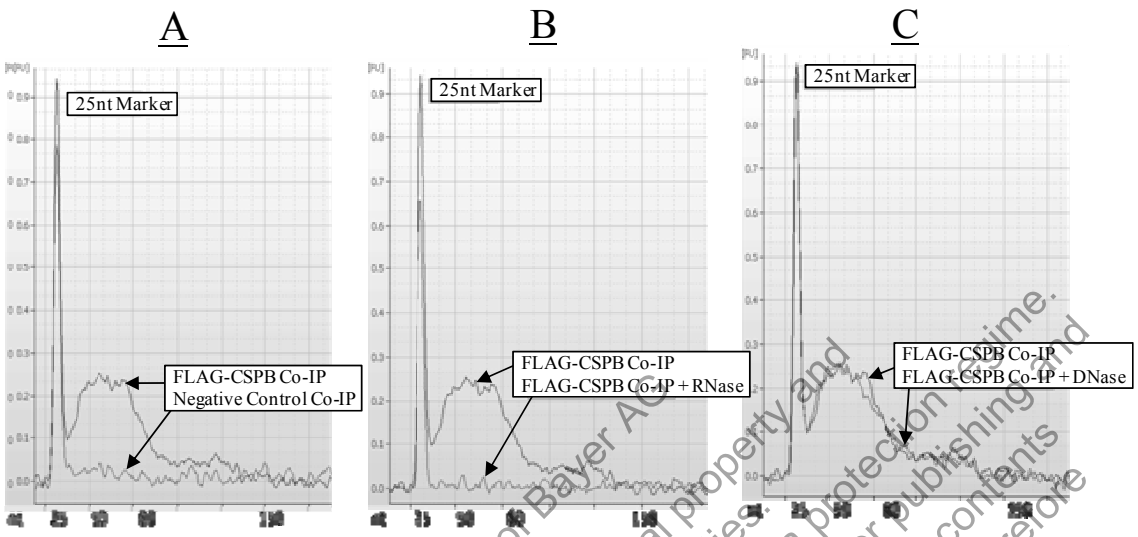


Figure 3. Co-Immunoprecipitation of CSPB-FLAG:RNA Complexes from Leaf Tissue

Leaf tissue from corn plants expressing a FLAG-tagged version of CSPB was ground and extracted. The FLAG-CSPB:RNA complexes were immunoprecipitated using anti-FLAG antibodies and analyzed on an Agilent Bioanalyzer. The results show the nucleic acid precipitated compared to a conventional leaf (A) and the digestion of the Co-IP sample with RNAse (B) and DNAse(C). The 25nt Marker shows where a 25 nucleotide (nt) sequence would elute. Results confirm that CSPB binds RNA in vitro but not DNA.

CSPB-FLAG corn plants are transgenic events containing the *cspB* coding region with an additional sequence which adds 24 nucleotides to the 3' end resulting in an eight peptide C-terminal addition, called FLAG, to the translated CSPB protein. This eight peptide group allows the FLAG-tagged protein to be co-immunoprecipitated with a commercial kit (Sigma, St. Louis, MO). The CSPB-FLAG corn expression construct contained the same promoter and terminator as those used to drive CSPB expression in MON 87460 (i.e., rice actin promoter and Tr7 terminator). The event used for the Co-IP experiments had a single copy transgene insertion and it expressed the expected protein as confirmed by western blot and MALDI-TOF mass spectrometry. Plants were kept well watered and samples were taken 20 days after planting by cutting the plant at the leaf collar of the V4 or V5 leaf, removing the leaf and harvesting the basal 1/8th of the next three youngest leaves from test and control plants.

In MON 87460, the expression of the CSPB protein is under control of the rice actin promoter which enables constitutive expression of the protein and decouples expression of CSPB protein from the cold shock response in bacteria. Using a CSPB specific-ELISA, the pattern of CSPB accumulation in MON 87460 leaf tissue and developing reproductive tissues was evaluated. CSPB accumulation is highest in rapidly growing areas of the leaf and declines as the tissue matures (Figure 4). CSPB concentrations tend to increase over time in developing ears and decline over time in silks. Likewise, CSPB concentrations increase over time in immature tassels and either remain the same or decline in pollen (Figure 5). Sub-cellular localization of CSPB was evaluated by immunohistochemistry. In MON 87460 coleoptiles, CSPB was distributed between cytoplasm and nucleus (Figure 6). Similar sub-cellular localization of CSPB was previously observed in corn protoplasts (Castiglioni et al., 2008).

Taken together, the data on RNA binding, CSPB accumulation and CSPB localization in MON 87460 are consistent with the pattern of RNA binding, accumulation, localization, and functions described for plant CSD-containing proteins (Fusaro et al. 2007; Sasaki et al., 2007; Chaikam and Karlson, 2008).

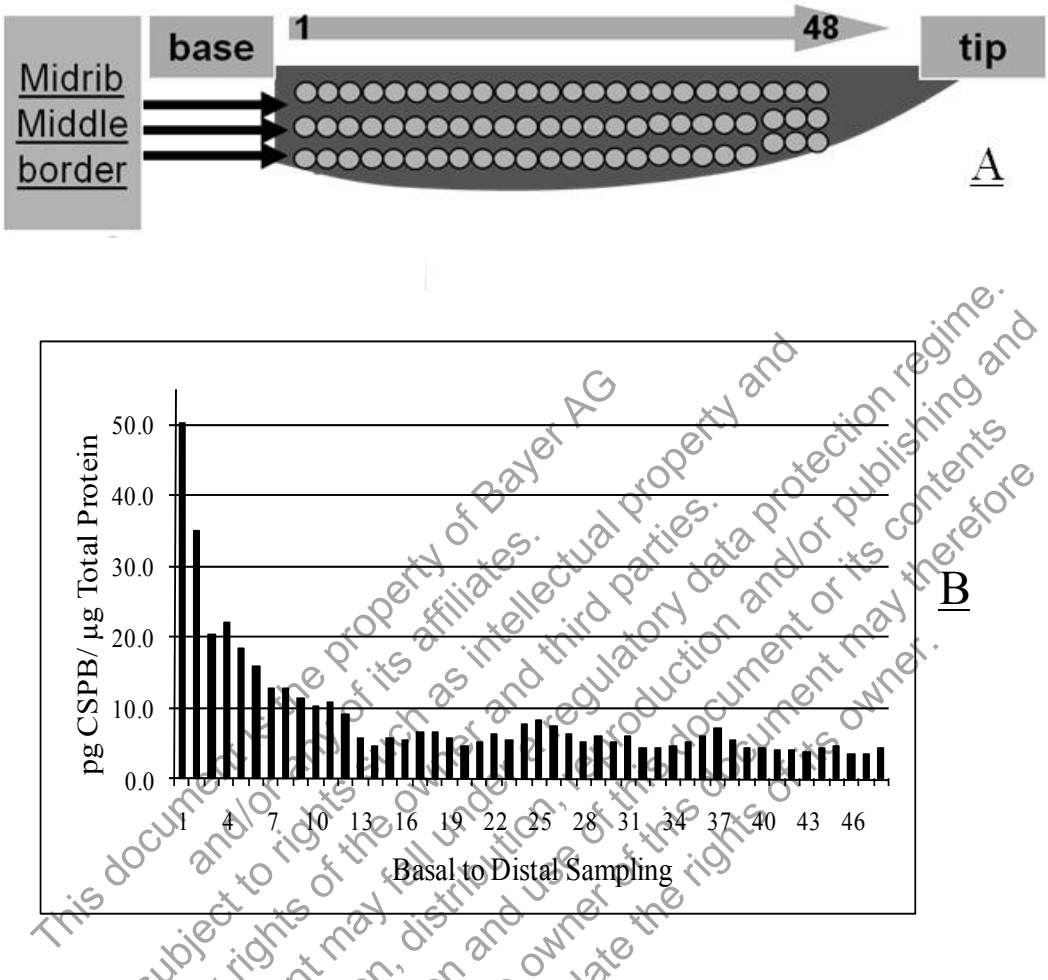


Figure 4. Differential Accumulation of CSPB Across Leaves of MON 87460

CSPB levels in different V10 leaf sections of corn MON 87460 sampled according to the leaf diagram (A) were determined using a validated CSPB-specific ELISA (B). The values represent the means of three leaf sections from two different V10 leaves. The sample locations from leaf base to leaf tip shown in panel A correspond with the numbers in the x axis of panel B. The basal, rapidly growing, portion of the leaf contained a significantly higher level of CSPB than the rest of the leaf segments, with the distal portion of the leaf having the lowest concentrations of CSPB.

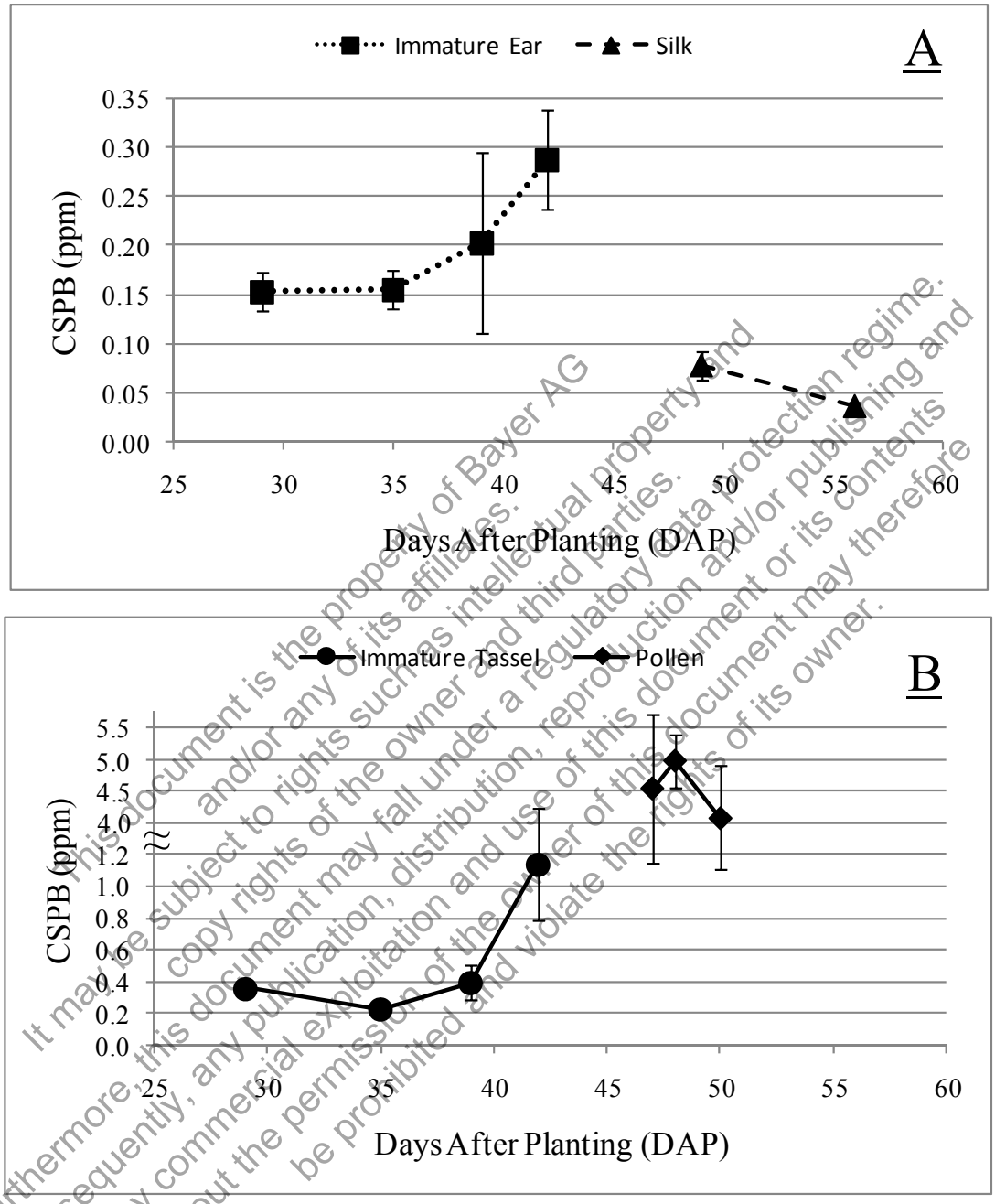


Figure 5. Expression of CSPB in Tissues of MON 87460 Grown in a Greenhouse

MON 87460 plants were grown under well-watered conditions in a greenhouse and tissues were sampled on different days after planting. CSPB levels were determined by a validated ELISA and are based on fresh weight of the tissue. (A) Immature ears and silks (B) immature tassels and pollen. CSPB levels increased in developing ears and tassels. Highest CSPB levels were detected in pollen whereas silks had the lowest levels.

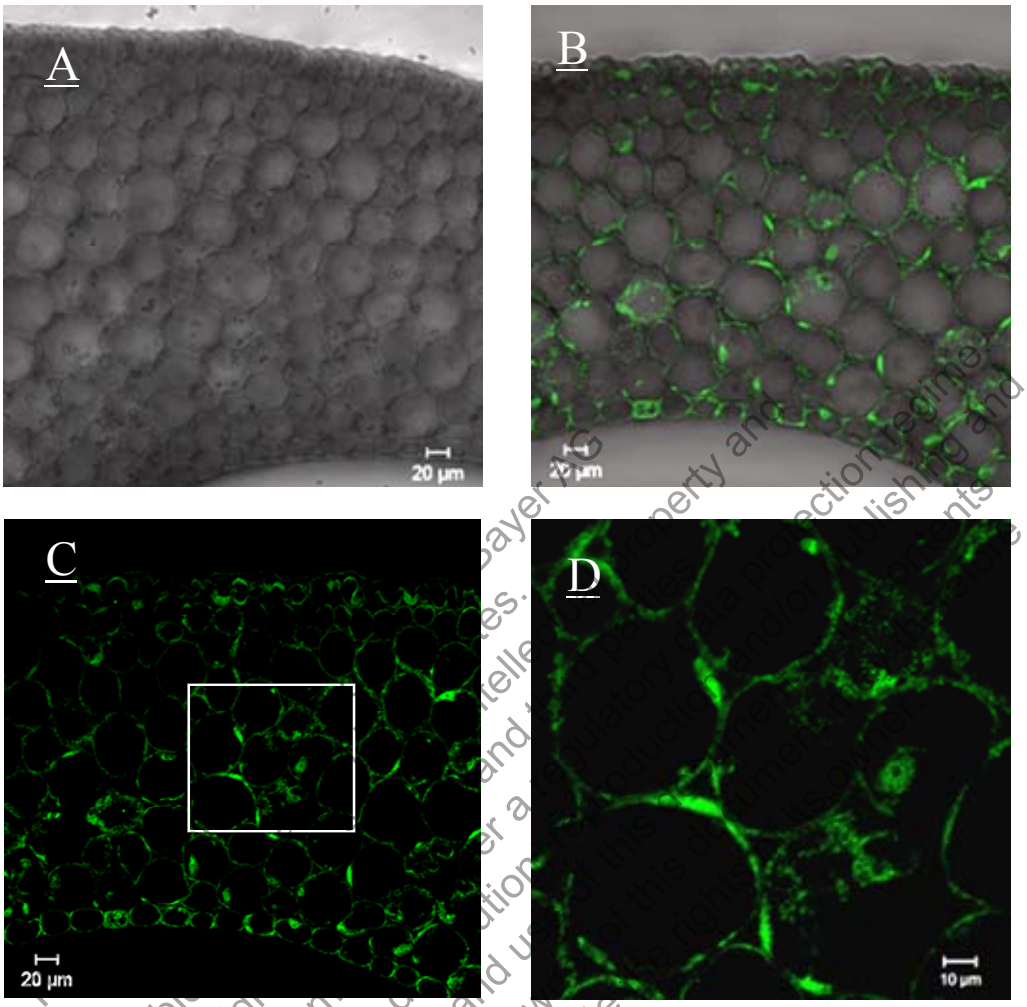


Figure 6. Immunohistochemical Localization of CSPB in MON 87460 Shoot Coleoptiles

Shoot (coleoptiles) sections from 3-day post-germination plants were incubated with affinity purified goat anti-CSPB. The CSPB antibody was detected using a fluorescently labeled small fragment secondary antibody. The control tissue (A) had no specific fluorescent signal, while in MON 87460 (B) the fluorescent signal can be clearly seen; both images are overlays with bright light to view the cells at 20X magnification. Observation of just the fluorescent signal under 20X (C) and 60X (D) magnification shows that the CSPB is localized in the nucleus and cytoplasm.

CSPB protein expression improves yield and vegetative productivity under water-limited conditions

In a greenhouse study conducted under water-limited conditions, young MON 87460 plants showed a trend toward overall better vegetative performance as evidenced by an advantage in carbon fixation rate compared to the control under water-limited conditions (Figure 7). Improved vegetative performance provides the physiological capacity necessary for the development of reproductive organs such as silks and pollen especially under drought stress. In turn, improvements in plant physiological capacity leads to increased numbers of kernels per ear and overall improved yield potential (Andrade et al., 2002; Bruce et al., 2002; Campos et al., 2006; Welcker et al., 2007; Fuad-Hassan et al., 2008; Chenu et al., 2008).

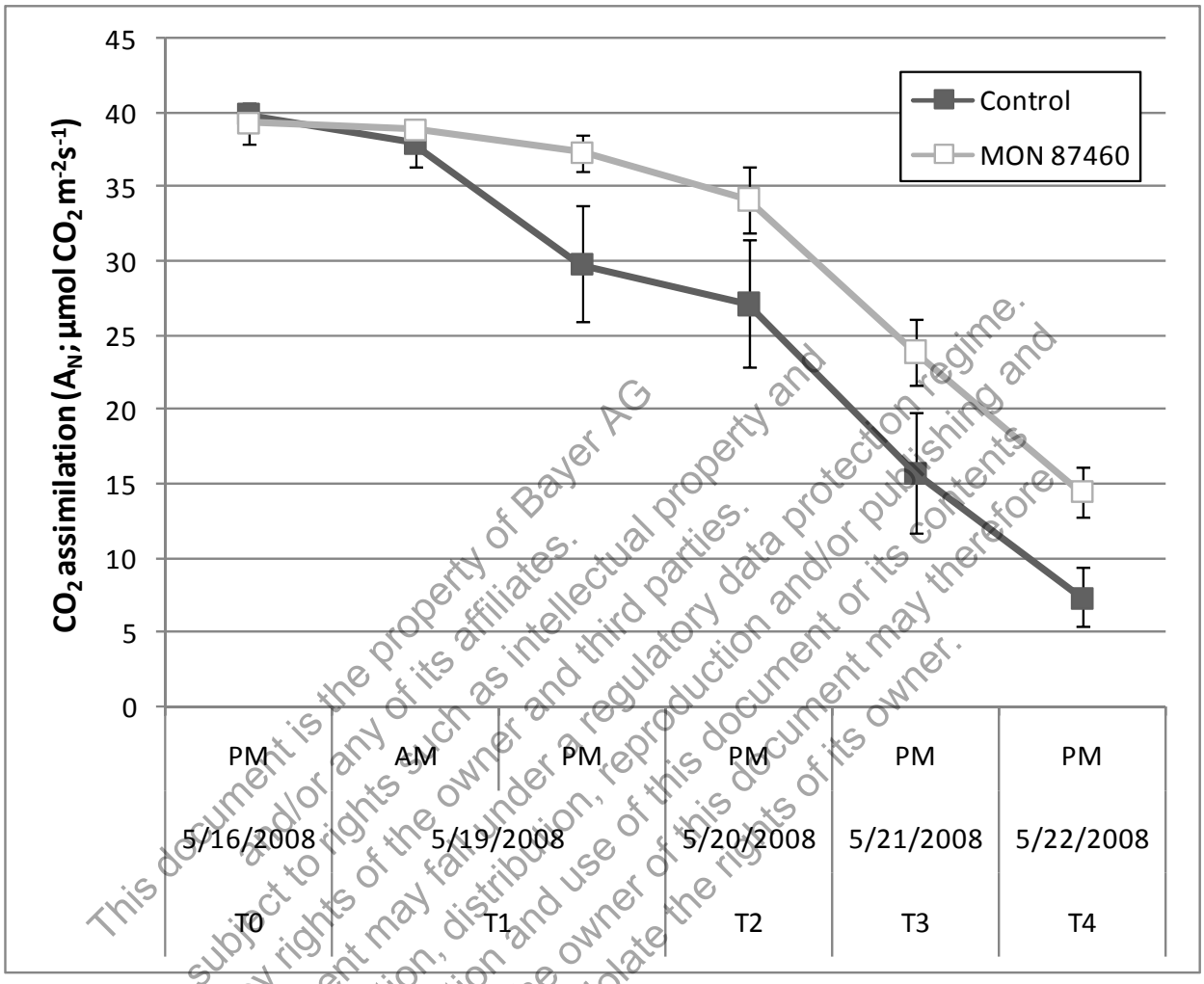


Figure 7. CO_2 Assimilation over Time for MON 87460 and the Control

Closed squares are the control, open squares are MON 87460.

Measurements of CO_2 assimilation (A_N) were obtained using a LICOR 6400 instrument. Measurements were made on small sections of mature V5 leaves midway between the base and the tip of the leaf that had reached steady-state in the measurement chamber. Gas exchange parameters were obtained under steady-state illumination. Results were obtained from six paired replicate plants of MON 87460 and the control that were subjected to six days of water-limited conditions based on pot weight beginning at the V5 growth stage.

Water limitation during the growing season can diminish corn productivity and yield, particularly during flowering and grainfill periods when corn yield potential is most sensitive to stress by disrupting fertilization and kernel development (Claassen and Shaw, 1970; Boyer and Westgate, 2004; Campos et al., 2006). Using a high through-put biotechnology approach, Castiglioni et al. (2008) demonstrated that bacterial CSPs can confer improved stress adaptation to multiple plant species. CSPB-containing events were evaluated in water-limited field trials in environments that received no rainfall during the 10 to 14 days immediately prior to flowering. The water-limited treatment resulted in an average reduction in growth rates to 50% of the well-watered rate. Using an across-event analysis, the CSPB-containing events demonstrated increases in leaf extension rates relative to the controls, improvements in chlorophyll content and improvements in photosynthetic rates. These measures of vegetative performance indicated that the CSPB protein has a positive impact on overall plant productivity and, therefore, yield potential. When plants were grown under well-watered conditions in both the greenhouse and field, no appreciable difference between CSPB-expressing lines and the control were detected.

In field trials under water-limited conditions, MON 87460 demonstrated improvements in yield and yield components through trends toward increased yield (16.5%), kernels per ear (13.1%), and kernel weight (3.9%) (Table 2). Similar results were observed a subsequent study, where differences were detected in yield (9.3%), kernels per ear (8.5%) and a trend toward increased kernel weight (2.5%) (Table 3). Results from these studies demonstrate that the major component contributing to the improved yield of MON 87460 under water-limited conditions is the increased number of kernels per ear, which is consistent with the current understanding of the effect of drought stress on corn yield potential (Westgate et al., 2004; Campos et al., 2006; Welcker et al., 2007). MON 87460 was chosen for development based on its yield advantage under water-limited conditions compared to the control and absence of negative pleiotropic effects on plant performance.

CSPB protein function: Conclusion

In summary, expression of the CSPB protein in MON 87460 results in reduced yield loss under drought conditions when compared to conventional corn grown under identical conditions. The major component contributing to the improved yield of MON 87460 under water-limited conditions is the increased number of kernels per plant, which is consistent with the current understanding of the effect of drought stress on corn yield potential. CSD-containing proteins moderate stress responses in bacteria and plants, primarily through stabilization of RNA. Like endogenous CSD-containing proteins found in plants, the CSPB protein in MON 87460 interacts with RNA, accumulates in rapidly growing tissues and localizes to the cytoplasm and nucleus, thereby helping to maintain normal cellular function in those tissues critical to yield.

Table 2. Yield Component and Physiology Data from a Kansas Field Trial in 2003

Endpoint	MON 87460	Control	Difference, MON 87460 minus control	Diff (%)	p-value
Yield (bu/ac)	80.0	68.7	11.4	16.5	0.153
Kernels per ear	289	256	33	13.1	0.233
200 kernel weight (g)	72.6	69.9	2.7	3.9	0.283
Ears per plot	33.8	33.5	0.3	1.0	0.834
Stomatal conductance ($mmol/m^2/s$)	262.7	235.8	26.9	11.4	0.064
Photosynthetic rate ($\mu mol\ CO^2/m^2/s$)	37.2	34.1	3.1	8.9	0.066
Transpiration rate ($mmol/m^2/s^1$)	6.1	5.8	0.3	5.6	0.126
Leaf extension rate (cm/5 d)	21.2	17.4	3.8	22.1	0.008

bu/ac – bushels per acre
Yield data were normalized to 15.5% moisture.
Kernel per ear measurements were collected from a subsample out of each plot.
Kernel weights were taken from 200 kernel samples from a subsample of ears.
The number of harvestable ears was counted from each of the plots at the end of the season.
MON 87460 and the control were planted in two row plots, 34 plants per row, at a density of 32,000 plants per acre. Twelve paired-plot replicates were planted in a randomized block design.
Photosynthesis measurements were made using a PP Systems Ciras-1 Portable Photosynthesis System.
Photosynthesis and leaf extension rate measurements were collected on six plants each of MON 87460 and the control.

Table 3. Yield Component and Growth Data from a California Field Trial in 2007

Endpoint	MON 87460	Control	Difference, MON 87460 minus control	Diff (%)	p-value
Well-watered yield (bu/ac)	297	295	2.3	0.8	0.568
Water-limited yield (bu/ac)	206	188	17.5	9.3	0.038
Water-limited kernels per ear	483.1	445.2	37.8	8.5	0.004
Water-limited 50 kernel weight (g)	17.4	16.9	0.426	2.5	0.068
Water-limited leaf extension rate (cm/8 d)	27.8	24.7	3.1	12.6	0.034
Water-limited plant height (cm)	220	215	4.8	2.2	0.046
Water-limited plant biomass (g)	24.0	22.0	2.0	9.1	0.376

bu/ac – bushels per acre
Yield data were normalized to 15.5% moisture.
Kernel per ear measurements were collected from a subsample out of each plot.
Kernel weights were taken from 50 kernel samples from a subsample of ears.
The well-watered treatment contained five replicates. The water-limited treatment contained 10 replicates. Each replicate consisted of four six-row plots with 20.5 foot rows planted with 50 kernels per row.
Leaf extension, plant height and plant biomass measurements were collected from three plants in each of the ten water-limited replicates.

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APPENDIX C. Materials and Methods used for Protein Characterization and Equivalence Studies

Part 1 – Cold Shock Protein B (CSPB)

Protein Purification

The MON 87460-produced CSPB protein was purified from the grain of MON 87460. The CSPB protein was purified at ~4 °C from an extract of ground grain using a combination of ammonium sulfate fractionation, anion exchange chromatography, immunoaffinity chromatography, and size exclusion chromatography. Protein purification records are archived at Monsanto Company under Orion lot 10000842, and the purification methods are described below.

The ground grain (10 kg) was mixed with a Tris-borate extraction buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) for 17 h at approximately a 1:10 sample weight to buffer volume ratio. To remove lipids, diatomaceous earth (Advanced Minerals Corp, Goleta, CA) was added to a final concentration of 7.5% (w/v) and mixed for 3 hours. The final slurry was filtered using an Ertel Alsop filter press (Kingston, NY) with Die 42 micro media filter pads and a Cuno filter (45115-12-90S, Hagedorn & Gannon Co., Inc). The filtrate was concentrated by diafiltration utilizing a polysulfone hollow fiber cartridge with a 3 kDa Molecular Weight Cut Off (MWCO) (surface area: 3.25 m^2, GE Healthcare, Piscataway, NJ). An ammonium sulfate precipitate was prepared by the addition of ammonium sulfate salt to the clarified extract to a final saturation of 40% and was allowed to dissolve overnight at 4 °C. After centrifugation, the ammonium sulfate pellet was discarded and the supernatant collected and diafiltered against 20 mM Tris-HCl pH 7.0, resulting in a final volume of 14 L.

The sample was loaded onto a 4.4 L (14 cm x 20 cm) Q Sepharose Fast Flow anion exchange resin column (GE Healthcare, Piscataway, NJ), which was equilibrated with AEC buffer A (20 mM Tris-HCl, pH 7.0). The bound CSPB was eluted with a linear salt gradient that increased from 0 M to 0.5 M sodium chloride (in AEC buffer A) over 44 L. Fractions containing MON 87460-produced CSPB protein were identified by western blot analysis and totaled 9.2 L. These fractions were pooled and concentrated using diafiltration to a final volume of ~0.6 L.

The concentrated sample containing MON 87460-produced CSPB protein was re-circulated over two AminoLink (Pierce, Rockford, IL) columns (2.4 ml: 1.6x1.2 cm; 4.4 ml: 1.6x2.2 cm) to which a monoclonal anti-CSPB antibody (Leinco Technologies Inc, St Louis, MO) had been conjugated. Bound CSPB protein was eluted using 100 mM triethylamine buffer and neutralized with 1/20th volume of 1 M sodium phosphate, pH 6.8. The process was repeated a total of 19 times to capture and elute most of the CSPB protein present in the concentrated AEC pool. After analysis of fractions by western blot, those containing CSPB protein were combined to a final volume of 205 ml. The pool was concentrated by diafiltration to approximately 27 ml and then divided into three 9 ml samples that were further purified by size exclusion chromatography on a 320 ml, 60 cm bed height, Sephacryl S-100 HR column (GE Healthcare, Piscataway, NJ) equilibrated in 20 mM Tris-HCL pH 7.0. The fractions containing CSPB protein were identified by western blot and a final pool of ~105 ml was concentrated by diafiltration with a mini cartridge to approximately 12 ml. Further concentration to 0.5 ml was accomplished by

placing the solution into a slide-A-lyzer dialysis cassette (MWCO: 3.5 kDa, size: 0.5-3 ml, Pierce, Rockford, IL) and removing the excess of solvent (~11.5 ml) by exposure to a water absorbing polymer powder (Aquacide I, EMD, Gibbstown, NJ). The concentrated pool of MON 87460-produced protein was submitted to the Analytical Protein Standard (APS) program as 23 aliquots that were assigned APS lot 1000842.

Protein Concentration

The concentration of the MON 87460-produced CSPB protein was estimated using quantitative densitometric analysis of silver stained SDS-PAGE. The *E. coli*-produced CSPB protein (amounts ranging from 10 to 60 ng) was used to create a standard curve. Aliquots of the MON 87460-produced CSPB protein and reference standard were diluted in 20 mM Tris-HCl pH 7.0 and 5× Laemmli buffer (5× LB), heated at 100.3 °C for 3 min, and applied to a pre-cast tricine 10 - 20% polyacrylamide gradient 18-well gel. A 50-fold diluted MON 87460-produced protein solution was mixed with 5× LB and three different amounts were loaded in duplicate on the gel. Electrophoresis was performed at a constant voltage of 200 V for 45 min. Pre-stained molecular weight markers (Invitrogen SeeBlue Plus2, Carlsbad, CA) were loaded in parallel.

The gel was stained using the Owl Silver Staining Kit Protocol (Owl Separation Systems, Portsmouth, NH). The following steps were performed during the gel staining procedure:

1. Fixing for 10 min in 150 ml of fixing solution (60 ml deionized water, 75 ml methanol, and 15 ml acetic acid);
2. 15 min incubation in 150 ml of a second fixing solution (82.5 ml deionized water, 45 ml methanol, 15 ml acetic acid and 7.5 ml Reagent Bottle 1);
3. 10 min incubation in 150 ml of a pretreatment solution (75 ml methanol, 7.5 ml Pretreatment Reagent, and 67.5 ml deionized water);
4. Washing for 5 min with 150 ml of deionized water;
5. 15 min staining in 150 ml of the silver staining solution (7.5 ml Staining Solution A, 7.5 ml Staining Solution B, and 135 ml deionized water);
6. Washing three times for 2 min each with 150 ml of deionized water:
7. Development of stained protein bands occurred in 150 ml of developer solution (7.5 ml Concentrated Developer and 142.5 ml deionized water) for 8 min, and was stopped by addition of 7.5 ml stopping solution for 10 min;
8. The gel was washed three times for 2 min each with 150 ml of deionized water and the gel was then changed to 50 ml of a 20% ethanol solution.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The raw data were exported to a Microsoft Excel (version 2002, SP3) file for the construction of the calibration curve and the final concentration determination of the MON 87460-produced CSPB concentration.

Immunoblot Analysis

Immunoblot analysis was performed to confirm the identity of the CSPB protein purified from MON 87460 and to compare the immunoreactivity of the MON 87460- and *E coli*-produced CSPB proteins.

The MON 87460- and *E. coli*-produced CSPB proteins were loaded onto the same gel at equal amounts of 3, 6, and 9 ng. Aliquots of each protein were diluted in 20 mM Tris-HCl pH 7.0 and mixed with 5× LB, heated at 100 °C for 3 min, and applied to a pre-cast tricine 10 - 20% polyacrylamide gradient 15-well gel. The three amounts of each protein were loaded in duplicate on the gel. Electrophoresis was performed at a constant voltage of 170 V for 70 min. Pre-stained molecular weight markers (SeeBlue Plus2 Prestained, Invitrogen, Carlsbad, CA) were loaded in parallel to verify electrotransfer of protein to the membrane and estimate the size of the immunoreactive bands observed. Electrotransfer to a 0.45 μm nitrocellulose membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 35 V.

The membrane was blocked for 1 hour with 5% (w/v) non-fat dry milk (NFDM) in 1× phosphate-buffered saline containing Tween-20 (PBST). The membrane was probed with a 1:1000 dilution of goat anti-CSPB antibody (Orion lot 10000798, aliquot # 101) in 5% (w/v) NFDM in PBST for 14 hr. Excess antibody was removed using three 10 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase-conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10000 in 5% (w/v) NFDM in PBST for 60 min. Excess HRP-conjugate was removed using three 10 min washes with PBST. All incubations were performed at room temperature, except for the primary antibody which was incubated at 4°C. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ) and exposed (10, 30, and 60 s) to BioMax XAR film (Eastman Kodak, Rochester, NY). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

The immunoreactive bands of the MON 87460-produced CSPB protein in each lane migrating to the same level as the reference standard protein were quantitated and compared to the signals corresponding to the CSPB reference standard protein. Quantitation of the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The raw data were exported to a Microsoft Excel (version 2002, SP3) file for the pair wise comparison of all the loads. An average percent difference was calculated for each comparison to determine the immunoreactivity equivalence.

N-Terminal Sequencing

N-terminal sequencing by Edman degradation was used to confirm the identity of the MON 87460-produced CSPB and to determine if the N-terminal methionine was present in the protein.

Protein Blot for N-Terminal Analysis

An aliquot of MON 87460-produced CSPB was removed from storage, diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB to a final concentration of 10 ng/□l, heated at 100.3 °C for 3 min, and loaded in duplicate at 250 ng per lane onto a tricine 10-20% gradient polyacrylamide 10-well gel. Pre-stained molecular weight markers (SeeBlue

Plus2 Prestained, Invitrogen, Carlsbad, CA) were loaded in parallel to verify electrotransfer of protein to the membrane and estimate the size of the stained bands observed. Electrophoresis was performed at a constant voltage of 170 V for 70 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 25 V. The blot was stained with Ponceau S (Sigma, St. Louis, MO) to visualize the markers and the CSPB protein.

N-Terminal Sequencing

The bands corresponding to MON 87460-produced CSPB protein were excised from the blot and N-terminal sequence analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapiller and Hood, 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise™ Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas[99] software (version 3.59a, LabSystems, Altrincham, Cheshire, England). A phenylthiohydantoin (PTH)-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to chromatographically calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 pmole β-lactoglobulin, Applied Biosystems) was analyzed before and after the analysis of the two CSPB protein bands that were analyzed as a single sample, to verify that the sequencer met performance criteria for repetitive yield and sequence identity.

MALDI-TOF Mass Analysis

MALDI-TOF mass spectrometry was used to confirm the molecular weight of the MON 87460-produced CSPB. Since the protein was determined to be very pure (97%) prior to this analysis, it was not deemed necessary to separate the protein by SDS-PAGE.

An aliquot of the MON 87460-produced CSPB protein was diluted ten-fold to a final concentration of ~12 µg/ml. Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 µl) from each of the tryptic samples were co-crystallized with 0.75 µl sinapinic acid on the analysis plate. The sample was analyzed in the 1000 to 25000 Da range using 200 shots at a laser intensity setting of 2603 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in linear mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical mass of the expected CSPB (plant) protein sequence based upon the nucleotide sequence. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ± 2 Da from the mass analyzed.

MALDI-TOF Tryptic Mass Map Analysis

MALDI-TOF mass spectrometry was used to confirm the identity of the MON 87460-produced CSPB protein. Since the protein was determined to be very pure (97%) prior to this analysis, it was not deemed necessary to separate the protein by SDS-PAGE.

An aliquot of the MON 87460-produced CSPB protein was diluted ten-fold to a final concentration of ~12 µg/ml. A 30 µl sample was transferred to a micro vial tube and evaporated to dryness in a Speed-Vac concentrator. The sample was digested for 16 hr at 37 °C with 660 ng of trypsin (Promega, Madison, WI) in 20 µl of a 25 mM ammonium bicarbonate buffer.

Ten µl of the trypsin digested sample was transferred to a separate micro vial for guanidination of the peptides using the ProteoMass™ guanidination kit (Sigma, St. Louis, MO). To the tube, 10 µl of guanidination reagent (O-methylisourea hemisulfate) solution and 10 µl of base (2.85 M NH_4OH) were added and the tube was vortexed. The tube was incubated at 65 °C for 30 min, then 10 µl of stop solution (10% trifluoroacetic acid (TFA)) was added.

Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture from a Sequazyme Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 µL) from each of the trypsin digested samples were co-crystallized with 0.75 µL α-cyano-4-hydroxycinnamic acid on the analysis plate. The sample was analyzed in the 500 to 5000 Da range using 100 shots at a laser intensity setting of 1783 and 2175 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected MON 87460-produced CSPB protein sequence based upon the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks in the 500 to 1000 Da range if there were two or more isotopically resolved peaks, and in the 1000 to 5000 Da range if there were three or more isotopically resolved peaks in the spectra. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ± 2 Da from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data. The tryptic mass map coverage was considered acceptable if ≥ 40 % of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments.

Molecular Weight and Purity Estimation – SDS-PAGE

Aliquots of the *E. coli*-produced reference standard and MON 87460-produced CSPB proteins were diluted with 20 mM Tris-HCl, pH 7.0 and mixed with 5× LB to a final protein concentration of 10 ng/µl. The MON 87460-produced protein was analyzed in duplicate at 50, 100, and 150 ng of total protein per lane. The *E. coli*-produced CSPB protein reference standard was analyzed at 100 ng of purity corrected full-length protein. All samples were heated in a thermo-block at 100.3 °C for 3 min and applied to a pre-cast tricine 10-20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Pre-stained molecular weight markers (Invitrogen SeeBlue Plus2, Carlsbad, CA) were loaded in parallel. Electrophoresis was performed at a constant voltage of 170 V for 70 min.

The gel was stained using the Owl Silver Staining Kit Protocol (Owl Separation Systems, Portsmouth, NH). The same procedure described previously in this appendix was

followed, though the solutions were proportionately adjusted to a final volume of 50 ml. Also, the development of protein bands occurred during incubation of the gel in the developer solution.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). The molecular weight markers were used to estimate the apparent molecular weight of the MON 87460-produced CSPB protein. For the purity evaluation, all visible bands within each lane were quantified. The purity and estimated molecular weight of the MON 87460-produced CSPB protein were reported as the average of the six values obtained by densitometric analysis.

Glycosylation Analysis

Glycosylation analysis was used to determine whether the MON 87460-produced CSPB protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 87460-produced CSPB protein, the *E. coli*-produced CSPB reference standard protein, and the positive controls, transferrin (~ 76 – 81kDa, Sigma-Aldrich, St. Louis, MO), and horseradish peroxidase (~ 40kDa, Pierce, Rockford, IL) were each diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB. These samples were heated at 100 °C for 3 min, cooled, and loaded on a tricine 10-20% polyacrylamide gradient 10-well mini-gel. Each sample was loaded at 25 and 50 ng per lane. SeeBlue Plus2 pre-stained protein molecular weight markers (Invitrogen) were loaded to verify electrotransfer of the proteins to the membrane and the CandyCane™ Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were loaded as positive/negative controls and markers for molecular weight. Electrophoresis was performed at a constant voltage of 170 V for 60 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen) was performed for 90 min at a constant voltage of 35 V.

Carbohydrate detection was performed directly on the PVDF membrane using the Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes). The manufacturer's protocol was followed. All steps were performed at room temperature. The PVDF membrane was fixed in 25 ml of a solution containing 50% methanol and 5% glacial acetic acid for 45 min, the solution was then changed and the membrane was incubated overnight. Two, 10 min washes (50 ml each) with 3% (v/v) glacial acetic acid (wash solution) were followed by a 20 min oxidation in 25 ml of an oxidizing solution containing periodic acid (Component C from kit). Membrane was washed three times, 10 min each, in 50 ml of wash solution. The blot was then incubated in 25 ml of Pro-Q Emerald Staining Solution that was prepared using the kit reagents. After 1 hr of staining in the dark, two 20 min, 50 ml wash cycles were followed by two 30 min, 50 ml wash cycles. The final wash cycles included two 50 ml, 1 min deionized water washes followed by three 5 min methanol washes(EMD, San Diego, CA). The blot was then scanned using the BioRad Molecular Imager FX using the Alexa 488 illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescently-labeled glycosylated proteins.

After glycosylation analysis the blot was stained to visualize the proteins present on the membrane. Proteins were stained using the SYPRO® Ruby Protein Blot Stain (Molecular Probes). Sections 2.4 to 2.6 of the manufacturer's instructions were followed and all steps were performed at room temperature and incubations were done on a shaking table.

The blot used for glycosylation was stained in 10 ml of the SYPRO staining solution for 15 min. The solution was discarded and the blot was washed twice for 5 min in 50 ml of deionized water. The blot was stored in 25 ml of deionized water. The blot was then scanned using the BioRad Molecular Imager FX using the SYPRO Ruby illumination setting (Quantity One software; version 4.6, build 036) in order to visualize the fluorescently-labeled proteins.

Functional Activity Assay

In order to assess the functional activity of the MON 87460-produced CSPB protein and to compare its activity to the *E. coli*-produced CSPB reference standard protein, aliquots of the MON 87460-produced CSPB protein and *E. coli*-produced CSPB reference standard protein were analyzed for their ability to unfold polynucleotide hairpin structures. Activity is expressed as the amount of DLP that is unfolded by CSPB. The probe consists of a custom synthesized 35-base oligonucleotide DNA fragment with a 6-FAM fluorescent label at the 5' end and a black hole quencher at the 3' end. The oligonucleotide probe forms a double strand stem of six base pairs due to complementary bases located at the 5' and 3' ends. The 23 nucleotides (dT) in the middle form a loop. and the binding of CSPB to the loop will separate the double strands of the probe, thus separating the fluorophore from the quencher, allowing fluorescence to be emitted and measured.

The assay was carried out on a micro titer plate. A calibration curve using the 6-FAM was constructed from serial dilutions of a 100 nM stock solution of the 6-FAM. The dilutions were done in Assay buffer (25 mM Tris-HCl, 100 mM NaCl, 2 mM EDTA, pH 7.5) and the final concentrations of the 6-FAM were 0.234, 0.468, 0.938, 1.875, 3.75, 7.50, and 15.00 pmoles/well. The sample wells were prepared by adding 175 µl of a reagent solution containing 0.34 µM DLP in the assay buffer. The plate was incubated at 30.1 °C for 30 min. Then, 25 µl of dilutions of each MON 87460- and *E.coli*-produced CSPB protein (3 µg total CSPB), in triplicate, were added to the test wells and the plate was incubated at 30 °C in a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, CA). The fluorescence was determined with an excitation wavelength of 485 nm and an emission wavelength of 520 nm using a template created within the SpectaMax Pro GxP software (version 5.0.1).

Storage Stability

The short-term stability of the MON 87460-produced CSPB protein during storage in a freezer set to maintain -80°C was evaluated by comparing the purity and molecular weight values obtained on day 0 to the purity and molecular weight values obtained on day 14 of storage. Day 0 stability analysis corresponds to the purity and molecular weight determination. On day 14, an aliquot was removed from a -80 °C freezer, diluted with 20 mM Tris-HCl pH 7.0 and mixed with 5× LB to a final concentration of 10 ng/µl, heated at 100 °C for 3 min, and loaded in duplicate (50, 100, and 150 ng per lane) onto a tricine 10 - 20% gradient polyacrylamide 10-well gel. Staining and densitometric analysis were performed as described for Molecular Weight and Purity Estimation-SDS-PAGE. The protein samples were considered to have undergone degradation if a > 10% decrease in purity and/or molecular weight was observed relative to the value determined on Day 0.

Part 2 – Neomycin Phosphotransferase II (NPTII)

Preparation of Protein Extracts from Leaf Samples

Frozen leaf samples from MON 87460 and conventional corn with a genetic background similar to that of the test material were extracted using phosphate saline buffer containing 0.1 % Triton X-100 (PBST) and Complete Mini (protease inhibitor) EDTA-free protein inhibitor (Roche, Indianapolis, IN) as the extraction buffer. Samples of leaf tissues (0.22 g of MON 87460 and 0.21 g of conventional control) were place in a polypropylene mesh bag from a Plant Protein Extraction kit (Pierce, Rockfold, IL) and the extraction buffer was added to the bag at a tissue to buffer ratio of 1:5 (w/v). With the open end of the bag upright and held closed, the lower portion of the bag was placed on a hard flat surface, and pressed and rubbed with the backend of a marker pen 15 times. The extracts were transferred to 1.5 ml labeled tubes and centrifuged for 5 min at approximately 15,000 × *g* at room temperature. Each supernatant was transferred to a clean, labeled 1.5 ml tube, stored on ice and used within the day for the analysis.

To produce a spiked assay control, 5 µl of a 0.5 mg/ml NPTII protein reference standard solution was mixed with 35 µl leaf extract from conventional corn. The resulting final concentration of NPTII protein was 0.0625 mg/ml.

SDS-PAGE and Immunoblotting

Aliquots of 40 µl of each sample were mixed with 10 µl of 5× loading buffer (5×LB) and heated for three min at 96.2 ºC. A pre-cast tris-glycine 4-20% gradient polyacrylamide SDS 12-well gel (Invitrogen, Carlsbad, CA) was loaded with the following samples:

5, 10, and 15 µl of leaf extract from MON 87460,

5, 10, and 15 µl of the spiked assay control,

10 µl of NPTII reference standard,

10 µl of leaf extract from conventional corn,

5 µl of Precision Plus Protein WesternC molecular weight markers in triplicate (Bio-Rad, Hercules, CA).

Electrophoresis was performed at a constant voltage of 125 V for 90 min. Proteins separated by SDS-PAGE were transferred to a nitrocellulose membrane (0.45 µm pore size, Invitrogen, Carlsbad, CA) at a constant voltage of 25 V for 90 min.

The membrane was blocked for 18 h at ~ 4 ºC with 5% (w/v) NFDM in PBST. From this point on, all incubations were performed at room temperature. The membrane was probed with a 1:2000 dilution of a rabbit anti-NPTII antibodies (Sigma. St. Louis, MO, Cat No N6412) in 1% (w/v) NFDM in PBST for 60 min. Excess antibodies were removed using three 5 min washes with PBST. The membrane was probed with HRP-conjugated goat anti-rabbit IgG (Vector lab, Burlingame, CA) secondary antibody at a dilution of 1:5000 in 1% (w/v) NFDM in PBST for 60 min. Precision Protein StrepTactin-HRP conjugates (Bio-Rad, Hercules, CA) were added to the secondary antibody incubation solution at a dilution of 1:50000 (a 10-fold dilution was made first with PBST, then a 1 to 5000 dilution was made in the incubation solution) to visualize the position of the WesternC protein molecular weight markers. Excess HRP-conjugates

were removed using three 5 min washes with PBST. Immunoreactive bands were visualized using the ECL detection system (GE healthcare, Piscataway, NJ) and films were exposed for 5, 10 and 20 s to Hyperfilm ECL high performance chemiluminescence film (GE Healthcare, GE healthcare, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Immunoblot Analysis

The 10 s exposure film was scanned using a Bio-Rad GS-800 densitometer (Hercules, CA) and used for the image analysis. The image analysis was performed using Quantity One software (Version 4.6, Bio-Rad, Hercules, CA). The apparent molecular weights of the MON 87460-produced NPTII protein and the NPTII reference standard in the spiked assay control were determined relative to the known values of the Precision Plus Protein WesternC molecular weight markers loaded on the gel. The apparent molecular weight was calculated as the average value for all loads of each sample and the average values were compared.

Equivalence criteria

The equivalence of the MON 87460- and *E. coli*-produced NPTII proteins was established by direct comparison of their apparent molecular weight and immunoreactivity with NPTII specific antibodies. The criteria for these tests were pre-established during developmental work taking into consideration the inherent variability of each analytical method. These criteria were as follows:

The immunoreactive band corresponding to the NPTII protein from the leaf extract of MON 87460 should migrate to the same position as the NPTII protein in the spiked assay control. No immunoreactive band with the same mobility as the NPTII protein reference standard should be observed in leaf extract from conventional corn.

The apparent molecular weight of the MON 87460-produced NPTII protein should be within ± 10% of the *E. coli*-produced NPTII protein in the spiked assay control.

References:

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APPENDIX D. Summary of the Tryptic Masses of the CSPB Protein Identified through MALDI-TOF MS

Table 1. Summary of the Tryptic Masses Identified for the MON 87460-Produced CSPB Protein Using MALDI-TOF Mass Spectrometry

Only experimental masses that matched expected masses are listed in the table.

Observed Mass (Da)		Expected Mass (Da)	Mass Difference (Da)	AA Position	Fragment Sequence
Crude Sample	Guanidinated Sample				
810.42	-	810.38	0.04	7-12	WFNSEK
-	852.51	852.38	0.13	7-12G	WFNSEK
885.54	-	885.48	0.06	56-64	GPQAANVTK
-	927.64	927.48	0.16	56-64G	GPQAANVTK
1878.11	-	1877.92	0.19	39-55	TLEEGQAVSFEIVEGNR
-	2903.32	2903.36	0.04	13-38G	GFGFIEVEGQDDVFVHFSAIQGEGFK

APPENDIX E. Materials and Methods used for the Estimation of CSPB and NPTII Protein Levels in Tissues of MON 87460 – U.S. 2006 and Chile 2006/2007 Studies

Tissue Processing and Protein Extraction

All samples produced at the field sites were shipped to Monsanto's processing facility in Creve Coeur, MO. During the processing step, dry ice was combined with the individual samples, and vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed samples were transferred into capped tubes and stored in a -80°C freezer until use.

CSPB and NPTII were extracted from all tissues by shaking tubes mounted in a Harbil mixer for a specified period of time. Each extraction tube contained eight ¼" diameter Chrome-steel beads, buffer and a tissue to buffer ratio as specified below.

Table 1. Protein Extraction Methods for First Season Tissue Samples

Protein	*Tissue*	*Extraction Buffer*	*T:B Ratio*[1]	*Shake Time (minutes)*	*Sample Clarification Method*
CSPB/NPTII	Leaf [2]	PBST/BSA[3]	1:100	7.0	Serum filter
CSPB/NPTII	Root[4]	PBST/BSA	1:20	7.0	Serum filter
CSPB/NPTII	Forage[5]	TB[6]	1:30	7.0	Serum filter
CSPB/NPTII	Grain	TB	1:25	10.5	Serum filter
CSPB	Silk	PBST/BSA	1:50	7.0	Serum filter
CSPB	Pollen	TB	1:50	10.5	Serum filter

[1]T:B Ratio – Tissue to buffer ratio
[2]Overseason leaf (OSL1, OSL2, OSL3, and OSL4)
[3]1x Phosphate Buffered Saline + 0.05% (w/v)Tween + 0.1%(w/v) Bovine Serum Albumin
[4]Overseason root (OSR1, OSR2, OSR3, and OSR4), forage root, and senescent root
[5]Forage, overseason whole plant (OSWP1, OSWP2, OSWP3, and OSWP4), and stover
[6]1x Tris borate buffer (0.1 M Tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20, pH 7.8).

Following shaking, insoluble material was removed from the extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The clarified extracts were aliquot, and stored frozen in a -80°C freezer until ELISA analysis.

CSPB Antibodies

Goat polyclonal CSPB-specific IgG was purified by Protein G-agarose affinity chromatography followed by affinity chromatography on AminoLink immobilized CSPB protein (lot G-812159). The concentration of the CSPB protein affinity purified IgG was determined to be 0.8 mg/ml by spectrophotometric methods. The purified antibody was stored in 137 mM NaCl, 10 mM $Na_2HPO_4 \cdot 7\ H_2O$, 1 mM KH_2PO_4, and 2.7 mM KCl, pH 7.4 (1X phosphate buffered saline (PBS)). CSPB protein affinity purified IgG was used as the well coating antibody.

Protein G agarose affinity purified goat polyclonal anti-CSPB was coupled with biotin (Sigma, St. Louis, MO) according to the manufacturer's instructions and assigned lot G-806080-2. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to HRP.

NPTII Antibodies

Rabbit polyclonal antibodies (lot G-805224) specific for the NPTII protein were purified using Protein-A agarose affinity chromatography by TechServ Associates (St. Louis, MO). The concentration of the purified IgG was determined to be 5.6 mg/ml by spectrophotometric methods. The purified antibody was stored in 20 mM potassium phosphate, 150 mM NaCl, pH 7.3 and preserved with 0.01% (w/v) sodium azide.

The purified NPTII antibodies were coupled with biotin (Sigma, St. Louis, MO) according to the manufacturer's instructions and assigned lot G-814147. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to HRP.

CSPB ELISA Method

Affinity-purified goat anti-CSPB capture antibodies were diluted in coating buffer (15 mM Na2CO3, 35 mM NaHCO3, pH 9.6) and immobilized onto 96-well microtiter plates at 2.0 µg/ml followed by incubation in a 4°C refrigerator for >8 h. Prior to each step in the assay, plates were washed with 1X PBS containing 0.05% (w/v) Tween-20 (1X PBST). Plates were blocked with the addition of 150 µl per well of 1X PBST with 1% BSA for 60 to 70 min at 37°C. CSPB protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37°C. The captured CSPB protein was detected by the addition of 100 µl per well of biotinylated goat anti-CSPB antibodies and NeutrAvidin-HRP (Pierce). Plates were developed by adding 100 µl per well of HRP substrate, 3,3',5,5'-tetramethylbenzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 3 M H3PO4. Quantitation of the CSPB protein was accomplished by interpolation from a CSPB protein standard curve that ranged from 0.05 – 1.6 ng/ml.

NPTII ELISA Method

Rabbit anti-NPTII capture antibodies were diluted in coating buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) and immobilized onto 96-well microtiter plates at 5.0 µg/ml followed by incubation in a 4°C refrigerator for ≥8 h. Prior to each step in the assay, plates were washed with 1X PBST. NPTII protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37°C. The captured NPTII protein was detected by the addition of 100 µl per well of biotinylated rabbit anti-NPTII antibodies and NeutrAvidin-HRP. Plates were developed by adding 100 µl per well of TMB. The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantitation of the NPTII protein was accomplished by interpolation from a NPTII protein standard curve that ranged from 0.094 – 3.0 ng/ml.

Moisture Analysis

All tissues were analyzed for moisture content using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared using the test and control samples of a given tissue type grown at a given site. These pools were prepared for all tissues in this study. The average percent

moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$DWCF = 1 - [Mean\ \%\ TSSP\ Moisture / 100]$$

The DWCF was used to convert protein levels assessed on a µg/g fresh weight (fwt) basis into levels reported on a µg/g dry weight (dwt) basis using the following calculation:

$$Protein\ Level\ in\ Dry\ Weight = \frac{(Protein\ Level\ Fresh\ Weight)}{(DWCF)}$$

The protein levels that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

Data Analyses

All CSPB and NPTII ELISA plates were analyzed on a SPECTRAmax Plus (Molecular Devices, Sunnyvale, CA) or a SPECTRAFluor Plus (Tecan, Research Triangle Park, NC) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO version 4.7.1 or SoftMax Pro GxP version 5.0.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a µg/g fwt basis. For all proteins, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values in µg/g fwt were also converted to µg/g dwt by applying the DWCF. Microsoft Excel 2002 (Version 10.6834.6830 SP3, Microsoft, Redmond, WA) was used to calculate the CSPB and NPTII protein levels in corn tissues.

APPENDIX F. Supplementary Data from the Chilean 2006/2007 Field Trial to Support Combined Site Analyses

Table 1. Comparison of Mean Reference Substance Phenotypic Characteristics of the Well-Watered and Water-Limited Treatments in a 2006/2007 Chilean Field Trial for Use in Site Selection

	CL[1]		CT[1]		LUM[1]		QUI[1]	
Phenotypic characteristic	**Well-Watered**	**Water-Limited**	**Well-Watered**	**Water-Limited**	**Well-Watered**	**Water-Limited**	**Well-Watered**	**Water-Limited**
Days to 50% silking	63.1	63.8	66.2	67.3	70.3	73.7*	67.7	67.1
Ear height (in)	63.4	50.9*	55.0	46.0	50.4	41.8*	63.5	63.4
Plant height (in)	110.7	79.7*	105.9	92.1	97.9	75.0*	112.0	112.8
Yield (bu/ac)	185.5	82.3*	236.5	152.3*	213.9	94.4*	203.1	196.3
Yield reduction (%)		56%		36%		56%		3%

* Indicates statistical difference within site between references in the well-watered and water-limited treatments ($p \leq .0.05$).
[1] Study sites: CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota.

An evaluation of the reference substances in the water-limited treatment at the CL, CT and LUM sites showed more than a 15% reduction in reference substance yields as a result of the water-limited treatment. Other indicators of stress were also observed. Thus, these sites are applicable to assessments requiring well-watered and water-limited comparisons. In contrast, reference substances at the QUI site did not exhibit indication of moisture stress in the water-limited treatment. Therefore, the QUI site is not applicable to assessments requiring well-watered and water-limited comparisons and was not included in any combined-site analyses.

APPENDIX G. Supplementary Protein Level Data

The appendix reports protein expression data for MON 87460 grown at an individual site (QUI) of the 2006/2007 Chile field production. As noted in Part VI, Section 2, this site was excluded from the combined-site data analysis because it did not meet the requirements specified by the intended water-limited conditions.

Table 1 presents results for CSPB and Table 2 presents results for NPTII. Evaluation of the overall data set confirmed expression levels were as expected. No unexpected values for either CSPB or NPTII were observed.

The QUI site results reported below do not impact the conclusion of protein expression studies of MON 87460 presented in Part VI.

Table 1. Summary of CSPB Protein Levels in Tissue Collected from MON 87460 Grown at the QUI Site During the 2006/2007 Chilean Growing Season

Tissue Type	Well-Watered		Water-Limited		LOQ / LOD (µg/g fwt)
	Mean (SD)[1] Range[2] (µg/g fwt.)[3]	Mean (SD) Range (µg/g dwt.)[4]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)	
OSL-1	0.46 (0.10) 0.36 - 0.56	3.1 (0.68) 2.4 - 3.7	0.47 (0.076) 0.39 - 0.53	2.5 (0.40) 2.0 - 2.8	0.015 / 0.0069
OSL-2	0.28 (0.037) 0.24 - 0.31	1.5 (0.19) 1.3 - 1.6	0.31 (0.015) 0.29 - 0.32	1.6 (0.077) 1.5 - 1.6	0.015 / 0.0069
OSL-3	0.11 (0.036) 0.084 - 0.15	0.50 (0.16) 0.37 - 0.67	0.11 (0.049) 0.061 - 0.16	0.46 (0.21) 0.26 - 0.68	0.015 / 0.0069
OSL-4	0.093 (0.0015) 0.092 - 0.095	0.44 (0.0073) 0.44 - 0.45	0.10 (0.031) 0.059 - 0.12	0.48 (0.16) 0.30 - 0.58	0.015 / 0.0069
OSR-1	0.12 (0.023) 0.10 - 0.14	1.2 (0.23) 0.95 - 1.4	0.10 (0.0063) 0.10 - 0.11	1.0 (0.063) 0.99 - 1.1	0.0020 / 0.0018
OSR-2	0.13 (0.044) 0.10 - 0.18	1.3 (0.44) 1.0 - 1.8	0.10 (0.022) 0.081 - 0.12	0.92 (0.20) 0.74 - 1.1	0.0020 / 0.0018
OSR-3	0.046 (0.010) 0.036 - 0.056	0.46 (0.10) 0.36 - 0.56	0.052 (0.023) 0.033 - 0.077	0.52 (0.23) 0.33 - 0.77	0.0020 / 0.0018
OSR-4	0.048 (0.014) 0.035 - 0.062	0.40 (0.11) 0.29 - 0.52	0.059 (0.0029) 0.056 - 0.062	0.45 (0.022) 0.43 - 0.47	0.0020 / 0.0018
OSWP-1	0.34 (0.0032) 0.34 - 0.35	3.1 (0.029) 3.1 - 3.1	0.31 (0.039) 0.29 - 0.36	2.8 (0.35) 2.6 - 3.2	0.0045 / 0.0043
OSWP-2	0.20 (0.023) 0.18 - 0.23	2.2 (0.26) 2.0 - 2.5	0.18 (0.019) 0.16 - 0.20	2.5 (0.28) 2.3 - 2.8	0.0045 / 0.0043
OSWP-3	0.082 (0.0079) 0.073 - 0.087	0.68 (0.066) 0.61 - 0.72	0.077 (0.0054) 0.071 - 0.081	0.70 (0.049) 0.64 - 0.74	0.0045 / 0.0043
OSWP-4	0.11 (0.017) 0.092 - 0.12	0.74 (0.11) 0.61 - 0.81	0.12 (0.0058) 0.11 - 0.13	0.79 (0.039) 0.76 - 0.84	0.0045 / 0.0043
Forage Root	0.0090 (0.0013) 0.0080 - 0.010	0.056 (0.0082) 0.050 - 0.066	0.0062 (0.0027) 0.0038 - 0.0092	0.041 (0.018) 0.025 - 0.061	0.0020 / 0.0018
Senescent Root	0.0051 (0.0041) 0.0021 - 0.010	0.032 (0.025) 0.013 - 0.061	0.0028 (N/A[5]) N/A	0.018 (N/A) N/A	0.0020 / 0.0018
Forage	0.023 (0.0030) 0.021 - 0.027	0.10 (0.013) 0.091 - 0.12	0.021 (0.0040) 0.017 - 0.025	0.091 (0.017) 0.073 - 0.11	0.0045 / 0.0043
Stover	0.014 (0.00059) 0.013 - 0.015	0.048 (0.0020) 0.046 - 0.050	0.012 (0.0041) 0.0082 - 0.016	0.039 (0.013) 0.026 - 0.053	0.0045 / 0.0043
Silk	0.053 (0.014) 0.036 - 0.066	0.66 (0.17) 0.45 - 0.82	0.040 (0.012) 0.032 - 0.054	0.40 (0.12) 0.32 - 0.54	0.0075 / 0.0047
Pollen	12 (1.1) 12 - 14	18 (1.7) 17 - 20	11 (0.84) 10 - 12	17 (1.2) 16 - 18	0.050 / 0.045
Grain	0.043 (0.010) 0.031 - 0.051	0.050 (0.012) 0.036 - 0.059	0.036 (0.0091) 0.029 - 0.046	0.042 (0.011) 0.034 - 0.054	0.0038 / 0.0017

[1]The mean and standard deviation were calculated across sites (n=3 for well-watered and n=3 for water-limited, except silk where n=4 for well-watered and senescent root where n=1 under water-limited).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis.
[4]Protein levels are expressed as µg/g on a dry weight basis. The dry weight values were calculated by dividing the fresh weight by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A – not applicable

Table 2. Summary of NPTII Protein Levels in Leaf, Root, Forage and Grain Samples Collected from MON 87460 Grown at the QUI Site During the 2006/2007 Chilean Growing Season

	Well-Watered		Water-Limited		
Tissue Type	**Mean (SD)[1] Range[2] (µg/g fwt.)[3]**	**Mean (SD) Range (µg/g dwt.)[4]**	**Mean (SD) Range (µg/g fwt.)**	**Mean (SD) Range (µg/g dwt.)**	**LOQ / LOD (µg/g fwt)**
OSL-1	0.42 (0.039) 0.38 - 0.45	2.8 (0.26) 2.5 - 3.0	0.50 (0.0093) 0.49 - 0.50	2.6 (0.049) 2.6 - 2.7	0.047/ 0.0090
OSR-1	0.044 (0.016) 0.025 - 0.055	0.44 (0.16) 0.25 - 0.55	0.036 (0.0094) 0.028 - 0.046	0.36 (0.094) 0.28 - 0.46	0.0075/ 0.0043
Forage	0.034 (0.0020) 0.032 - 0.036	0.15 (0.0088) 0.14 - 0.15	0.031 (0.0023) 0.028 - 0.033	0.13 (0.010) 0.12 - 0.14	0.0056/ 0.0024
Grain	<LOQ (N/A[5]) N/A	N/A (N/A) N/A	<LOQ (N/A) <LOD-<LOQ	N/A (N/A) N/A	0.0047 / 0.0024

[1]The mean and standard deviation were calculated across sites (n=3 for well-watered and n=3 for water-limited, except silk where n=4 for well-watered and senescent root where n=1 under water-limited).
[2]Minimum and maximum values were determined for each tissue type across sites.
[3]Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis.
[4]Protein levels are expressed as µg/g on a dry weight basis. The dry weight values were calculated by dividing the fresh weight by the dry weight conversion factors obtained from moisture analysis data.
[5]N/A – not applicable

APPENDIX H. Materials and Methods Used in Safety Assessment of CSPB and NPTII

1. Digestibility Assessment of CSPB in Simulated Gastric and Intestinal Fluids

1.1. Test Substance

The test substance was the *E. coli*-produced CSPB protein (historical APS lot 20-100125, current Orion lot 10000802), which was purified from the fermentation of *E. coli* transformed with plasmid pMON 106651. The DNA sequence encoding the CSPB protein was confirmed both prior to and following fermentation of *E. coli*. Records pertaining to the purification of this *E. coli*-produced CSPB protein are archived under APS lot 20-100125. The CSPB protein is stored in a -80°C freezer in a buffer containing 20mM Tris-HCl pH 8.0.

1.2. Characterization of Test Substance

The characterization of the physicochemical and functional properties of the test substance was performed under characterization plan 20-100125 and they are described in the Certificate of Analysis. The CSPB protein has a concentration of 6.7 mg/ml, a purity of 100%, and an apparent molecular weight of 6.5 kDa as determined by SDS-PAGE. The N-terminal sequence of the CSPB protein was also confirmed during characterization.

1.2. Test Systems

1.2.1. Simulated Gastric Fluid (SGF)

SGF contained the proteolytic enzyme pepsin in a buffer adjusted to an acidic pH. The SGF was prepared using a highly purified form of pepsin (Catalog number P-6887, Sigma Company, St. Louis, MO). The SGF was formulated so that ten units of pepsin activity per µg of the CSPB protein would be present in the digestion reactions. The amount of pepsin powder used to prepare SGF was calculated from the specific activity reported on the product label. Activity was assessed using a SGF activity assay, where one unit of activity is defined as a change in $A_{280\ nm}$ of 0.001 per min at 37 °C, measured as trichloroacetic acid (TCA) soluble products using hemoglobin as the substrate. The assay was used to confirm the activity before initiating the digestion of the CSPB protein. The digestion of the protein was monitored by SDS-PAGE stained gels and western blot analysis using a CSPB specific antibody.

1.2.2. Justification for Selection of the SGF Test System

In vitro digestion models are used widely to assess the nutritional value of ingested proteins based on their amino acid bioavailability. The correlation between protein allergenicity and protein stability in an *in vitro* pepsin digestion assay has been previously established (Astwood et al., 1996). The pepsin digestibility assay protocol that was used in this study was standardized by the International Life Sciences Institute (ILSI) in a multi-laboratory test and the results demonstrated that the *in vitro* pepsin digestion assay is reproducible when a common protocol is followed (Thomas et al., 2004).

1.2.3. Simulated Intestinal Fluid (SIF)

SIF contained a mixture of enzymes, known as pancreatin, in a buffer adjusted to neutral pH. SIF was prepared according to the method described in The United States Pharmacopoeia (USP, 1995). The pancreatin used for the preparation of SIF was obtained from Sigma Company (Catalog number P1500, St. Louis, MO). The SIF was formulated so that 55.3 µg of pancreatin powder would be present per µg of CSPB protein in the digestion reactions. Activity of the SIF was confirmed prior to initiating the digestion of the CSPB protein using an SIF activity assay. The digestion of the CSPB protein was monitored by western blot analysis using a CSPB specific antibody.

1.2.4. Justification for selection of the SIF Test System

In vitro digestion models are used widely to assess the digestibility of ingested substances. SIF is frequently used for *in vitro* studies to assess the digestibility of food components (Yagami et al., 2000; Okunuki et al., 2002).

1.3. Experimental Design

1.3.1. Digestibility of the CSPB Protein in SGF

Digestion of the CSPB protein in SGF was evaluated over time by analyzing specimens from targeted incubation time points. A numerical code using the numbers 0 through 7 was used to distinguish incubation time points according to the following:

Targeted Incubation Time Point	Designation(s)
0 min	SGF T0, SGF P0, SGF N0
0.5 min	SGF T1
2 min	SGF T2
5 min	SGF T3
10 min	SGF T4
20 min	SGF T5
30 min	SGF T6
60 min	SGF T7, SGF P7, SGF N7

SGF for the digestion was prepared to contain approximately 2072 U/ml of pepsin activity , by dilution of a stock SGF solution with SGF buffer lacking pepsin (10 mM HCl, 2 mg/ml NaCl, pH ~1.2). The digestion mixture was prepared by adding 27 µl of the CSPB protein to a tube containing 873 µl of pre-heated (36.9 ºC, 10 min) SGF which corresponds to 180.9 µg of CSPB protein and 1809 U of pepsin, respectively. The tube contents were mixed by vortexing and immediately placed in a 36.9 ºC water bath. Specimens (100 µl) were removed at 0.5, 2, 5, 10, 20, 30 and 60 min (corresponding to specimen time points SGF T1 through SGF T7). Each 100 µl specimen was immediately placed in a tube containing the quenching mixture, consisting of 35 µl of 0.7 M sodium carbonate buffer (0.7 M Na_2CO_3, pH 11), and 35 µl of 5× Laemmli Buffer (5× LB, 312.5 mM Tris-HCl, 25% (v/v) 2-mercaptoethanol, 10% (w/v) sodium dodecyl sulfate, 0.025% (w/v) Bromophenol Blue, and 50% (v/v) glycerol, pH 6.8).

The SGF T0 incubation time point was prepared in a separate tube. Ninety seven µl of SGF (201 U of pepsin) was quenched by the addition of 35 µl of 0.7 M sodium carbonate

buffer (0.7 M Na_2CO_3, pH 11), and 35 µl of 5× LB prior to the addition of 3 µl (20.1 µg) of the CSPB protein.

All quenched specimens, were heated to 75-100 ºC for 5-10 min, frozen on dry ice, and stored in a –80 ºC freezer until analyzed.

Experimental controls were prepared to determine the stability of the CSPB protein in the test system buffer lacking pepsin (10 mM HCl, 2 mg/ml NaCl, pH ~1.2). These experimental controls were prepared in a similar manner as described above for SGF T0, but the targeted incubation times were limited to 0 (SGF P0) and 60 min (SGF P7).

Experimental controls were also prepared to determine the stability of the test system lacking the CSPB protein. Protein storage buffer (20 mM Tris, pH 8.0) was added to SGF in place of the CSPB protein. These experimental controls were prepared in a similar manner as described above in Section 6.1 for SGF T0, but the targeted incubation times were limited to 0 (SGF N0) and 60 min (SGF N7).

All quenched specimens, were heated to 75-100 ºC for 5-10 min, frozen on dry ice, and stored in a –80 ºC freezer until analyzed.

1.3.2. Digestibility of the CSPB Protein in SGF followed by SIF

Stability of the CSPB protein was assessed by digestion in SGF followed by digestion in SIF. The digestions were evaluated by SDS-PAGE stained gels and western blot analysis using a CSPB specific antibody.

Digestion of the CSPB protein in SGF followed by SIF was evaluated over time by analyzing specimens at targeted incubation time points. A numerical code using the numbers 0 through 7 were used to distinguish incubation time points according to the following:

Targeted Incubation Time Point	Designation(s)
SGF system	
0 min	**SEQ 0min**
2 min	**SEQ 2min**
SIF system	
0 min	**SEQ T0, SEQ P0, SEQ N0**
0.5 min	**SEQ T1**
2 min	**SEQ T2**
5 min	**SEQ T3**
10 min	**SEQ T4**
30 min	**SEQ T5**
1 h	**SEQ T6**
2 h	**SEQ T7, SEQ P7, SEQ N7**

The SGF was prepared to contain approximately 2632 U/ml of pepsin activity. The digestion in SGF was prepared by adding 30 µl of the CSPB protein to a tube containing 764 µl of pre-heated (36.8 ºC, 6 min) SGF, corresponding to 201 µg of CSPB protein and 2010 U of pepsin, respectively. The tube contents were mixed by vortexing and immediately placed in a 36.9 ºC water bath. The tube was removed after 2 min, and the

reaction was immediately quenched by adding 277 µl of 0.7 M sodium carbonate buffer (0.7 M Na_2CO_3, pH 11). After quenching, an aliquot of 120 µl was removed for analysis, and mixed with 30 µl of 5× LB, and heated to 75-100 ºC for 5-10 min and designated as SEQ 2min.

For digestion in SIF, 526 µl of the quenched SGF reaction mixture was added to 550 µl of pre-heated (36.2 ºC, 10 min) SIF, corresponding to 100 µg SGF digested and quenched CSPB protein (based on the pre-digested concentration) and 5.5 mg of pancreatin. The tube contents were mixed by vortexing and immediately placed in a 36.5 ºC water bath. Digestion specimens (100 µl) were removed from the tube at 30 s, 2, 5, 10, 30 min, 1, and 2 h (corresponding to specimen time points SEQ T1 through SEQ T7) and immediately placed in a tube containing 25 µl of 5× LB, heated to 75-100 ºC for 5-10 min, and frozen on dry ice for complete quenching.

The zero incubation time point for the SGF digestion phase (SEQ 0min) was prepared in a separate tube by first quenching 76 µl of SGF (201 U of pepsin) with 27 µl of sodium carbonate buffer, and 27 µl of 5× LB buffer and heating to 75-100 ºC for 5-10 min prior to the addition of 3 µl (20.1 µg) of the CSPB protein.

The zero incubation time point for the SIF digestion phase (SEQ T0) was prepared in a separate tube by first quenching 83 µl of SIF (0.83 mg) with 40 µl of 5× LB buffer and heating to 75-100 ºC for 5-10 min prior to the addition of 79 µl (15 µg, based on the pre digestion concentration) of the SGF digested and quenched CSPB protein.

Experimental controls for the SIF digestion phase were prepared to determine the stability of the CSPB protein fragment in the SIF test system buffer lacking pancreatin (50 mM potassium phosphate monobasic, pH adjusted to 7.5 with sodium hydroxide). These experimental controls were prepared in a similar manner as described above for SEQ T0, but the targeted incubation times were limited to 0 (SEQ P0) and 2 h (SEQ P7).

Experimental controls were also prepared to characterize the test system (SIF) lacking the SGF digested and quenched CSPB protein. Protein storage buffer (20 mM Tris, pH 8.0) was added to SIF in place of the SGF digested CSPB protein. These experimental controls were prepared in a similar manner as described above for SEQ T0, but the targeted incubation times were limited to 0 (SEQ N0) and 2 h (SEQ N7).

All quenched specimens, were heated to 75-100 ºC for 5-10 min, frozen on dry ice, and stored in a –80 ºC freezer until analyzed.

1.3.3. Digestibility of the CSPB Protein in SIF

Digestion of the CSPB protein in SIF was evaluated over time by analyzing specimens at targeted incubation time points. A numerical code using the numbers 0 through 9 was used to distinguish incubation time points according to the following:

Targeted Incubation Time Point	Designation(s)
0 min	SIF T0, SIF P0, SIF N0
5 min	SIF T1
15 min	SIF T2
30 min	SIF T3
1 h	SIF T4
2 h	SIF T5
4 h	SIF T6
8 h	SIF T7
12 h	SIF T8
24 h	SIF T9, SIF P9, SIF N9

The digestion was prepared by adding 50 µl of the test substance to a tube containing 1.85 ml of pre-heated (36.6 ºC, 5 min) SIF, corresponding to 335 µg of the CSPB protein and 18.5 mg of pancreatin, respectively. The tube contents were mixed by vortexing and immediately placed in a 36.0 ºC water bath. Digestion specimens (100 µl) were removed at 5, 15, 30 min, 1, 2, 4, 8, 12, and 24 h (corresponding to specimen time points SIF T1 through SIF T9) and immediately placed in a tube containing 25 µl of 5× LB, heated to 75-100 ºC for 5-10 min, and frozen on dry ice for complete quenching.

The zero incubation time point (SIF T0) was prepared in a separate tube by first quenching 110 µl of SIF (1.1 mg) with 29 µl of 5× LB buffer and heating to 75-100 ºC for 5-10 min prior to the addition of 3 µl (20.1 µg) of the CSPB protein.

Experimental controls were prepared to determine the stability of the CSPB protein in the test system buffer lacking pancreatin (50 mM potassium phosphate, pH 7.5). These experimental controls were prepared in a similar manner as described for SIF T0, but the targeted incubation times were limited to 0 (SIF P0) and 24 h (SIF P7).

Experimental controls were also prepared to characterize the test system (SIF) lacking the CSPB protein. Protein storage buffer (20 mM Tris, pH 8.0) was added to SIF in place of the CSPB protein. These experimental controls were prepared in a similar manner as described above for SIF T0, but the targeted incubation times were limited to 0 (SIF N0) and 24 h (SIF N7).

All quenched specimens, were heated to 75-100 ºC for 5-10 min, frozen on dry ice, and stored in a –80 ºC freezer until analyzed.

1.4. Specimen Retention

All specimens will be retained in a –80 °C freezer for one year, after which they will no longer afford analytical evaluation and may be discarded.

1.5. Analytical Methods

Activity of the SGF and SIF were assessed using pepsin and pancreatin activity assays respectively. The digestibility of the CSPB protein in SGF, and in SGF followed by SIF was assessed using stained SDS-PAGE and western blot analysis. The digestibility of the CSPB protein in SIF was assessed using western blot analysis. The lower limit of detection (LOD) of the CSPB protein was determined for stained SDS-PAGE and western blots. The identity of a transiently stable fragment of ~2.5 kDa in SGF digestion was determined by N-terminal sequencing of that fragment.

1.6. SGF Activity Assays

The SGF activity assay was used to confirm the suitability of the test system before its use with the CSPB protein according to the current version of SOP BR-ME-0460. The assay is based on the ability of pepsin to digest denatured hemoglobin. Undigested hemoglobin was precipitated with TCA, and the amount of soluble peptides was estimated by measuring the absorbance at 280 nm. The amount of soluble peptides is directly proportional to the amount of protease activity. One unit of pepsin activity in this assay is defined as the amount of pepsin that will produce a change in absorbance at 280 nm of 0.001 per min at pH 1.2-2.0 at 37 ± 2 ºC. The SGF solution was formulated to contain 0.03 mg of powder per ml of SGF buffer. Acceptable specific activity (units/mg pepsin powder) for the SGF was equal to the specific activity determined by the manufacturer, ±1000 units/mg.

Since digestion of the CSPB protein in SGF and in SGF followed by SIF were performed on two separate days, two separate activity assays were performed. Both SGF solutions were diluted to 0.03 mg of solid material (pepsin) per ml of SGF [the dilution factor (DF) was 26.7]. Acidified hemoglobin [2% (w/v), 5 ml] was added to each of three replicates of the test sample and blank samples and pre-warmed at 37 ± 2 °C for 5-10 min prior to starting the reactions. Diluted SGF (1 ml) was added to each replicate of test samples and both test and blank samples were incubated at 37.1 and 36.9 °C for pepsin activity 1 and pepsin activity 2, respectively, for an additional 10 min. The reactions were stopped by the addition of 10 ml of 5% (v/v) chilled TCA to the test and blank samples. Diluted SGF (1 ml) was then added to the blank samples. Samples were mixed and then incubated for another 5-10 min at 36.9 and 37.1 °C for pepsin activity 1 and pepsin activity 2, respectively. Precipitated protein was removed by filtering the test and the blank samples using 0.8 µm syringe filters. Samples of the clarified test and blank samples were read at 280 nm in a Beckman DU-650 Spectrophotometer (Beckman Coulter, Inc., Fullerton, CA) The activities of pepsin were calculated using the following equation:

$$\frac{Mean\,Test_{A280nm} - Mean\,Blank_{A280nm}}{0.001 \times 10\,\text{min} \times 1\,ml} \times DF$$

where 0.001 is the change in the absorbance at 280 nm per min at pH 1.2-2.0 and 37 ± 2 ºC produced by one unit of pepsin activity; 10 min is the reaction time, 1 ml is the amount of SGF added to the reaction; and, DF is the dilution factor for the SGF.

1.7. SIF Activity Assay

The SIF activity assay was used to confirm the suitability of the test system before its use with the CSPB protein according to the current version of SOP BR-ME-0461. One unit of pancreatin activity in this assay is defined as an increase in the absorbance at 574 nm of 0.001 per min at 37 ± 2 ºC. An acceptable specific activity for the SIF was defined as 11,000 ± 3,000 U/ml.

The assay is based on the estimation of the amount of soluble peptides present in a TCA solution after pancreatin digestion of resorufin-labeled casein (Roche Molecular Biochemicals, Mannheim, Germany). Undigested resorufin-labeled casein is precipitated with TCA and the amount of soluble peptide is estimated in the supernatant by measuring the absorbance at 574 nm. The amount of soluble peptide is directly proportional to the amount of proteolytic activity.

Three activity replicates were incubated with 0.05× SIF (1× SIF was diluted to 0.05× SIF before the activity assay was initiated) for 15 min at 35.9 ºC. Three blank replicates were incubated with 50 mM KH_2PO_4, pH 7.5 in place of SIF. The reaction was quenched by addition of chilled 5% (v/v) TCA to activity and blank replicates. The supernatants recovered after centrifugation were neutralized by the addition of assay buffer (500 mM Tris-HCl, pH 8.8), and the absorbance of the clarified activity and blank replicates was read at 574 nm using a Beckman DU-650 spectrophotometer. The activity of SIF was calculated using the following equation:

$$\frac{Mean\,Activity_{A574nm} - Mean\,Blank_{A574nm}}{0.001 \times 15\,\text{min} \times 0.1\,ml \times 0.05}$$

where 0.001 is the change in the absorbance at 574 nm per min at 37 ± 2 ºC produced by one unit of pancreatin activity, 15 min is the reaction time, 0.1 ml is the amount of 0.05× SIF added to the reaction, and 0.05 is the SIF dilution factor.

1.7. SDS-PAGE and Colloidal Brilliant Blue G Staining

Specimens containing 1× LB from the SGF, and SGF followed by SIF *in vitro* digestions of the CSPB protein were separated by SDS-PAGE using pre-cast tricine 10-20% polyacrylamide gradient mini-gels and tricine running buffer (Invitrogen, Carlsbad, CA) according to the current version of SOP BR-ME-0388. The CSPB protein was loaded at 0.8 µg per lane based on pre-digestion total protein concentration. All experimental controls were loaded at the same volumes as those containing CSPB protein so that they would be comparable. All specimens were heated at 95.4 ºC for 5 min prior to loading on the gels. Mark 12 molecular weight markers (Invitrogen, Carlsbad, CA) were loaded in parallel to estimate the relative molecular weight of proteins and peptides visualized by staining. Electrophoresis was performed at a constant voltage of 125 V for 81 and 90 min for SGF and SGF followed by SIF digestions of the CSPB protein, respectively. After electrophoresis, proteins were visualized by staining the gels with colloidal Brilliant Blue G (Sigma, St. Louis, MO).

The colloidal Brilliant Blue G staining method was selected because it is an effective method for detecting nanogram quantities of a protein in a gel (Neuhoff et al., 1988). After separation of the proteins, the gels were fixed in a solution containing 7% (v/v) acetic acid and 40% (v/v) methanol for 30 min and stained for 16 h 50 min and 16 h 20 min for SGF and SGF followed by SIF digestions of the CSPB protein, respectively, in 1× Brilliant Blue G-colloidal stain solution containing 20% (v/v) methanol. The gels were destained for 25 s in 10% (v/v) acetic acid, 25% (v/v) methanol and then completely destained for ~23 h in a 25% (v/v) methanol solution. Images were captured using a Bio-Rad GS-800 densitometer (BioRad, Hercules, CA). The results of the *in vitro* digestibility of CSPB in SGF and SGF followed by SIF were determined by visual examination of the stained gels.

The LOD of the CSPB protein was determined using the colloidal Brilliant Blue G staining procedure. Various dilutions of the SGF zero time point (SGF T0) digestion specimen were loaded onto a separate gel that was run concurrently with the gel used to assess CSPB protein digestibility in SGF. Aliquots of the SGF T0 digestion specimen representing approximately 0.8, 0.4, 0.1, 0.05, 0.02, 0.01, 0.005, 0.0025, 0.001, and 0.0005 µg total protein per lane were used for the stained LOD gel.

1.8. Western Blot Analysis

Specimens from the SGF, SGF followed by SIF, and SIF *in vitro* digestions of the CSPB protein were separated by SDS-PAGE using pre-cast tricine 10-20% polyacrylamide gradient mini-gels with tricine running buffer. The protein loaded in each lane was based on pre-digestion concentrations of the CSPB protein. The digestion samples were diluted with 1× LB to a concentration of ~2 ng/µl, and ~10 ng of the CSPB protein digestion specimens were loaded in each lane. The experimental controls were loaded in the same volumes as the digestion specimens. All samples were heated to 101.3, 102.7 and 95.4 °C for 5, 3, and 5 min for the SGF, SIF and SGF followed by SIF digestions of the CSPB protein, respectively, prior to loading on the gels. Electrophoresis was performed at 125 V for 85, 83, and 90 min for SGF, SIF and SGF followed by SIF digestions of the CSPB protein, respectively. After electrophoresis, the proteins were electrotransferred onto nitrocellulose membranes with a pore size of 0.45 µm (Invitrogen, Carlsbad, CA) for 90 min at a constant voltage of 25 V. Prestained molecular weight markers (Precision Plus Dual color Protein Standards, Bio-Rad, Hercules, CA) were used to verify electrotransfer of the proteins to the membranes.

Proteins transferred to nitrocellulose membranes were analyzed by western blot. The membranes were blocked for 16 h at ~4 ºC with 5% (w/v) NFDM in a phosphate buffered saline - Tween® 20 (PBST) buffer. All subsequent incubations were performed at room temperature. Goat anti–CSBP affinity purified antibody (lot 10000866) was incubated with the membranes for 60 min at a dilution of 1:1000 in 1% (w/v) NFDM in PBST. Excess antibody was removed by three 10 min washes with PBST. The membranes were incubated with HRP–conjugated rabbit anti-goat IgG (lot HC993069, Pierce, Rockford, IL) at a dilution of 1:5000 in 1% (w/v) NFDM in PBST for 60 min, and washed three times for 20 min with PBST. Immunoreactive bands were visualized using the ECL detection system (GE Healthcare, Piscataway, NJ) and exposed to Hyperfilm ECL high performance chemiluminescence film (GE Healthcare, Piscataway, NJ). Films were developed using a Konica SRX101A automated film processor (Konica, Tokyo, Japan).

The films were scanned using a Bio-Rad GS-800 densitometer to produce electronic images to be used as figures for reporting purposes.

The approximate molecular weights of the proteins observed on the western blot were visually determined relative to the positions of the molecular weight markers.

The LOD for the western blot analysis procedure was determined for the CSPB protein by loading various dilutions of the SGF and SIF zero time point (SGF T0 and SIF T0, respectively) digestion specimens on separate gels. These gels were run concurrently with the SGF and SIF digestion western blot gels, respectively, and subjected to the same western blot procedure as described above. The following approximate total protein loadings of the SGF T0 and SIF T0 were used for the western blot LOD analysis: 10, 5, 2.5, 1, 0.5, 0.2, 0.1, 0.05, and 0.025 ng per lane.

1.9. N-Terminal Sequencing

N-terminal sequencing by Edman degradation was used to determine the N-terminal sequence of the transiently stable fragment with an apparent molecular weight of ~2.5 kDa .

1.10. Protein Blot for N-Terminal Sequence Analysis

The specimen SGF T1 was used to further characterize the transiently stable fragment of ~2.5 kDa. This specimen corresponds to the 30 s digestion of the CSPB protein which provided sufficient amount of the fragment for sequencing.

The SGF T1 specimen was heated at 102.7 °C for 3 min, and loaded in triplicate at 4.8 µg per lane onto a tricine 10-20% polyacrylamide gradient 10-well gel. Precision Plus prestained molecular weight markers were loaded in parallel to verify electrotransfer of the protein to the membrane and estimate the size of the stained bands observed. Electrophoresis was performed at a constant voltage of 125 V for 100 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 90 min at a constant voltage of 25 V. The blot was stained with Coomassie Blue R-250 stain (Bio-Rad, Hercules, CA) and then destained for ≥ 5 min with Coomassie Blue R-250 destain (Bio-Rad, Hercules, CA) to visualize the markers, the CSPB protein, and the stable fragment. The blot was scanned using a Bio-Rad GS-800 densitometer to produce an electronic image.

1.11. N-Terminal Sequencing

The bands corresponding to the transiently stable fragment of ~2.5 kDa were excised from the blot and used as a single sample. N-terminal sequence analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar and Hood, 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise™ Control Software (version 1.1a) were used. Chromatographic data were collected using Atlas[99] software (version 3.59a, LabSystems, Altrincham, Cheshire, England). A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to chromatographically calibrate the instrument for the analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (10 picomole β-lactoglobulin,

Applied Biosystems, Foster City, CA) was analyzed before and after the analysis of the three stable fragment bands to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity of the stable fragment was established by comparing the sequence data of 12 amino acids (15 cycles were run, only 12 amino acids were reported due to loss of repetitive yield) to the expected CSPB protein sequence.

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APPENDIX I. Materials and Methods used for Compositional Analysis of MON 87460 in U.S. 2006 and Chile 2006/2007 Studies

1.0. Test, control and reference substances

1.1. Test substance

The test substance was MON 87460. Forage and grain tissues of corn MON 887460 were evaluated in this study.

1.2. Control substance

The control substance was conventional corn hybrid with genetic background similar to MON 87460. The forage and grain tissues of the control substance were evaluated in this study.

1.3. Reference substances

The reference substances were 15 conventional commercial corn hybrids. A single replicate of the forage and grain tissues from each reference substance was evaluated in this study. The following conventional corn hybrids were analyzed:

Table 1. Reference substances for U.S. 2006 Study

Material Name	Seed Lot No.	Field Code
DKC 61-42	GLP-0603-16998-S	IAE
DKC 60-15	GLP-0604-17072-S	IAE
DKC 63-78	GLP-0604-17073-S	IAE
H8991	GLP-0603-16996-S	IAW
DKC 61-50	GLP-0603-16999-S	IAW
33N29	GLP-0604-17088-S	IAW
33K39	GLP-0604-17076-S	IL
M-3744	GLP-0604-17077-S	IL
M-3765	GLP-0604-17078-S	IL
BT-6512	GLP-0604-17079-S	IN
B-625	GLP-0604-17083-S	IN
B-645	GLP-0604-17084-S	IN
S-2721	GLP-0604-17146-S	KS
32B33	GLP-0604-17147-S	KS
33H25	GLP-0604-17071-S	KS
G-8424	GLP-0604-17089-S	NE
NC+4822	GLP-0604-17090-S	NE
34N43	GLP-0604-17091-S	NE

Table 2. Reference substances for Chile 2006/2007 Study

Material Name	Seed Lot No.	Field Code
33D11	GLP-0604-17075-S	CL
BT 6011	GLP-0610-17684-S	CL
Garst 8424	GLP-0610-17687-S	CL
DKC62-30	GLP-0609-17618-S	CL
33N09	GLP-0610-17691-S	CT
33K39	GLP-0604-17076-S	CT
BT 6613	GLP-0610-17683-S	CT
DKC63-78	GLP-0609-17613-S	CT
33N29	GLP-0604-17088-S	LUM
Garst 8445	GLP-0610-17688-S	LUM
DKC61-50	GLP-0609-17612-S	LUM
RX 715	GLP-0609-17615-S	LUM
34N43[1]	GLP-0604-17091-S	QUI[1]
BT 6610[1]	GLP-0610-17685-S	QUI[1]
Garst 8545[1]	GLP-0609-17689-S	QUI[1]
DKC60-15[1]	GLP-0609-17610-S	QUI[1]

2.0. Test, control and reference substance characterization

The identities of the forage and grain samples from each test, control, and reference substance were verified by the Study Director by confirming the chain-of-custody documentation supplied with the forage and grain collected from the plots. The grain of the test, control, and reference substances were also characterized, by event-specific PCR analysis, for the presence of the *cspB* coding region.

3.0. Field trial description

U.S. 2006

Seed was planted in the spring of 2006 at six sites (IAE, IAW, IL, IN, KS, and NE) in the United States. Locations of the field sites are as follows: IAE, Benton County, Iowa; IAW, Greene County, Iowa; IL, Stark County, Illinois; IN, Parke County, Indiana; KS, Pawnee County, Kansas, and NE, York County, Nebraska. At each field site, the T/C/R seed starting substances were planted in a randomized complete block design with three replicates per block. Each block (replicate) consisted of five plots with one plot for each test, control, and reference substance. Production was managed according to normal agronomic field practices. Grain and forage samples were harvested from all plots at ambient temperature and forwarded to Monsanto Company (St. Louis, MO). A sub-sample for compositional analysis was obtained from each tissue sample harvested. These sub-samples were then ground and stored in a freezer set to maintain a temperature of -20$_{o}$C until their shipment on dry ice to Covance Laboratories Inc. (Madison, WI) for analysis.

Chile 2006/2007

Seed was planted in the winter of 2006 at four replicated field sites (CL, CT, LUM, and QUI) in Chile. Locations of the field sites are as follows: CL, Colina, Region

Metropolitana; CT, Calera de Tango, Region Metropolitana; LUM, Lumbreras, Region Metropolitana, and QUI, Quillota, "V". The test and control substances were grown at all field sites. Four different conventional reference substances were also grown at each of the field sites. The field design incorporated a strip-plot design. The whole plot factor was irrigation treatment. Well-watered was irrigation management for optimal yield. Water-limited was irrigation management to target replacement of 55-65% of water evapotranspiration starting at plant growth stage ~V10 and continuing through ~R2. The design for the whole plot factor was a randomized complete block design. The strip-plot factor consisted of the test, control, and reference substances.

Grain and forage samples were harvested from all plots at ambient temperature and forwarded to Monsanto Company (St. Louis, MO). A sub-sample for compositional analysis was obtained from each tissue sample collected. These sub-samples were then ground and stored in a freezer set to maintain a temperature of -20°C until their shipment on dry ice to Covance Laboratories, Inc. (Madison, WI) for analysis.

4.0. Analytical methods

Components assessed in forage samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. Components assessed in grain samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, ADF, NDF, total detergent fiber (TDF), total amino acid composition, fatty acid composition, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc), vitamins (vitamin B1 [thiamine], vitamin B2 [riboflavin], vitamin B6 [pyridoxine], vitamin E, niacin, folic acid), furfural, raffinose, phytic acid, *p*-coumaric acid, and ferulic acid.

All compositional analyses were performed at Covance Laboratories, Inc. (Madison, Wisconsin). Methods for analysis were based on internationally-recognized procedures and literature publications. Brief descriptions of the methods utilized for the analyses are described below.

4.1 Moisture

Sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation was 0.100%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 926.08 and 925.09, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.2 Ash

Sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of quantitation was 0.100%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 923.03, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.3 Protein

Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The limit of quantitation was 0.100%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 955.04 and 979.09, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

Bradstreet, R. B., *The Kjeldahl Method for Organic Nitrogen*, Academic Press: New York, New York, (1965).

4.4 Fat by Acid Hydrolysis (Forage Analysis)

Forage sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steambath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again on a steambath under nitrogen, dried, and weighed. The limit of quantitation was 0.100%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 922.06 and 954.02, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

4.5 Fat by Soxhlet Extraction (Grain Analysis)

The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of quantitation was 0.100%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 960.39 and 948.22, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005)

4.6 Carbohydrate (CHO)

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

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

The limit of quantitation was 0.100%.

United States Department of Agriculture, "Energy Value of Foods", *Agriculture Handbook No. 74,* pp. 2-11, (1973).

4.7 Acid Detergent Fiber

Sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

Forage and Fiber Analyses, Agriculture Handbook No.379, United States Department of Agriculture, Washington, D.C. (1970).

4.8 Neutral Detergent Fiber

Sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically. The limit of quantitation was 0.100%.

Approved Methods of the American Association of Cereal Chemists, 9th Ed., Method 32.20, (1998).

Forage and Fiber Analyses, Agriculture Handbook No. 379, United States Department of Agriculture, (1970).

4.9 Total Dietary Fiber

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values. The limit of quantitation was 1.0%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 985.29, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.10 Mineral Analysis by ICP Emission Spectrometry

The sample was dried, precharred, and ashed overnight in a muffle set to maintain 500°C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of reference standards.

Table 3. Reference Calibration Ranges and Limits of Quantitation

Mineral	Reference Calibration Range (µg/ml)	Limit of Quantitation (ppm)
Calcium	200, 1000	20.0
Copper	2, 10	0.50
Iron	10, 50	2.00
Magnesium	50, 250	20.0
Manganese	2, 10	0.30
Phosphorus	200, 1000	20.0
Potassium	200, 1000	100
Sodium	200, 1000	100
Zinc	10, 50	0.40

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 984.27 and 985.01, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.11 Amino Acid Composition

Samples were assayed by three methods to obtain the full profile. 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 acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The limit of quantitation was 0.100 mg/g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 982.30, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.12 Fatty Acid Composition

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in 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. The limit of quantitation was 0.00400%.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 996.06, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

Official Methods and Recommended Practices of the AOCS, 5th Ed., Method Ce 1-62, American Oil Chemists' Society: Champaign, Illinois, (1997).

4.13 Folic acid

Sample was hydrolyzed in a potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis by autoclaving, the sample was treated with a chicken-pancreas enzyme and incubated approximately 18 hours to liberate the bound folic acid. The amount of folic acid was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*,

with the growth response of a folic acid standard. This response was measured turbidimetrically. The limit of quantitation was 0.060 µg/g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 960.46 and 992.05, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

Methods of Analysis for Infant Formulas, Infant Formula Council, Atlanta, Georgia, Section C-2, (1985).

4.14 Niacin

Sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interferences. The amount of niacin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum,* with the growth response of a niacin standard. This response was measured turbidimetrically. The limit of quantitation was 0.300 µg/g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 944.13, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.15 Thiamine Hydrochloride

Sample was autoclaved under weak acid conditions to extract the thiamine. The resulting solution was incubated with a buffered enzyme solution to release any bound thiamine. The solution was purified on a cation-exchange column. An aliquot was reacted with potassium ferricyanide to convert thiamine to thiochrome. The thiochrome was extracted into isobutyl alcohol, measured on a fluorometer, and quantitated by comparison to a known standard. The limit of quantitation was 0.01 mg/100g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 942.23, 953.17, and 957.17, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

4.16 Vitamin B_2 (Riboflavin)

Sample was hydrolyzed with dilute hydrochloric acid and the pH was adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of multipoint riboflavin standards. The growth response was measured turbidimetrically. The limit of quantitation was 0.200 µg/g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Methods 940.33 and 960.46, AOAC INTERNATIONAL, Gaithersburg, Maryland, (2005).

The United States Pharmacopeia, Twenty-Ninth Revision, p. 1913, United States Pharmacopeial Convention, Inc.: Rockville, Maryland, (2005).

4.17 Pyridoxine Hydrochloride

The sample was hydrolyzed with dilute sulfuric acid in the autoclave and the pH was adjusted to remove interferences. The amount of pyridoxine was determined by comparing the growth response of the sample, using the yeast *Saccharomyces*

carlsbergensis, with the growth response of a pyridoxine standard. The response was measured turbidimetrically. The limit of quantitation was 0.070 µg/g.

Official Methods of Analysis of AOAC INTERNATIONAL, 18th Ed., Method 961.15, AOAC INTERNATIONAL: Gaithersburg, Maryland, (2005).

Atkins, L., Schultz, A. S., Williams, W. L., and Frey, C. N., "Yeast Microbiological Methods for Determination of Vitamins," *Industrial and Engineering Chemistry, Analytical Edition,* 15:141-144, (1943).

4.18 Vitamin E

The product was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column. The limit of quantitation for this study was approximately 0.500 mg/100g.

Cort, W. M., Vincente, T. S., Waysek, E.H., and Williams, B. D., "Vitamin E Content of Feedstuffs Determined by High-Performance Liquid Chromatographic Fluorescence," *Journal of Agricultural Food Chemistry, 31*:1330-1333, (1983).

Speek, A. J., Schijver, J., and Schreurs, W. H. P., "Vitamin E Composition of Some Seed Oils as Determined by High-Performance Liquid Chromatography with Fluorometric Quantitation," *Journal of Food Science, 50*(1):121-124, (1985).

McMurray, C. H., Blanchflower, W. J., and Rice, D. A., "Influence of Extraction Techniques on Determination of α-Tocopherol in Animal Feedstuffs," *Journal of the Association of Official Analytical Chemists,* 63(6):1258-1261, (1980).

4.19 p-Coumaric Acid and Ferulic Acid

Sample was extracted with methanol using ultrasonication, hydrolyzed using 4N sodium hydroxide, buffered using acetic acid/sodium hydroxide, acidified with 3N hydrochloric acid, and filtered. The levels of p-coumaric and ferulic acids in the extract were determined by reverse phase high-performance liquid chromatography with ultraviolet detection. The limit of quantitation was approximately 50.0 ppm.

Hagerman, A. E. and Nicholson, R. L., "High-Performance Liquid Chromatographic Determination of Hydroxycinnamic Acids in Maize Mesocotyl," *Journal of Agricultural and Food Chemistry*, 30 (No. 6):1098-1102, (1982).

4.20 Phytic Acid

Sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1, 5µm (150 x 4.1mm) with a refractive index detector. The limit of quantitation was approximately 0.100%.

Lehrfeld, Jacob, "HPLC Separation and Quantitation of Phytic Acid and Some Inositol Phosphates in Foods: Problem and Solutions," *Journal of Agricultural and Food Chemistry*, 42:2726-2731, (1994).

Lehrfeld, Jacob, "High-Performance Liquid Chromatography Analysis of Phytic Acid on a pH-Stable, Macroporous Polymer Column," *Cereal Chemistry*, 66(6):510-515, (1989).

4.21 Raffinose

Sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-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 using a flame ionization detector. The limit of quantitation was 0.0500%.

Brobst, K. M., "Gas-Liquid Chromatography of Trimethylsilyl Derivatives," *Methods in Carbohydrate Chemistry*, Volume 6, Academic Press: New York, New York, (1972).

Mason, B. S., and Slover, H. T., "A Gas Chromatographic Method for the Determination of Sugars in Foods," *Journal of Agricultural and Food Chemistry*, 19(3):551-554, (1971).

4.22 2-Furaldehyde (Furfural)

Ground sample was extracted with 4% trichloroacetic acid and injected directly on a high-performance liquid chromatography system for quantitation of free furfurals by ultraviolet detection. The limit of quantitation was 0.500 ppm.

Albala-Hurtado S., Veciana-Nogues, M. T., Izquierdo-Pulido, M., and Vidal-Carou, M. C., "Determination of Free and Total Furfural Compounds In Infant Milk Formulas By High-Performance Liquid Chromatography," *Journal of Agricultural and Food Chemistry*, 45:2128-2133, (1997).

4.23 Sugar and Sugar Alcohols (SGAL)

Sugars and sugar alcohols were extracted from the sample with water. Aliquots were dried under inert gas and reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl- β -D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoracetic acid (TFA) treatment and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation for this study was 0.0500%.

Mason, B. S. and Slover, H. T., "A Gas Chromatographic Method for the Determination of Sugars in Foods," Journal of Agricultural and Food Chemistry, 1971.

Brosbt, K., "Gas Liquid Chromatography of Trimethylsilyl Derivations," Methods in Carbohydrate Chemistry, 6:3-8, Academic Press, New York, NY. 1972.

4.24 Free Proline

The sample was extracted in acid. Determination was by high-performance liquid chromatography (HPLC) with fluorescence or diode array detection. Primary amino acids were derivitized with o-phthalaldehyde and the secondary amino acids were derivatized with fluorenylmethyl chloroformate before injection. The limit of quantitation for this study was 0.0100 mg/g.

R. Schuster, "Determination of Amino Acids in Biological, Pharmaceutical, Plant and Food Samples by Automated Precolumn Derivitization and HPLC", Journal of Chromatography, 1988, 431, 271-284

Henderson, J. W., Ricker, R. D., Bidlingmeyer, B. A., Woodward, C., "Rapid Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids, Amino Acid Analysis Using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC," Agilent Publication, 2000.

4.25 Glycerol

Glycerol was extracted from the sample with water. A portion of the extract was passed through glass microfiber filter paper and an appropriate dilution was made. The sample was injected onto a high performance anion exchange chromatograph (HPAEC) equipped with a Pulsed Amerdperomtric Detector (PAD). The amount of glycerol present was quantitated relative to an external standard curve using regression analysis. The limit of quantitation for this study was 20 ppm.

Hanko, V. P. and Rohrer, J. S., "Determination of Carbohydrates, Sugar Alcohols, and Glycols in Cell Cultures and Fermentation Broths Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection", Analytical Biochemistry, 283:192-199, (2000).

4.26 Glycine Betaine, Choline, Salicylic acid and Abscisic Acid

Internal standard and extraction solvent (0.1% formic acid in 50:50 methanol:water) were added to the sample. After centrifugation and filtration samples were analyzed by Liquid chromatography using MS/MS for detection. Specific precursor-fragment transitions were monitored for each analyte using the multiple reaction monitoring (MRM) technique. The analytes were identified by comparison to reference standards using the retention time of the specific precursor-fragment response.

5.0. Control of bias

The test, control, and reference substances from each respective plot within the field sites were produced under similar agronomic conditions. To control and/or minimize bias, the samples were analyzed in the order specified by a computer-generated randomized sample list. The Study Director generated the randomized sample list and forwarded it to Covance Laboratories, Inc. prior to analysis.

6.0. Statistical analysis

6.1. Data processing

After compositional analyses were performed at Covance Laboratories, Inc., data spreadsheets were sent to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. for statistical analysis. A statistical sub-report was generated by Certus and sent to Monsanto Company. The following formulas were used for re-expression of the data for statistical analysis:

Table 4. Unit Conversions

Component	From (X)	To	Formula
Proximates (excluding moisture), Fiber, Antinutrients	% FW	% DW	X/d
Calcium, Phosphorus, Magnesium, Potassium, Sodium	ppm FW	% DW	$(X/d) \times 10^{-4}$
Copper, Iron, Manganese, Zinc	ppm FW	mg/kg DW	X/d
Secondary Metabolites	ppm FW	µg/g DW	X/d
Thiamine HCl	mg/100g FW	mg/kg DW	10 (X/d)
Vitamin E	mg/g FW	mg/kg DW	10^3 (X/d)
Folic Acid, Niacin, Riboflavin, Pyridoxine HCl/Vitamin B6	µg/g FW	mg/kg DW	X/d
Amino Acids (AA)	mg/g FW	% DW	X/(10*d)
Fatty Acids (FA)	% FW	% Total FA	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA
'X' is the individual sample value; 'd' is the fraction of the sample that is dry matter.			

6.1.1. U.S. 2006 Data Processing

In order to complete a statistical analysis for a compositional constituent in this study, at least 50% of the values for an analyte had to be greater than the assay LOQ. Analytes with greater than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 15 analytes with greater than 50% of observations below the assay LOQ were excluded from statistical analysis: 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, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid, sodium, and furfural. These components naturally occur at very low levels in corn.

For individual measurements below the assay's LOQ, where fewer than 50% of the total values were below the LOQ, results were assigned a value equal to half the quantitation limit. The following analytes were assigned values:

		Obs. Below LOQ				
Component	**Units**	**N**	**(%)**	**Total N**	**LOQ**	**Value Assigned**
Grain Fatty Acid						
16:1 Palmitoleic	% FW	13	24.1	54	0.0040	0.0020
22:0 Behenic	% FW	1	1.9	54	0.0040	0.0020

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A predicted residual sums of squares (PRESS) residual is the difference between any value and its predicted value from a statistical model that excludes the datum point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. For this study, no results had a PRESS residual value ouside of the ± 6 studentized PRESS residual range.

6.1.2. Chile 2006/2007 Data Processing

In order to complete a statistical analysis for a compositional constituent in this study, at least 50% of the values for an analyte had to be greater than the assay LOQ. Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 16 analytes with more than 50% of observations below the assay LOQ were excluded from statistical analysis: 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 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural.

Otherwise, results below the LOQ were assigned a value equal to half the quantitation limit. The following analytes were assigned values:

		Obs. Below LOQ				
Component	**Units**	**N**	**(%)**	**Total N**	**LOQ**	**Value Assigned**
Forage Proximate						
Total Fat	% FW	9	7.9	114	0.10	0.050
Grain Fatty Acid						
22:0 Behenic	% FW	30	26.5	113	0.0040	0.0020
Grain Vitamin						
Vitamin E	mg/g FW	5	4.4	113	0.0050	0.0025
Grain Antinutrients						
Raffinose	% FW	2	1.8	113	0.050	0.025

Individual samples assigned a value are represented in Listing 2 of the Statistical Sub-report.

PRESS residuals were used to identify outliers. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the datum point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme datum points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. The following result had a PRESS residual value outside of ± 6 range:

Site	Rep	Description	Analyte	ID	Sent Value	Value	PRESS Std Residual
Grain Mineral							
CL	1	DM1718	Copper	0645B302-00804	12	13.5287	17.1470

The copper value was considered an outlier and was removed from further analysis. The outlier test procedure was reapplied to all remaining copper data to detect potential outliers that were masked in the first analysis. No further PRESS residuals were outside of ± 6 range.

6.2. Statistical methodology for U.S. 2006

At the field sites, the test, control, and reference substances were grown in single plots randomly assigned within each of three replication blocks. The compositional components for the test and control substances were statistically analyzed using a mixed model analysis of variance. The data from the six replicated sites were analyzed separately and as a combined data set.

Individual replicated site analyses used the model:

$$Y_{ij} = U + T_i + B_j + e_{ij},$$

where Y_{ij} = unique individual observation, U = overall mean, T_i = hybrid effect, B_j = random block effect, and e_{ij} = residual error.

Combined site analyses 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 = hybrid effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by hybrid interaction effect, and e_{ijk} = residual error. For each compositional component, the forage and grain from the test substance was compared to the conventional control.

A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component,

99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial references (George et al., 2004; Ridley et al., 2002). Each tolerance interval estimate was based upon one observation per unique reference substance. Individual substances with multiple observations were summarized within sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS® software was used to generate all summary statistics and perform all analyses (SAS® Software Release 9.1, 2002-2003). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

6.3. Statistical methodology for Chile 2006/2007

All T/C/R substances were grown in single plots randomly assigned within each of three replication blocks. All corn compositional analysis components were statistically analyzed using a mixed model analysis of variance. The three replicated sites were analyzed both separately and combined. Individual replicated site analyses used model (1).

(1) $Y_{ijk} = U + B_i + T_j + BT_{ij} + S_k + BS_{ik} + TS_{jk} + e_{ijk}$,

where Y_{ijk} = unique individual observation, U = overall mean, B_i = random block effect, T_j = irrigation treatment effect, BT_{ij} = random block by treatment interaction effect, S_k = substance effect, BS_{ik} = random block by substance effect, TS_{jk} = treatment by substance interaction effect and e_{ijk} = residual error.

Combined site analyses used model (2).

(2) $Y_{ijkl} = U + L_i + B(L)_{ij} + T_k + LT_{ik} + TB(L)_{ijk} + S_l + SB(L)_{ijl} + TS_{kl} + LS_{il} + LTS_{ikl} + e_{ijkl}$,

where Y_{ijkl} = unique individual observation, U = overall mean, L_i = random location effect, $B(L)_{ij}$ = random block within location effect, T_k = irrigation treatment effect, LT_{ik} = random location by treatment interaction effect, $TB(L)_{ijk}$ = random treatment by block within location interaction effect, S_l = substance effect, $SB(L)_{ijl}$ = random substance by block within location interaction effect, TS_{kl} = treatment by substance interaction effect, LS_{il} = random location by substance interaction effect, LTS_{ikl} = random location by treatment by substance interaction effect and e_{ijkl} = residual error.

For each component analysis, mean comparison tests of each test substance versus the conventional control substance within each irrigation treatment were conducted.

A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component within each irrigation treatment, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial conventional substances. Each tolerance interval estimate was based upon one

observation per unique reference substance within each treatment. For each treatment, data were first summarized by substance within site and then by substance across sites. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS® programming was used to generate all summary statistics and perform all analyses (Version 9.1.3, SAS Institute, Inc. 2002-2003). Report tables present p-values from SAS as either <0.001 or the actual value truncated to three decimal places.

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APPENDIX J. Compositional Analyses Data for Individual Sites

This appendix contains the compositional analysis tables for the six individual sites from the 2006 U.S. production and 2006/2007 Chile production as follows:

Site No. (State)	Table
U.S. 1 (IAE)	1
U.S. 2 (IAW)	2
U.S. 3 (IL)	3
U.S. 4 (IN)	4
U.S. 5 (KS)	5
U.S. 6 (NE)	6
Chile 1 (CL) well-watered	7
Chile 1 (CL) water-limited	8
Chile 2 (CT) well-watered	9
Chile 2 (CT) water-limited	10
Chile 3 (LUM) well-watered	11
Chile 3 (LUM) water-limited	12

Table 1. Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.77 (0.024) [0.75 - 0.79]	0.79 (0.024) [0.73 - 0.83]	-0.019 (0.022) [-0.048 - 0.024]	-0.11, 0.076	0.479	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.45 (0.012) [0.43 - 0.46]	0.43 (0.012) [0.40 - 0.45]	0.018 (0.016) [-0.0042 - 0.055]	-0.053, 0.089	0.392	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.64 (0.013) [0.63 - 0.65]	0.65 (0.013) [0.61 - 0.67]	-0.0048 (0.013) [-0.018 - 0.021]	-0.060, 0.050	0.741	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.23 (0.0063) [0.21 - 0.24]	0.23 (0.0063) [0.22 - 0.24]	-0.0011 (0.0028) [-0.0060 - 0.0037]	-0.013, 0.011	0.720	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	1.99 (0.064) [1.94 - 2.02]	2.05 (0.064) [1.89 - 2.17]	-0.065 (0.059) [-0.15 - 0.050]	-0.32, 0.19	0.387	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.38 (0.0065) [0.38 - 0.39]	0.38 (0.0065) [0.37 - 0.39]	0.00091 (0.0057) [-0.0097 - 0.010]	-0.024, 0.026	0.888	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.31 (0.0068) [0.30 - 0.32]	0.32 (0.0068) [0.31 - 0.33]	-0.0087 (0.0016) [-0.011 - -0.0057]	-0.016, -0.0017	0.032	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.37 (0.013) [0.35 - 0.38]	0.39 (0.013) [0.36 - 0.41]	-0.016 (0.0041) [-0.023 - -0.0088]	-0.033, 0.0018	0.061	(0.30 - 0.41) [0.22, 0.49]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.35 (0.047) [1.30 - 1.38]	1.40 (0.047) [1.28 - 1.49]	-0.050 (0.040) [-0.11 - 0.026]	-0.22, 0.12	0.334	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.30 (0.0044) [0.29 - 0.30]	0.29 (0.0044) [0.28 - 0.30]	0.0022 (0.0051) [-0.0081 - 0.0075]	-0.020, 0.024	0.709	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.21 (0.0057) [0.20 - 0.21]	0.20 (0.0057) [0.19 - 0.21]	0.0050 (0.00097) [0.0031 - 0.0061]	0.00081, 0.0091	0.035	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.54 (0.015) [0.52 - 0.54]	0.55 (0.015) [0.51 - 0.57]	-0.011 (0.014) [-0.027 - 0.016]	-0.070, 0.048	0.494	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	0.99 (0.030) [0.95 - 1.01]	1.03 (0.030) [0.95 - 1.08]	-0.039 (0.023) [-0.074 - 0.0029]	-0.14, 0.058	0.224	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.51 (0.0089) [0.50 - 0.51]	0.50 (0.0089) [0.48 - 0.51]	0.0094 (0.013) [-0.0056 - 0.038]	-0.045, 0.064	0.534	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.36 (0.0071) [0.36 - 0.37]	0.36 (0.0071) [0.34 - 0.37]	0.0078 (0.0084) [-0.0033 - 0.024]	-0.028, 0.044	0.452	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.065 (0.0027) [0.062 - 0.067]	0.066 (0.0027) [0.060 - 0.072]	-0.00066 (0.0038) [-0.0098 - 0.0065]	-0.017, 0.016	0.876	(0.047 - 0.070) [0.037, 0.081]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.32 (0.029) [0.31 - 0.33]	0.27 (0.029) [0.19 - 0.31]	0.053 (0.042) [-0.0057 - 0.14]	-0.13, 0.23	0.331	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.51 (0.015) [0.48 - 0.52]	0.53 (0.015) [0.49 - 0.55]	-0.020 (0.0034) [-0.024 - -0.013]	-0.034, -0.0049	0.028	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.67 (0.056) [11.60 - 11.75]	11.80 (0.056) [11.67 - 11.88]	-0.12 (0.079) [-0.28 - 0.00050]	-0.46, 0.22	0.257	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.16 (0.0021) [0.16 - 0.16]	0.16 (0.0021) [0.16 - 0.16]	-0.0022 (0.0022) [-0.0061 - 0.0015]	-0.012, 0.0073	0.428	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	1.89 (0.021) [1.88 - 1.90]	1.85 (0.021) [1.80 - 1.90]	0.045 (0.025) [0.0023 - 0.090]	-0.064, 0.15	0.219	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	19.85 (0.29) [19.32 - 20.66]	19.89 (0.29) [19.81 - 19.96]	-0.044 (0.41) [-0.65 - 0.85]	-1.82, 1.73	0.925	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	64.46 (0.31) [63.73 - 65.07]	64.29 (0.31) [64.01 - 64.65]	0.17 (0.43) [-0.91 - 1.05]	-1.69, 2.04	0.726	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.22 (0.021) [1.17 - 1.25]	1.25 (0.021) [1.22 - 1.27]	-0.031 (0.0064) [-0.042 - -0.020]	-0.058, -0.0031	0.040	(0.89 - 1.56) [0.39, 1.85]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

			Difference (Test minus Control)			
Analytical Component (Units)[1]	**Test Mean (S.E.) [Range]**	**Control Mean (S.E.) [Range]**	**Mean (S.E.) [Range]**	**95% CI (Lower, Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.39 (0.0047) [0.39 - 0.39]	0.38 (0.0047) [0.37 - 0.39]	0.012 (0.0066) [-0.0018 - 0.019]	-0.016, 0.040	0.210	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.18 (0.0051) [0.18 - 0.19]	0.19 (0.0051) [0.18 - 0.20]	-0.0089 (0.0069) [-0.022 - 0.00015]	-0.039, 0.021	0.327	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.17 (0.026) [0.14 - 0.22]	0.19 (0.026) [0.14 - 0.23]	-0.022 (0.030) [-0.082 - 0.015]	-0.15, 0.11	0.546	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	3.86 (0.50) [2.48 - 4.63]	3.23 (0.50) [3.11 - 3.45]	0.62 (0.63) [-0.63 - 1.32]	-2.08, 3.33	0.425	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	9.39 (0.17) [9.33 - 9.43]	8.38 (0.17) [7.99 - 8.83]	1.01 (0.25) [0.50 - 1.44]	-0.051, 2.07	0.054	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	12.23 (0.22) [11.94 - 12.52]	11.60 (0.22) [11.24 - 12.11]	0.63 (0.11) [0.41 - 0.77]	0.15, 1.10	0.029	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0049 (0.00008) [0.0048 - 0.0051]	0.0049 (0.00008) [0.0048 - 0.0050]	0.00004 (0.00002) [0 - 0.00008]	-0.00005, 0.00013	0.184	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	2.56 (0.74) [1.51 - 4.61]	2.09 (0.74) [1.82 - 2.50]	0.47 (0.82) [-0.43 - 2.11]	-3.06, 4.00	0.624	(1.14 - 2.56) [0.39, 3.21]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	20.61 (1.60) [18.44 - 24.88]	19.20 (1.60) [17.86 - 20.37]	1.41 (2.26) [-1.92 - 7.02]	-8.30, 11.12	0.596	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.11 (0.0039) [0.11 - 0.11]	0.11 (0.0039) [0.10 - 0.12]	-0.0041 (0.0055) [-0.010 - 0.0068]	-0.028, 0.019	0.533	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	6.40 (0.38) [6.09 - 6.79]	6.69 (0.38) [5.70 - 7.27]	-0.30 (0.39) [-0.97 - 0.39]	-1.99, 1.39	0.527	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.29 (0.011) [0.28 - 0.29]	0.30 (0.011) [0.27 - 0.32]	-0.012 (0.014) [-0.028 - 0.015]	-0.071, 0.047	0.469	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.37 (0.0087) [0.36 - 0.39]	0.38 (0.0087) [0.36 - 0.39]	-0.0062 (0.0098) [-0.025 - 0.0066]	-0.048, 0.036	0.592	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	20.10 (0.64) [19.35 - 21.28]	20.67 (0.64) [19.40 - 21.69]	-0.57 (0.73) [-2.02 - 0.35]	-3.73, 2.58	0.516	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.52 (0.084) [1.33 - 1.71]	1.41 (0.084) [1.33 - 1.49]	0.11 (0.12) [-0.069 - 0.38]	-0.40, 0.63	0.447	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	84.47 (0.27) [84.42 - 84.55]	84.22 (0.27) [83.71 - 84.97]	0.25 (0.39) [-0.54 - 0.72]	-1.42, 1.92	0.586	(82.11 - 87.06) [80.32, 89.92]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Moisture (% FW)	9.78 (0.092) [9.57 - 10.00]	9.22 (0.092) [9.17 - 9.29]	0.56 (0.13) [0.28 - 0.83]	0.0014, 1.12	0.049	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	10.23 (0.24) [9.91 - 10.50]	10.60 (0.24) [10.03 - 11.00]	-0.37 (0.12) [-0.50 - -0.12]	-0.90, 0.16	0.095	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.79 (0.091) [3.57 - 3.95]	3.78 (0.091) [3.66 - 3.87]	0.0084 (0.13) [-0.24 - 0.29]	-0.55, 0.56	0.954	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.33 (0.0099) [0.32 - 0.36]	0.34 (0.0099) [0.33 - 0.35]	-0.012 (0.014) [-0.033 - 0.025]	-0.072, 0.049	0.485	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	20.32 (0.79) [19.22 - 22.16]	18.84 (0.79) [17.73 - 19.84]	1.48 (1.11) [-0.27 - 4.43]	-3.30, 6.26	0.313	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	5.96 (0.23) [5.63 - 6.57]	6.12 (0.23) [5.91 - 6.26]	-0.16 (0.30) [-0.63 - 0.38]	-1.43, 1.11	0.644	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.49 (0.17) [1.29 - 1.65]	1.46 (0.17) [1.03 - 1.74]	0.035 (0.24) [-0.30 - 0.50]	-1.00, 1.07	0.896	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	3.44 (0.065) [3.32 - 3.55]	3.41 (0.065) [3.30 - 3.52]	0.021 (0.092) [-0.10 - 0.24]	-0.37, 0.42	0.837	(2.43 - 4.17) [1.84, 4.94]

Table 1 (cont). Statistical Summary of Site IAE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

			Difference (Test minus Control)			
Analytical Component (Units)[1]	**Test Mean (S.E.) [Range]**	**Control Mean (S.E.) [Range]**	**Mean (S.E.) [Range]**	**95% CI (Lower, Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg DW)	13.67 (0.41) [12.67 - 14.18]	13.92 (0.41) [13.45 - 14.42]	-0.25 (0.57) [-1.76 - 0.71]	-2.72, 2.23	0.709	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.78 (0.044) [0.77 - 0.81]	0.81 (0.044) [0.69 - 0.88]	-0.031 (0.056) [-0.11 - 0.078]	-0.27, 0.21	0.640	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.20 (0.0029) [0.19 - 0.20]	0.15 (0.0029) [0.15 - 0.16]	0.042 (0.0041) [0.035 - 0.050]	0.024, 0.060	0.009	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1754.87 (54.40) [1677.78 - 1883.87]	1604.55 (54.40) [1530.67 - 1673.46]	150.32 (76.93) [4.32 - 353.20]	-180.67, 481.32	0.189	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	124.88 (3.91) [122.75 - 128.89]	132.18 (3.91) [124.57 - 142.02]	-7.30 (3.27) [-13.13 - -1.83]	-21.37, 6.77	0.155	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 2. Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.83 (0.050) [0.80 - 0.88]	0.82 (0.050) [0.70 - 0.89]	0.00057 (0.057) [-0.096 - 0.10]	-0.24, 0.24	0.992	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.46 (0.020) [0.43 - 0.48]	0.46 (0.020) [0.41 - 0.48]	-0.0018 (0.016) [-0.024 - 0.029]	-0.070, 0.066	0.920	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.66 (0.029) [0.64 - 0.69]	0.67 (0.029) [0.59 - 0.71]	-0.0023 (0.029) [-0.050 - 0.050]	-0.13, 0.12	0.943	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.23 (0.0088) [0.22 - 0.24]	0.24 (0.0088) [0.21 - 0.25]	-0.0017 (0.0078) [-0.015 - 0.012]	-0.035, 0.032	0.851	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	2.12 (0.13) [2.05 - 2.26]	2.11 (0.13) [1.76 - 2.31]	0.0098 (0.15) [-0.24 - 0.28]	-0.65, 0.67	0.954	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.40 (0.012) [0.39 - 0.41]	0.39 (0.012) [0.36 - 0.41]	0.0034 (0.011) [-0.012 - 0.026]	-0.046, 0.053	0.795	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.33 (0.016) [0.32 - 0.34]	0.32 (0.016) [0.28 - 0.34]	0.0057 (0.018) [-0.023 - 0.040]	-0.073, 0.084	0.784	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.40 (0.023) [0.39 - 0.42]	0.38 (0.023) [0.32 - 0.41]	0.022 (0.027) [-0.021 - 0.070]	-0.093, 0.14	0.502	(0.30 - 0.41) [0.22, 0.49]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.45 (0.10) [1.38 - 1.56]	1.44 (0.10) [1.17 - 1.59]	0.010 (0.12) [-0.20 - 0.22]	-0.52, 0.54	0.941	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.30 (0.0072) [0.29 - 0.30]	0.30 (0.0072) [0.28 - 0.31]	-0.0013 (0.0048) [-0.0088 - 0.0076]	-0.022, 0.019	0.805	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.23 (0.0069) [0.22 - 0.23]	0.23 (0.0069) [0.21 - 0.24]	-0.0039 (0.0070) [-0.014 - 0.0093]	-0.034, 0.026	0.633	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.57 (0.036) [0.55 - 0.61]	0.57 (0.036) [0.48 - 0.62]	0.0015 (0.041) [-0.067 - 0.074]	-0.17, 0.18	0.974	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	1.07 (0.057) [1.04 - 1.12]	1.03 (0.057) [0.88 - 1.12]	0.037 (0.072) [-0.080 - 0.17]	-0.27, 0.35	0.660	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.53 (0.031) [0.51 - 0.57]	0.56 (0.031) [0.48 - 0.60]	-0.027 (0.034) [-0.089 - 0.026]	-0.17, 0.12	0.505	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.37 (0.016) [0.36 - 0.39]	0.38 (0.016) [0.34 - 0.41]	-0.0091 (0.015) [-0.034 - 0.018]	-0.074, 0.055	0.604	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.068 (0.0043) [0.063 - 0.072]	0.072 (0.0043) [0.060 - 0.078]	-0.0040 (0.0034) [-0.0093 - 0.0023]	-0.018, 0.010	0.357	(0.047 - 0.070) [0.037, 0.081]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.32 (0.023) [0.28 - 0.35]	0.34 (0.023) [0.29 - 0.36]	-0.022 (0.013) [-0.047 - -0.0035]	-0.078, 0.034	0.232	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.54 (0.026) [0.52 - 0.56]	0.51 (0.026) [0.44 - 0.55]	0.024 (0.029) [-0.020 - 0.079]	-0.10, 0.15	0.491	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.99 (0.085) [11.83 - 12.09]	11.77 (0.085) [11.65 - 11.95]	0.21 (0.051) [0.14 - 0.31]	-0.0099, 0.43	0.054	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.18 (0.011) [0.17 - 0.18]	0.19 (0.011) [0.17 - 0.22]	-0.0067 (0.013) [-0.033 - 0.0097]	-0.064, 0.051	0.666	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.03 (0.042) [2.00 - 2.06]	1.98 (0.042) [1.90 - 2.09]	0.049 (0.041) [-0.027 - 0.11]	-0.13, 0.23	0.357	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	20.56 (0.22) [20.49 - 20.61]	20.59 (0.22) [20.16 - 21.18]	-0.027 (0.31) [-0.70 - 0.43]	-1.36, 1.30	0.937	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	63.13 (0.31) [63.05 - 63.27]	63.36 (0.31) [62.49 - 63.86]	-0.23 (0.40) [-0.67 - 0.57]	-1.95, 1.49	0.622	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.30 (0.016) [1.28 - 1.31]	1.31 (0.016) [1.27 - 1.33]	-0.0098 (0.010) [-0.023 - 0.010]	-0.053, 0.033	0.432	(0.89 - 1.56) [0.39, 1.85]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.40 (0.0094) [0.39 - 0.41]	0.40 (0.0094) [0.38 - 0.42]	-0.0039 (0.011) [-0.017 - 0.017]	-0.050, 0.043	0.752	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.19 (0.0074) [0.19 - 0.19]	0.20 (0.0074) [0.19 - 0.22]	-0.0092 (0.010) [-0.035 - 0.0053]	-0.054, 0.036	0.470	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.24 (0.018) [0.20 - 0.25]	0.21 (0.018) [0.17 - 0.23]	0.027 (0.0035) [0.021 - 0.033]	0.012, 0.042	0.015	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	4.02 (0.43) [3.28 - 4.94]	2.53 (0.43) [1.94 - 3.17]	1.49 (0.60) [0.66 - 3.00]	-1.11, 4.09	0.132	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	10.07 (0.56) [9.29 - 11.63]	8.02 (0.56) [7.82 - 8.30]	2.05 (0.64) [1.36 - 3.32]	-0.70, 4.79	0.084	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	13.76 (0.59) [13.50 - 13.95]	12.88 (0.59) [11.53 - 14.39]	0.88 (0.79) [-0.44 - 2.29]	-2.52, 4.28	0.382	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0059 (0.00016) [0.0057 - 0.0061]	0.0061 (0.00016) [0.0057 - 0.0063]	-0.00013 (0.00023) [-0.00059 - 0.00038]	-0.0011, 0.00087	0.636	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	1.69 (0.41) [1.47 - 2.12]	2.35 (0.41) [1.72 - 3.43]	-0.65 (0.33) [-1.31 - -0.24]	-2.08, 0.77	0.186	(1.14 - 2.56) [0.39, 3.21]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	17.91 (0.96) [16.82 - 19.40]	16.32 (0.96) [14.17 - 17.94]	1.58 (1.01) [-0.44 - 2.65]	-2.77, 5.94	0.258	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.12 (0.0035) [0.12 - 0.13]	0.12 (0.0035) [0.11 - 0.13]	0.00064 (0.0049) [-0.0088 - 0.013]	-0.020, 0.022	0.907	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	6.41 (0.18) [6.31 - 6.51]	6.12 (0.18) [5.63 - 6.40]	0.29 (0.20) [0.012 - 0.68]	-0.58, 1.16	0.285	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.33 (0.010) [0.32 - 0.34]	0.33 (0.010) [0.30 - 0.34]	-0.0036 (0.014) [-0.024 - 0.034]	-0.065, 0.058	0.825	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.38 (0.0058) [0.37 - 0.39]	0.37 (0.0058) [0.36 - 0.37]	0.010 (0.0081) [-0.0077 - 0.026]	-0.025, 0.045	0.330	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	19.34 (0.58) [19.06 - 19.71]	18.73 (0.58) [17.41 - 20.15]	0.61 (0.67) [-0.44 - 1.85]	-2.25, 3.48	0.454	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.63 (0.074) [1.52 - 1.83]	1.52 (0.074) [1.46 - 1.56]	0.11 (0.10) [-0.047 - 0.30]	-0.32, 0.54	0.391	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	83.83 (0.59) [83.38 - 84.05]	84.08 (0.59) [82.98 - 85.63]	-0.25 (0.76) [-1.57 - 1.07]	-3.53, 3.03	0.773	(82.11 - 87.06) [80.32, 89.92]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Moisture (% FW)	9.88 (0.21) [9.65 - 10.30]	9.97 (0.21) [9.69 - 10.40]	-0.087 (0.30) [-0.75 - 0.48]	-1.39, 1.21	0.801	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	11.00 (0.58) [10.59 - 11.52]	10.76 (0.58) [9.24 - 11.75]	0.24 (0.64) [-0.86 - 1.35]	-2.51, 2.99	0.746	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.55 (0.023) [3.53 - 3.57]	3.64 (0.023) [3.60 - 3.70]	-0.096 (0.032) [-0.16 - -0.055]	-0.23, 0.041	0.094	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.28 (0.011) [0.25 - 0.30]	0.27 (0.011) [0.26 - 0.28]	0.015 (0.011) [-0.0066 - 0.031]	-0.033, 0.062	0.319	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	16.42 (0.87) [15.84 - 16.95]	17.66 (0.87) [15.41 - 19.42]	-1.24 (1.23) [-2.93 - 1.53]	-6.51, 4.03	0.418	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	5.64 (0.20) [5.32 - 6.02]	5.41 (0.20) [5.21 - 5.80]	0.23 (0.28) [-0.48 - 0.80]	-0.99, 1.44	0.506	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.55 (0.13) [1.29 - 1.72]	1.76 (0.13) [1.49 - 1.94]	-0.21 (0.051) [-0.31 - -0.14]	-0.43, 0.0058	0.052	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	2.85 (0.062) [2.77 - 2.90]	2.48 (0.062) [2.33 - 2.57]	0.37 (0.039) [0.31 - 0.44]	0.20, 0.54	0.011	(2.43 - 4.17) [1.84, 4.94]

Table 2 (cont). Statistical Summary of Site IAW Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

			Difference (Test minus Control)			
Analytical Component (Units)[1]	**Test Mean (S.E.) [Range]**	**Control Mean (S.E.) [Range]**	**Mean (S.E.) [Range]**	**95% CI (Lower, Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg DW)	14.42 (1.03) [12.15 - 16.28]	15.36 (1.03) [14.30 - 16.94]	-0.94 (0.63) [-2.15 - -0.013]	-3.67, 1.78	0.275	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.89 (0.036) [0.85 - 0.96]	0.86 (0.036) [0.80 - 0.92]	0.029 (0.050) [-0.073 - 0.16]	-0.19, 0.25	0.624	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.16 (0.0069) [0.15 - 0.18]	0.18 (0.0069) [0.16 - 0.19]	-0.011 (0.0098) [-0.036 - 0.0022]	-0.053, 0.031	0.367	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1753.10 (78.24) [1661.13 - 1914.78]	1847.90 (78.24) [1760.60 - 1997.77]	-94.80 (5.95) [-101.93 - -82.99]	-120.38, -69.22	0.003	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	110.78 (8.71) [99.45 - 122.86]	136.30 (8.71) [121.98 - 156.25]	-25.52 (6.82) [-33.39 - -11.94]	-54.84, 3.81	0.064	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 3. Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.76 (0.030) [0.74 - 0.77]	0.79 (0.030) [0.73 - 0.87]	-0.032 (0.038) [-0.10 - 0.027]	-0.19, 0.13	0.485	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.41 (0.015) [0.39 - 0.43]	0.43 (0.015) [0.40 - 0.46]	-0.016 (0.020) [-0.037 - 0.024]	-0.10, 0.069	0.509	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.61 (0.017) [0.61 - 0.62]	0.63 (0.017) [0.60 - 0.68]	-0.018 (0.024) [-0.065 - 0.020]	-0.12, 0.086	0.536	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.22 (0.0048) [0.21 - 0.22]	0.22 (0.0048) [0.22 - 0.23]	-0.0079 (0.0047) [-0.015 - 0.0013]	-0.028, 0.013	0.237	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	1.95 (0.080) [1.92 - 1.98]	2.02 (0.080) [1.87 - 2.24]	-0.077 (0.096) [-0.26 - 0.068]	-0.49, 0.33	0.503	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.37 (0.0060) [0.37 - 0.37]	0.37 (0.0060) [0.36 - 0.39]	-0.0054 (0.0085) [-0.024 - 0.0080]	-0.042, 0.031	0.592	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.30 (0.0051) [0.30 - 0.30]	0.31 (0.0051) [0.30 - 0.32]	-0.0087 (0.0068) [-0.022 - 0.00057]	-0.038, 0.021	0.330	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.36 (0.0085) [0.35 - 0.36]	0.38 (0.0085) [0.36 - 0.40]	-0.019 (0.011) [-0.041 - -0.0060]	-0.067, 0.028	0.220	(0.30 - 0.41) [0.22, 0.49]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.33 (0.060) [1.31 - 1.36]	1.39 (0.060) [1.28 - 1.55]	-0.059 (0.067) [-0.19 - 0.037]	-0.35, 0.23	0.468	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.28 (0.0030) [0.28 - 0.29]	0.29 (0.0030) [0.28 - 0.29]	-0.0023 (0.0043) [-0.013 - 0.0074]	-0.021, 0.016	0.645	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.20 (0.0045) [0.19 - 0.20]	0.20 (0.0045) [0.20 - 0.21]	-0.0078 (0.0035) [-0.012 - -0.00087]	-0.023, 0.0073	0.156	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.52 (0.020) [0.52 - 0.54]	0.55 (0.020) [0.51 - 0.60]	-0.024 (0.021) [-0.066 - 0.0050]	-0.12, 0.068	0.378	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	0.95 (0.033) [0.93 - 0.98]	0.99 (0.033) [0.92 - 1.06]	-0.041 (0.026) [-0.079 - 0.0083]	-0.15, 0.070	0.253	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.50 (0.026) [0.48 - 0.52]	0.52 (0.026) [0.48 - 0.59]	-0.012 (0.027) [-0.064 - 0.029]	-0.13, 0.10	0.701	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.35 (0.012) [0.34 - 0.36]	0.36 (0.012) [0.34 - 0.39]	-0.0052 (0.017) [-0.036 - 0.023]	-0.078, 0.068	0.785	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.061 (0.0018) [0.059 - 0.064]	0.064 (0.0018) [0.060 - 0.067]	-0.0022 (0.0025) [-0.0055 - 0.0043]	-0.013, 0.0087	0.476	(0.047 - 0.070) [0.037, 0.081]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.27 (0.041) [0.21 - 0.32]	0.30 (0.041) [0.21 - 0.37]	-0.024 (0.053) [-0.096 - 0.079]	-0.25, 0.20	0.697	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.48 (0.0086) [0.48 - 0.49]	0.50 (0.0086) [0.49 - 0.53]	-0.021 (0.012) [-0.044 - -0.0066]	-0.072, 0.029	0.211	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.76 (0.088) [11.72 - 11.80]	11.83 (0.088) [11.65 - 12.06]	-0.075 (0.098) [-0.26 - 0.070]	-0.50, 0.35	0.525	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.16 (0.0017) [0.15 - 0.16]	0.15 (0.0017) [0.15 - 0.16]	0.0010 (0.0024) [-0.0029 - 0.0070]	-0.0092, 0.011	0.705	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.06 (0.021) [2.05 - 2.07]	2.05 (0.021) [2.00 - 2.10]	0.0071 (0.025) [-0.041 - 0.043]	-0.10, 0.11	0.801	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	19.75 (0.22) [19.67 - 19.89]	20.10 (0.22) [19.50 - 20.43]	-0.35 (0.26) [-0.67 - 0.17]	-1.47, 0.77	0.311	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	64.25 (0.33) [64.11 - 64.34]	63.82 (0.33) [63.11 - 64.70]	0.43 (0.41) [-0.36 - 1.00]	-1.32, 2.18	0.403	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.26 (0.015) [1.23 - 1.27]	1.26 (0.015) [1.23 - 1.28]	-0.0044 (0.021) [-0.046 - 0.044]	-0.097, 0.088	0.855	(0.89 - 1.56) [0.39, 1.85]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.41 (0.0098) [0.40 - 0.42]	0.41 (0.0098) [0.39 - 0.42]	0.0025 (0.0059) [-0.0062 - 0.014]	-0.023, 0.028	0.720	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.18 (0.0055) [0.17 - 0.18]	0.19 (0.0055) [0.18 - 0.20]	-0.0064 (0.0078) [-0.026 - 0.0066]	-0.040, 0.027	0.496	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.18 (0.025) [0.15 - 0.22]	0.18 (0.025) [0.15 - 0.24]	-0.0031 (0.036) [-0.083 - 0.075]	-0.16, 0.15	0.938	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	2.50 (0.34) [2.44 - 2.63]	2.92 (0.34) [2.06 - 3.71]	-0.42 (0.48) [-1.27 - 0.37]	-2.46, 1.63	0.472	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	7.84 (0.69) [6.45 - 9.32]	8.75 (0.69) [8.03 - 9.72]	-0.91 (0.87) [-1.98 - 0.82]	-4.67, 2.85	0.406	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	11.47 (0.41) [10.42 - 12.04]	11.13 (0.41) [10.76 - 11.59]	0.35 (0.54) [-0.60 - 1.28]	-1.99, 2.68	0.587	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0053 (0.00014) [0.0051 - 0.0054]	0.0053 (0.00014) [0.0051 - 0.0056]	-0.00002 (0.00014) [-0.00023 - 0.00024]	-0.00063, 0.00058	0.890	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	1.73 (0.084) [1.58 - 1.94]	1.65 (0.084) [1.57 - 1.75]	0.084 (0.12) [-0.17 - 0.31]	-0.43, 0.60	0.554	(1.14 - 2.56) [0.39, 3.21]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	18.03 (0.21) [17.83 - 18.28]	18.87 (0.21) [18.50 - 19.40]	-0.84 (0.30) [-1.57 - -0.21]	-2.15, 0.46	0.108	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.11 (0.0039) [0.11 - 0.11]	0.11 (0.0039) [0.11 - 0.12]	-0.0010 (0.0043) [-0.0097 - 0.0033]	-0.020, 0.018	0.835	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	6.43 (0.23) [6.24 - 6.64]	6.75 (0.23) [6.33 - 7.34]	-0.32 (0.31) [-0.92 - 0.060]	-1.63, 1.00	0.407	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.30 (0.010) [0.29 - 0.31]	0.31 (0.010) [0.29 - 0.34]	-0.0061 (0.011) [-0.029 - 0.0084]	-0.056, 0.043	0.647	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.38 (0.011) [0.36 - 0.39]	0.37 (0.011) [0.35 - 0.38]	0.0061 (0.0077) [-0.0081 - 0.018]	-0.027, 0.039	0.511	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	19.40 (0.48) [19.05 - 19.96]	20.84 (0.48) [20.18 - 22.07]	-1.44 (0.68) [-3.02 - -0.22]	-4.37, 1.48	0.167	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.50 (0.088) [1.34 - 1.74]	1.37 (0.088) [1.32 - 1.40]	0.13 (0.11) [0.015 - 0.36]	-0.36, 0.62	0.373	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	84.70 (0.35) [84.36 - 85.12]	84.64 (0.35) [83.79 - 85.33]	0.062 (0.25) [-0.21 - 0.57]	-1.04, 1.16	0.831	(82.11 - 87.06) [80.32, 89.92]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Moisture (% FW)	9.94 (0.18) [9.71 - 10.30]	10.43 (0.18) [10.20 - 10.80]	-0.49 (0.058) [-0.59 - -0.39]	-0.74, -0.24	0.013	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	9.82 (0.32) [9.70 - 9.92]	10.23 (0.32) [9.57 - 11.08]	-0.41 (0.42) [-1.25 - 0.13]	-2.23, 1.40	0.430	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.98 (0.049) [3.85 - 4.06]	3.76 (0.049) [3.75 - 3.78]	0.22 (0.069) [0.068 - 0.32]	-0.075, 0.52	0.084	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.28 (0.0054) [0.27 - 0.29]	0.27 (0.0054) [0.27 - 0.28]	0.011 (0.0022) [0.0074 - 0.015]	0.0022, 0.021	0.033	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	19.69 (1.17) [19.05 - 20.07]	18.84 (1.17) [15.83 - 21.41]	0.86 (1.32) [-1.35 - 3.22]	-4.83, 6.54	0.583	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	5.62 (0.35) [5.03 - 6.29]	5.74 (0.35) [5.29 - 6.41]	-0.12 (0.50) [-1.38 - 1.00]	-2.27, 2.03	0.835	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.45 (0.19) [1.03 - 1.87]	1.13 (0.19) [1.02 - 1.33]	0.32 (0.17) [-0.0011 - 0.55]	-0.39, 1.04	0.190	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	3.26 (0.16) [2.88 - 3.55]	3.20 (0.16) [3.01 - 3.34]	0.057 (0.099) [-0.13 - 0.21]	-0.37, 0.48	0.625	(2.43 - 4.17) [1.84, 4.94]

Table 3 (cont). Statistical Summary of Site IL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Vitamin						
Vitamin E (mg/kg DW)	13.25 (0.89) [11.09 - 14.84]	14.40 (0.89) [13.23 - 15.03]	-1.15 (1.26) [-3.95 - 0.60]	-6.59, 4.29	0.459	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.84 (0.053) [0.82 - 0.86]	0.84 (0.053) [0.71 - 0.96]	-0.00028 (0.070) [-0.10 - 0.13]	-0.30, 0.30	0.997	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.22 (0.0056) [0.21 - 0.22]	0.20 (0.0056) [0.19 - 0.21]	0.020 (0.0079) [-0.0012 - 0.034]	-0.014, 0.054	0.131	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1676.73 (56.18) [1561.63 - 1774.03]	1696.88 (56.18) [1603.14 - 1772.58]	-20.14 (79.45) [-210.94 - 91.40]	-361.99, 321.70	0.823	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	107.79 (2.88) [104.66 - 111.37]	120.96 (2.88) [114.70 - 127.09]	-13.17 (4.07) [-22.43 - -7.37]	-30.70, 4.36	0.083	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 4. Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.63 (0.015) [0.60 - 0.67]	0.65 (0.015) [0.64 - 0.66]	-0.026 (0.019) [-0.060 - 0.0068]	-0.11, 0.057	0.312	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.37 (0.015) [0.33 - 0.40]	0.40 (0.015) [0.38 - 0.41]	-0.030 (0.021) [-0.071 - -0.0089]	-0.12, 0.059	0.284	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.54 (0.012) [0.52 - 0.56]	0.56 (0.012) [0.54 - 0.57]	-0.024 (0.014) [-0.051 - -0.0030]	-0.085, 0.037	0.235	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.19 (0.0029) [0.19 - 0.20]	0.20 (0.0029) [0.20 - 0.21]	-0.011 (0.0023) [-0.016 - -0.0078]	-0.021, -0.0014	0.039	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	1.60 (0.041) [1.52 - 1.71]	1.67 (0.041) [1.64 - 1.69]	-0.074 (0.055) [-0.17 - 0.022]	-0.31, 0.16	0.307	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.33 (0.0066) [0.33 - 0.35]	0.34 (0.0066) [0.34 - 0.35]	-0.0097 (0.0083) [-0.024 - 0.0043]	-0.045, 0.026	0.364	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.26 (0.0063) [0.25 - 0.28]	0.28 (0.0063) [0.27 - 0.28]	-0.015 (0.0071) [-0.029 - -0.0048]	-0.046, 0.015	0.165	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.29 (0.0070) [0.28 - 0.31]	0.32 (0.0070) [0.31 - 0.33]	-0.027 (0.0080) [-0.042 - -0.015]	-0.061, 0.0076	0.078	(0.30 - 0.41) [0.22, 0.49]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.07 (0.029) [1.01 - 1.14]	1.13 (0.029) [1.11 - 1.14]	-0.056 (0.038) [-0.12 - 0.0072]	-0.22, 0.11	0.276	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.26 (0.0058) [0.26 - 0.28]	0.27 (0.0058) [0.26 - 0.28]	-0.012 (0.0060) [-0.023 - -0.0036]	-0.037, 0.014	0.193	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.17 (0.0028) [0.16 - 0.17]	0.18 (0.0028) [0.17 - 0.18]	-0.010 (0.0039) [-0.019 - -0.0044]	-0.027, 0.0068	0.124	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.43 (0.011) [0.41 - 0.46]	0.46 (0.011) [0.45 - 0.46]	-0.023 (0.015) [-0.050 - 0.0028]	-0.089, 0.043	0.275	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	0.81 (0.017) [0.78 - 0.85]	0.85 (0.017) [0.83 - 0.86]	-0.040 (0.022) [-0.082 - -0.011]	-0.13, 0.053	0.205	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.43 (0.013) [0.40 - 0.46]	0.43 (0.013) [0.43 - 0.44]	-0.0013 (0.018) [-0.038 - 0.036]	-0.078, 0.075	0.950	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.31 (0.0062) [0.30 - 0.33]	0.32 (0.0062) [0.31 - 0.32]	-0.0036 (0.0087) [-0.021 - 0.015]	-0.041, 0.034	0.717	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.058 (0.0028) [0.054 - 0.064]	0.060 (0.0028) [0.055 - 0.063]	-0.0021 (0.0022) [-0.0063 - 0.0013]	-0.012, 0.0075	0.443	(0.047 - 0.070) [0.037, 0.081]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.23 (0.026) [0.16 - 0.27]	0.27 (0.026) [0.26 - 0.28]	-0.040 (0.037) [-0.12 - 0.00087]	-0.20, 0.12	0.390	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.41 (0.0095) [0.40 - 0.44]	0.45 (0.0095) [0.43 - 0.45]	-0.032 (0.011) [-0.053 - -0.017]	-0.079, 0.015	0.097	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.85 (0.040) [11.83 - 11.88]	12.03 (0.040) [11.93 - 12.10]	-0.18 (0.056) [-0.27 - -0.082]	-0.42, 0.056	0.080	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.15 (0.0016) [0.15 - 0.15]	0.15 (0.0016) [0.15 - 0.16]	-0.0033 (0.0023) [-0.0067 - 0.0012]	-0.013, 0.0064	0.283	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.05 (0.022) [2.01 - 2.11]	1.99 (0.022) [1.98 - 2.01]	0.056 (0.031) [-0.0016 - 0.12]	-0.080, 0.19	0.217	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	20.05 (0.095) [19.96 - 20.13]	20.63 (0.095) [20.47 - 20.88]	-0.58 (0.095) [-0.75 - -0.42]	-0.99, -0.17	0.025	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	63.81 (0.12) [63.67 - 63.95]	63.03 (0.12) [62.75 - 63.28]	0.78 (0.13) [0.52 - 0.92]	0.22, 1.34	0.026	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.28 (0.0073) [1.28 - 1.28]	1.30 (0.0073) [1.28 - 1.32]	-0.016 (0.010) [-0.035 - 0.0019]	-0.061, 0.028	0.250	(0.89 - 1.56) [0.39, 1.85]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.42 (0.0055) [0.41 - 0.43]	0.41 (0.0055) [0.40 - 0.42]	0.0086 (0.0077) [-0.011 - 0.021]	-0.025, 0.042	0.381	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.19 (0.0021) [0.18 - 0.19]	0.19 (0.0021) [0.19 - 0.20]	-0.0088 (0.0030) [-0.016 - -0.0029]	-0.022, 0.0043	0.101	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.20 (0.018) [0.16 - 0.23]	0.25 (0.018) [0.23 - 0.27]	-0.049 (0.025) [-0.099 - -0.012]	-0.16, 0.059	0.188	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	2.55 (0.15) [2.52 - 2.60]	3.63 (0.15) [3.32 - 4.03]	-1.08 (0.21) [-1.50 - -0.80]	-1.99, -0.17	0.036	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	9.20 (0.65) [8.30 - 9.86]	9.16 (0.65) [8.06 - 10.69]	0.031 (0.68) [-0.83 - 1.37]	-2.89, 2.95	0.967	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	12.69 (1.06) [11.30 - 14.57]	12.37 (1.06) [10.90 - 14.62]	0.32 (1.50) [-3.32 - 3.67]	-6.14, 6.78	0.849	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0053 (0.00010) [0.0052 - 0.0054]	0.0053 (0.00010) [0.0050 - 0.0055]	0.00004 (0.00015) [-0.00026 - 0.00042]	-0.00060, 0.00068	0.815	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	1.59 (0.081) [1.49 - 1.67]	1.72 (0.081) [1.54 - 1.89]	-0.14 (0.11) [-0.40 - 0.13]	-0.63, 0.36	0.354	(1.14 - 2.56) [0.39, 3.21]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	15.90 (0.50) [15.02 - 16.62]	16.79 (0.50) [15.73 - 17.35]	-0.89 (0.71) [-2.34 - 0.33]	-3.93, 2.15	0.335	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.097 (0.0022) [0.095 - 0.099]	0.098 (0.0022) [0.095 - 0.10]	-0.0011 (0.0030) [-0.0089 - 0.0030]	-0.014, 0.012	0.751	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	5.28 (0.14) [5.02 - 5.62]	5.67 (0.14) [5.50 - 5.77]	-0.39 (0.17) [-0.71 - -0.15]	-1.11, 0.33	0.143	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.27 (0.0045) [0.27 - 0.28]	0.28 (0.0045) [0.28 - 0.30]	-0.013 (0.0063) [-0.030 - -0.0044]	-0.040, 0.014	0.175	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.39 (0.0028) [0.38 - 0.39]	0.38 (0.0028) [0.38 - 0.39]	0.0013 (0.0040) [-0.0033 - 0.010]	-0.016, 0.018	0.777	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	18.74 (0.57) [18.24 - 19.37]	19.05 (0.57) [18.05 - 20.47]	-0.31 (0.80) [-2.22 - 0.75]	-3.75, 3.14	0.737	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.38 (0.020) [1.33 - 1.41]	1.45 (0.020) [1.42 - 1.47]	-0.062 (0.028) [-0.13 - -0.011]	-0.18, 0.060	0.161	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	86.64 (0.20) [86.20 - 87.04]	85.88 (0.20) [85.61 - 86.05]	0.75 (0.28) [0.15 - 1.43]	-0.45, 1.96	0.115	(82.11 - 87.06) [80.32, 89.92]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Moisture (% FW)	9.64 (0.24) [9.12 - 10.10]	10.04 (0.24) [9.71 - 10.30]	-0.39 (0.33) [-1.18 - 0]	-1.83, 1.04	0.359	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	8.40 (0.12) [8.19 - 8.71]	8.88 (0.12) [8.77 - 8.97]	-0.48 (0.17) [-0.78 - -0.19]	-1.21, 0.25	0.105	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.58 (0.092) [3.44 - 3.68]	3.79 (0.092) [3.59 - 3.96]	-0.21 (0.13) [-0.52 - 0.085]	-0.77, 0.35	0.243	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.29 (0.015) [0.26 - 0.32]	0.26 (0.015) [0.24 - 0.29]	0.023 (0.014) [-0.0033 - 0.041]	-0.035, 0.082	0.227	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	21.54 (0.40) [21.02 - 22.23]	21.01 (0.40) [20.38 - 21.85]	0.53 (0.24) [0.22 - 1.00]	-0.49, 1.55	0.153	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	5.87 (0.37) [5.52 - 6.42]	6.25 (0.37) [5.57 - 7.07]	-0.37 (0.52) [-1.55 - 0.31]	-2.60, 1.85	0.543	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.52 (0.073) [1.45 - 1.57]	1.09 (0.073) [0.94 - 1.27]	0.43 (0.089) [0.30 - 0.60]	0.051, 0.81	0.039	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	2.95 (0.11) [2.67 - 3.19]	3.08 (0.11) [2.99 - 3.12]	-0.13 (0.16) [-0.44 - 0.070]	-0.80, 0.55	0.510	(2.43 - 4.17) [1.84, 4.94]

Table 4 (cont). Statistical Summary of Site IN Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Vitamin						
Vitamin E (mg/kg DW)	13.65 (1.08) [12.52 - 15.68]	11.25 (1.08) [9.47 - 13.38]	2.40 (1.53) [-0.61 - 4.77]	-4.18, 8.98	0.256	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.63 (0.013) [0.60 - 0.66]	0.77 (0.013) [0.75 - 0.78]	-0.13 (0.012) [-0.15 - -0.11]	-0.19, -0.083	0.007	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.19 (0.0051) [0.18 - 0.20]	0.17 (0.0051) [0.16 - 0.17]	0.022 (0.0072) [0.012 - 0.042]	-0.0089, 0.053	0.091	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1866.47 (104.11) [1779.76 - 1916.05]	1526.49 (104.11) [1245.83 - 1683.39]	339.99 (97.86) [220.22 - 533.93]	-81.07, 761.04	0.073	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	118.43 (5.63) [115.18 - 122.36]	110.11 (5.63) [94.77 - 118.51]	8.31 (7.96) [-3.32 - 27.59]	-25.95, 42.58	0.406	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 5. Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.00 (0.045) [0.95 - 1.04]	0.97 (0.045) [0.86 - 1.04]	0.022 (0.039) [-0.041 - 0.094]	-0.15, 0.19	0.633	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.52 (0.016) [0.50 - 0.54]	0.50 (0.016) [0.46 - 0.52]	0.026 (0.0074) [0.016 - 0.041]	-0.0057, 0.058	0.071	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.76 (0.030) [0.73 - 0.79]	0.74 (0.030) [0.67 - 0.78]	0.020 (0.021) [-0.0099 - 0.060]	-0.069, 0.11	0.439	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.26 (0.0023) [0.26 - 0.27]	0.26 (0.0023) [0.26 - 0.26]	-0.00054 (0.0032) [-0.0042 - 0.0058]	-0.014, 0.013	0.880	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	2.55 (0.12) [2.43 - 2.66]	2.50 (0.12) [2.20 - 2.67]	0.054 (0.10) [-0.11 - 0.24]	-0.38, 0.49	0.646	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.44 (0.011) [0.43 - 0.45]	0.42 (0.011) [0.39 - 0.43]	0.019 (0.0082) [0.0070 - 0.035]	-0.016, 0.055	0.140	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.37 (0.012) [0.35 - 0.38]	0.35 (0.012) [0.32 - 0.37]	0.016 (0.0099) [0.0051 - 0.036]	-0.026, 0.059	0.241	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.47 (0.023) [0.44 - 0.50]	0.45 (0.023) [0.39 - 0.48]	0.023 (0.013) [0.0047 - 0.048]	-0.032, 0.079	0.212	(0.30 - 0.41) [0.22, 0.49]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.77 (0.088) [1.69 - 1.85]	1.74 (0.088) [1.51 - 1.87]	0.037 (0.077) [-0.085 - 0.18]	-0.29, 0.37	0.680	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.33 (0.0086) [0.31 - 0.34]	0.31 (0.0086) [0.29 - 0.33]	0.014 (0.0031) [0.011 - 0.020]	0.00071, 0.027	0.045	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.26 (0.0050) [0.26 - 0.28]	0.26 (0.0050) [0.25 - 0.26]	0.0076 (0.0062) [-0.0022 - 0.019]	-0.019, 0.034	0.346	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.69 (0.032) [0.66 - 0.72]	0.67 (0.032) [0.59 - 0.72]	0.016 (0.028) [-0.027 - 0.067]	-0.10, 0.13	0.615	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	1.19 (0.049) [1.12 - 1.23]	1.13 (0.049) [1.01 - 1.21]	0.055 (0.027) [0.018 - 0.11]	-0.061, 0.17	0.178	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.63 (0.024) [0.62 - 0.64]	0.64 (0.024) [0.57 - 0.67]	-0.0051 (0.026) [-0.037 - 0.046]	-0.12, 0.11	0.862	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.43 (0.017) [0.42 - 0.45]	0.43 (0.017) [0.38 - 0.45]	0.0050 (0.018) [-0.026 - 0.037]	-0.074, 0.084	0.810	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.076 (0.0051) [0.069 - 0.088]	0.077 (0.0051) [0.072 - 0.085]	-0.0012 (0.0072) [-0.014 - 0.016]	-0.032, 0.030	0.880	(0.047 - 0.070) [0.037, 0.081]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.41 (0.020) [0.38 - 0.43]	0.40 (0.020) [0.35 - 0.43]	0.012 (0.0094) [-0.00024 - 0.030]	-0.028, 0.052	0.326	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.62 (0.026) [0.58 - 0.64]	0.59 (0.026) [0.52 - 0.62]	0.030 (0.014) [0.011 - 0.058]	-0.032, 0.092	0.172	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	13.42 (0.64) [12.43 - 15.21]	12.35 (0.64) [12.32 - 12.38]	1.07 (0.89) [0.082 - 2.84]	-2.75, 4.89	0.351	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.19 (0.0049) [0.17 - 0.20]	0.18 (0.0049) [0.18 - 0.19]	0.0038 (0.0069) [-0.012 - 0.015]	-0.026, 0.033	0.638	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.17 (0.063) [2.07 - 2.34]	2.01 (0.063) [1.97 - 2.04]	0.16 (0.088) [0.031 - 0.33]	-0.22, 0.54	0.209	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	20.45 (0.18) [20.15 - 20.72]	20.69 (0.18) [20.39 - 21.03]	-0.24 (0.18) [-0.56 - 0.078]	-1.03, 0.55	0.322	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	61.69 (0.65) [59.90 - 62.78]	62.72 (0.65) [62.36 - 62.97]	-1.03 (0.92) [-3.07 - 0.42]	-5.00, 2.94	0.380	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.31 (0.057) [1.20 - 1.46]	1.24 (0.057) [1.22 - 1.26]	0.069 (0.069) [-0.016 - 0.20]	-0.23, 0.36	0.422	(0.89 - 1.56) [0.39, 1.85]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.44 (0.0050) [0.42 - 0.44]	0.44 (0.0050) [0.44 - 0.45]	-0.0053 (0.0061) [-0.017 - 0.0042]	-0.032, 0.021	0.481	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.18 (0.0034) [0.17 - 0.18]	0.19 (0.0034) [0.19 - 0.20]	-0.015 (0.0033) [-0.020 - -0.0088]	-0.029, -0.0010	0.043	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.17 (0.0039) [0.16 - 0.17]	0.18 (0.0039) [0.17 - 0.18]	-0.012 (0.0055) [-0.021 - 0.0032]	-0.036, 0.012	0.160	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	2.90 (0.34) [2.09 - 3.32]	2.40 (0.34) [2.13 - 2.91]	0.50 (0.36) [-0.074 - 1.15]	-1.04, 2.03	0.297	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	8.99 (0.83) [8.15 - 9.63]	10.30 (0.83) [8.46 - 12.22]	-1.31 (1.17) [-4.07 - 1.17]	-6.34, 3.73	0.380	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	12.54 (0.82) [11.17 - 13.56]	13.08 (0.82) [11.92 - 14.87]	-0.54 (0.75) [-1.32 - 0.96]	-3.77, 2.69	0.545	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0057 (0.00011) [0.0056 - 0.0060]	0.0060 (0.00011) [0.0058 - 0.0061]	-0.00025 (0.00016) [-0.00047 - 0.00016]	-0.00093, 0.00043	0.256	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	2.01 (0.22) [1.70 - 2.63]	1.62 (0.22) [1.57 - 1.71]	0.40 (0.31) [-0.0092 - 1.06]	-0.95, 1.74	0.333	(1.14 - 2.56) [0.39, 3.21]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	17.67 (0.30) [17.15 - 18.11]	18.60 (0.30) [18.20 - 19.22]	-0.93 (0.42) [-2.07 - -0.091]	-2.74, 0.89	0.159	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.13 (0.0016) [0.13 - 0.13]	0.13 (0.0016) [0.12 - 0.13]	-0.0018 (0.0022) [-0.0050 - 0.0035]	-0.011, 0.0077	0.509	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	8.47 (0.11) [8.19 - 8.64]	8.22 (0.11) [8.15 - 8.34]	0.25 (0.15) [-0.15 - 0.49]	-0.41, 0.92	0.243	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.35 (0.0073) [0.35 - 0.35]	0.36 (0.0073) [0.34 - 0.37]	-0.0082 (0.010) [-0.022 - 0.015]	-0.053, 0.037	0.514	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.38 (0.0025) [0.38 - 0.39]	0.39 (0.0025) [0.38 - 0.39]	-0.0034 (0.0035) [-0.010 - 0.0049]	-0.019, 0.012	0.436	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	23.88 (0.58) [22.68 - 24.75]	24.08 (0.58) [23.39 - 25.11]	-0.20 (0.81) [-2.43 - 1.37]	-3.70, 3.30	0.831	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.63 (0.069) [1.57 - 1.70]	1.62 (0.069) [1.48 - 1.79]	0.0078 (0.098) [-0.22 - 0.22]	-0.41, 0.43	0.943	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	82.29 (0.52) [81.40 - 83.29]	81.99 (0.52) [81.31 - 82.95]	0.30 (0.74) [-0.77 - 1.98]	-2.89, 3.48	0.727	(82.11 - 87.06) [80.32, 89.92]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Moisture (% FW)	9.84 (0.10) [9.61 - 10.00]	10.03 (0.10) [9.90 - 10.20]	-0.19 (0.15) [-0.38 - 0.10]	-0.82, 0.44	0.326	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	12.35 (0.53) [11.28 - 13.21]	12.75 (0.53) [11.76 - 13.33]	-0.40 (0.75) [-2.05 - 0.79]	-3.62, 2.83	0.650	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.73 (0.050) [3.66 - 3.85]	3.64 (0.050) [3.57 - 3.70]	0.092 (0.071) [-0.040 - 0.28]	-0.21, 0.40	0.323	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.33 (0.0089) [0.31 - 0.35]	0.34 (0.0089) [0.34 - 0.34]	-0.011 (0.013) [-0.031 - 0.012]	-0.065, 0.042	0.457	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	16.38 (1.09) [15.71 - 17.21]	18.19 (1.09) [15.26 - 19.87]	-1.81 (1.54) [-3.73 - 1.95]	-8.42, 4.80	0.359	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	6.80 (0.24) [6.41 - 7.43]	7.20 (0.24) [6.97 - 7.36]	-0.40 (0.23) [-0.69 - 0.065]	-1.40, 0.61	0.232	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.66 (0.25) [0.95 - 2.04]	1.71 (0.25) [1.63 - 1.83]	-0.049 (0.36) [-0.88 - 0.42]	-1.60, 1.50	0.904	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	3.84 (0.13) [3.78 - 3.89]	3.70 (0.13) [3.34 - 3.89]	0.14 (0.19) [-0.11 - 0.54]	-0.66, 0.94	0.527	(2.43 - 4.17) [1.84, 4.94]

Table 5 (cont). Statistical Summary of Site KS Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

			Difference (Test minus Control)			
Analytical Component (Units)[1]	**Test Mean (S.E.) [Range]**	**Control Mean (S.E.) [Range]**	**Mean (S.E.) [Range]**	**95% CI (Lower, Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Vitamin						
Vitamin E (mg/kg DW)	17.71 (0.88) [15.98 - 20.02]	17.97 (0.88) [17.37 - 18.44]	-0.26 (0.93) [-1.39 - 1.58]	-4.26, 3.74	0.804	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.98 (0.058) [0.95 - 1.00]	0.95 (0.058) [0.81 - 1.09]	0.031 (0.081) [-0.14 - 0.19]	-0.32, 0.38	0.737	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.20 (0.0053) [0.20 - 0.20]	0.21 (0.0053) [0.20 - 0.22]	-0.0078 (0.0075) [-0.023 - 0.0091]	-0.040, 0.024	0.405	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1896.76 (44.92) [1803.30 - 1966.67]	1863.66 (44.92) [1786.90 - 1926.50]	33.10 (63.52) [-74.27 - 179.76]	-240.22, 306.41	0.654	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	124.95 (7.61) [103.88 - 136.67]	141.53 (7.61) [137.62 - 144.77]	-16.58 (10.76) [-38.32 - -0.96]	-62.88, 29.72	0.263	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 6. Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.85 (0.011) [0.83 - 0.87]	0.86 (0.011) [0.85 - 0.87]	-0.0094 (0.0096) [-0.023 - 0.0092]	-0.051, 0.032	0.431	(0.60 - 0.91) [0.43, 1.08]
Arginine (% DW)	0.48 (0.020) [0.44 - 0.50]	0.44 (0.020) [0.41 - 0.48]	0.043 (0.029) [-0.036 - 0.087]	-0.080, 0.17	0.268	(0.34 - 0.51) [0.24, 0.60]
Aspartic Acid (% DW)	0.69 (0.0074) [0.67 - 0.70]	0.69 (0.0074) [0.69 - 0.71]	-0.0081 (0.0041) [-0.016 - -0.0019]	-0.026, 0.0096	0.188	(0.52 - 0.72) [0.39, 0.84]
Cystine (% DW)	0.24 (0.0030) [0.24 - 0.24]	0.24 (0.0030) [0.23 - 0.25]	-0.0015 (0.0020) [-0.0048 - 0.0020]	-0.010, 0.0070	0.527	(0.19 - 0.24) [0.15, 0.27]
Glutamic Acid (% DW)	2.19 (0.031) [2.13 - 2.23]	2.19 (0.031) [2.15 - 2.25]	0.00057 (0.041) [-0.044 - 0.082]	-0.17, 0.18	0.990	(1.54 - 2.32) [1.06, 2.76]
Glycine (% DW)	0.41 (0.0051) [0.41 - 0.42]	0.41 (0.0051) [0.40 - 0.42]	0.0029 (0.0059) [-0.0073 - 0.013]	-0.022, 0.028	0.674	(0.33 - 0.42) [0.26, 0.47]
Histidine (% DW)	0.33 (0.0048) [0.32 - 0.34]	0.33 (0.0048) [0.32 - 0.33]	0.0057 (0.0047) [-0.0016 - 0.014]	-0.015, 0.026	0.350	(0.25 - 0.33) [0.20, 0.36]
Isoleucine (% DW)	0.40 (0.0093) [0.39 - 0.42]	0.40 (0.0093) [0.38 - 0.42]	0.0036 (0.0016) [0.00066 - 0.0060]	-0.0031, 0.010	0.147	(0.30 - 0.41) [0.22, 0.49]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.48 (0.030) [1.43 - 1.52]	1.48 (0.030) [1.43 - 1.54]	-0.00099 (0.042) [-0.056 - 0.088]	-0.18, 0.18	0.983	(1.02 - 1.55) [0.68, 1.90]
Lysine (% DW)	0.32 (0.0048) [0.31 - 0.32]	0.30 (0.0048) [0.29 - 0.31]	0.013 (0.0067) [0.0020 - 0.027]	-0.016, 0.042	0.189	(0.27 - 0.32) [0.22, 0.36]
Methionine (% DW)	0.23 (0.0042) [0.22 - 0.24]	0.23 (0.0042) [0.23 - 0.24]	0.0038 (0.0060) [-0.0057 - 0.015]	-0.022, 0.030	0.595	(0.17 - 0.24) [0.14, 0.28]
Phenylalanine (% DW)	0.59 (0.011) [0.57 - 0.60]	0.58 (0.011) [0.57 - 0.60]	0.0049 (0.013) [-0.0097 - 0.031]	-0.051, 0.061	0.742	(0.43 - 0.61) [0.30, 0.74]
Proline (% DW)	1.09 (0.019) [1.05 - 1.13]	1.09 (0.019) [1.07 - 1.11]	-0.00075 (0.017) [-0.031 - 0.027]	-0.072, 0.071	0.968	(0.74 - 1.01) [0.56, 1.19]
Serine (% DW)	0.56 (0.011) [0.54 - 0.58]	0.57 (0.011) [0.55 - 0.58]	-0.0058 (0.015) [-0.036 - 0.014]	-0.070, 0.058	0.732	(0.39 - 0.60) [0.27, 0.70]
Threonine (% DW)	0.39 (0.0064) [0.38 - 0.40]	0.39 (0.0064) [0.37 - 0.39]	0.0017 (0.0090) [-0.011 - 0.027]	-0.037, 0.041	0.865	(0.29 - 0.40) [0.22, 0.46]
Tryptophan (% DW)	0.070 (0.0024) [0.067 - 0.076]	0.069 (0.0024) [0.067 - 0.071]	0.0014 (0.0019) [-0.0012 - 0.0052]	-0.0069, 0.0098	0.534	(0.047 - 0.070) [0.037, 0.081]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.34 (0.043) [0.31 - 0.36]	0.24 (0.043) [0.15 - 0.35]	0.10 (0.061) [-0.036 - 0.21]	-0.16, 0.37	0.234	(0.13 - 0.37) [0.0046, 0.54]
Valine (% DW)	0.54 (0.011) [0.53 - 0.57]	0.54 (0.011) [0.52 - 0.56]	0.0073 (0.0019) [0.0047 - 0.011]	-0.00093, 0.015	0.062	(0.42 - 0.54) [0.33, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	12.04 (0.15) [12.00 - 12.11]	11.88 (0.15) [11.45 - 12.12]	0.16 (0.19) [-0.048 - 0.55]	-0.67, 0.99	0.488	(8.80 - 13.33) [6.35, 16.03]
16:1 Palmitoleic (% Total FA)	0.19 (0.013) [0.19 - 0.19]	0.19 (0.013) [0.17 - 0.23]	-0.0049 (0.018) [-0.042 - 0.014]	-0.082, 0.072	0.811	(0.059 - 0.15) [0, 0.21]
18:0 Stearic (% Total FA)	2.09 (0.027) [2.04 - 2.12]	1.99 (0.027) [1.96 - 2.05]	0.098 (0.033) [0.065 - 0.16]	-0.043, 0.24	0.095	(1.36 - 2.14) [1.00, 2.51]
18:1 Oleic (% Total FA)	20.89 (0.28) [20.63 - 21.08]	21.05 (0.28) [20.56 - 21.77]	-0.16 (0.39) [-1.13 - 0.52]	-1.84, 1.51	0.718	(21.17 - 33.71) [11.92, 39.78]
18:2 Linoleic (% Total FA)	62.68 (0.42) [62.49 - 62.90]	62.85 (0.42) [61.88 - 63.91]	-0.17 (0.60) [-1.41 - 1.02]	-2.74, 2.41	0.804	(49.31 - 62.94) [45.91, 72.47]
18:3 Linolenic (% Total FA)	1.30 (0.012) [1.29 - 1.30]	1.26 (0.012) [1.24 - 1.29]	0.032 (0.015) [0.0048 - 0.058]	-0.034, 0.098	0.170	(0.89 - 1.56) [0.39, 1.85]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:0 Arachidic (% Total FA)	0.41 (0.0064) [0.40 - 0.41]	0.40 (0.0064) [0.38 - 0.41]	0.0083 (0.0081) [-0.0010 - 0.024]	-0.027, 0.043	0.413	(0.30 - 0.49) [0.23, 0.56]
20:1 Eicosenoic (% Total FA)	0.18 (0.0037) [0.18 - 0.19]	0.18 (0.0037) [0.17 - 0.19]	0.0019 (0.0038) [-0.0046 - 0.0086]	-0.015, 0.018	0.672	(0.20 - 0.29) [0.15, 0.33]
22:0 Behenic (% Total FA)	0.22 (0.035) [0.16 - 0.27]	0.19 (0.035) [0.15 - 0.26]	0.033 (0.023) [0.0085 - 0.078]	-0.064, 0.13	0.281	(0.069 - 0.28) [0, 0.37]
Fiber						
Acid Detergent Fiber (% DW)	2.36 (0.51) [1.57 - 3.24]	3.41 (0.51) [2.36 - 4.08]	-1.06 (0.72) [-2.51 - 0.87]	-4.15, 2.03	0.278	(1.82 - 4.48) [0.62, 5.72]
Neutral Detergent Fiber (% DW)	8.35 (0.29) [7.80 - 9.14]	9.11 (0.29) [8.96 - 9.21]	-0.76 (0.41) [-1.35 - 0.18]	-2.53, 1.01	0.207	(6.51 - 12.28) [3.45, 15.08]
Total Dietary Fiber (% DW)	12.86 (0.37) [12.46 - 13.64]	11.82 (0.37) [11.15 - 12.26]	1.04 (0.42) [0.21 - 1.59]	-0.78, 2.85	0.132	(10.65 - 16.26) [8.11, 17.95]
Mineral						
Calcium (% DW)	0.0050 (0.00017) [0.0047 - 0.0054]	0.0050 (0.00017) [0.0049 - 0.0051]	-0.00003 (0.00024) [-0.00037 - 0.00056]	-0.0011, 0.0010	0.921	(0.0036 - 0.0068) [0.0019, 0.0076]
Copper (mg/kg DW)	1.73 (0.027) [1.71 - 1.75]	1.76 (0.027) [1.69 - 1.79]	-0.024 (0.037) [-0.082 - 0.054]	-0.19, 0.14	0.586	(1.14 - 2.56) [0.39, 3.21]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Iron (mg/kg DW)	19.31 (0.42) [18.41 - 20.09]	20.05 (0.42) [19.40 - 20.58]	-0.73 (0.60) [-1.75 - 0.69]	-3.31, 1.84	0.345	(16.89 - 23.40) [13.28, 26.47]
Magnesium (% DW)	0.12 (0.0028) [0.11 - 0.12]	0.12 (0.0028) [0.11 - 0.12]	-0.00077 (0.0040) [-0.0063 - 0.0086]	-0.018, 0.017	0.865	(0.091 - 0.14) [0.059, 0.16]
Manganese (mg/kg DW)	7.74 (0.16) [7.31 - 8.07]	7.86 (0.16) [7.79 - 7.94]	-0.12 (0.22) [-0.55 - 0.13]	-1.05, 0.81	0.633	(4.83 - 8.05) [2.27, 9.92]
Phosphorus (% DW)	0.31 (0.0086) [0.29 - 0.33]	0.32 (0.0086) [0.31 - 0.33]	-0.0077 (0.012) [-0.025 - 0.021]	-0.060, 0.045	0.590	(0.24 - 0.36) [0.20, 0.40]
Potassium (% DW)	0.38 (0.0094) [0.36 - 0.39]	0.37 (0.0094) [0.35 - 0.39]	0.0029 (0.013) [-0.020 - 0.038]	-0.055, 0.060	0.848	(0.29 - 0.37) [0.26, 0.42]
Zinc (mg/kg DW)	23.67 (0.53) [22.85 - 24.24]	24.05 (0.53) [23.08 - 25.20]	-0.37 (0.75) [-1.26 - 1.17]	-3.60, 2.85	0.666	(16.78 - 28.17) [11.61, 32.63]
Proximate						
Ash (% DW)	1.57 (0.033) [1.49 - 1.62]	1.38 (0.033) [1.33 - 1.41]	0.19 (0.017) [0.17 - 0.22]	0.12, 0.26	0.007	(1.17 - 2.01) [0.55, 2.30]
Carbohydrates (% DW)	83.43 (0.26) [83.10 - 83.87]	83.77 (0.26) [83.31 - 84.27]	-0.35 (0.19) [-0.65 - 0.0075]	-1.17, 0.48	0.212	(82.11 - 87.06) [80.32, 89.92]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

			Difference (Test minus Control)			
Analytical Component (Units)[1]	**Test Mean (S.E.) [Range]**	**Control Mean (S.E.) [Range]**	**Mean (S.E.) [Range]**	**95% CI (Lower, Upper)**	**p-Value**	**Commercial (Range) [99% Tolerance Int.[2]]**
Proximate						
Moisture (% FW)	10.58 (0.33) [9.84 - 11.00]	10.87 (0.33) [10.30 - 11.20]	-0.29 (0.47) [-1.36 - 0.60]	-2.30, 1.73	0.602	(8.74 - 11.30) [7.58, 12.13]
Protein (% DW)	11.20 (0.26) [10.79 - 11.45]	11.20 (0.26) [10.65 - 11.70]	-0.0078 (0.17) [-0.35 - 0.19]	-0.75, 0.73	0.967	(8.27 - 11.50) [6.26, 13.45]
Total Fat (% DW)	3.81 (0.059) [3.72 - 3.96]	3.65 (0.059) [3.59 - 3.68]	0.16 (0.075) [0.033 - 0.29]	-0.16, 0.48	0.167	(2.95 - 4.40) [2.08, 5.12]
Vitamin						
Folic Acid (mg/kg DW)	0.31 (0.0093) [0.29 - 0.32]	0.30 (0.0093) [0.28 - 0.32]	0.0085 (0.013) [-0.0079 - 0.039]	-0.048, 0.065	0.585	(0.19 - 0.31) [0.13, 0.38]
Niacin (mg/kg DW)	17.19 (0.88) [15.53 - 19.10]	16.60 (0.88) [15.30 - 17.61]	0.59 (1.24) [-1.36 - 3.80]	-4.76, 5.94	0.681	(15.07 - 32.38) [4.67, 36.68]
Pyridoxine HCl/Vitamin B6 (mg/kg DW)	6.71 (0.43) [6.29 - 7.49]	6.69 (0.43) [5.83 - 7.41]	0.018 (0.60) [-1.12 - 1.66]	-2.58, 2.61	0.978	(4.93 - 7.53) [3.12, 8.09]
Riboflavin (mg/kg DW)	1.58 (0.086) [1.41 - 1.80]	1.48 (0.086) [1.42 - 1.55]	0.10 (0.075) [-0.0017 - 0.25]	-0.22, 0.42	0.313	(0.95 - 2.42) [0.047, 2.91]
Thiamine HCl (mg/kg DW)	3.50 (0.058) [3.37 - 3.60]	3.40 (0.058) [3.34 - 3.49]	0.10 (0.043) [0.023 - 0.17]	-0.084, 0.29	0.144	(2.43 - 4.17) [1.84, 4.94]

Table 6 (cont). Statistical Summary of Site NE Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Vitamin						
Vitamin E (mg/kg DW)	15.69 (0.74) [13.71 - 17.06]	15.22 (0.74) [14.72 - 15.64]	0.47 (1.05) [-1.93 - 2.34]	-4.05, 4.99	0.699	(5.96 - 17.70) [0, 26.07]
Antinutrient						
Phytic Acid (% DW)	0.87 (0.038) [0.77 - 0.94]	0.81 (0.038) [0.79 - 0.84]	0.053 (0.054) [-0.078 - 0.14]	-0.18, 0.28	0.428	(0.69 - 0.98) [0.50, 1.11]
Raffinose (% DW)	0.17 (0.0051) [0.16 - 0.19]	0.16 (0.0051) [0.15 - 0.16]	0.017 (0.0072) [0.00094 - 0.029]	-0.014, 0.048	0.136	(0.079 - 0.19) [0.039, 0.26]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1685.40 (76.01) [1563.89 - 1840.63]	1619.62 (76.01) [1527.31 - 1756.76]	65.78 (107.50) [-192.87 - 313.32]	-396.75, 528.30	0.602	(1205.75 - 2873.05) [395.96, 3485.38]
p-Coumaric Acid (µg/g DW)	108.85 (6.64) [100.00 - 117.85]	118.25 (6.64) [106.02 - 132.88]	-9.41 (9.39) [-24.19 - 11.83]	-49.80, 30.98	0.421	(128.21 - 327.39) [7.61, 408.53]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table 7. Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.66 (0.038) [0.63 - 0.72]	0.69 (0.038) [0.68 - 0.71]	-0.029 (0.051) [-0.077 - 0.032]	-0.14, 0.080	0.580	(0.66 - 0.89) [0.44, 1.06]
Arginine (% DW)	0.35 (0.019) [0.32 - 0.38]	0.37 (0.019) [0.32 - 0.40]	-0.018 (0.026) [-0.030 - -0.0012]	-0.074, 0.037	0.496	(0.34 - 0.46) [0.23, 0.55]
Aspartic Acid (% DW)	0.57 (0.027) [0.54 - 0.61]	0.59 (0.027) [0.58 - 0.60]	-0.021 (0.038) [-0.057 - 0.029]	-0.10, 0.058	0.579	(0.58 - 0.77) [0.39, 0.88]
Cystine (% DW)	0.21 (0.0068) [0.21 - 0.22]	0.22 (0.0068) [0.21 - 0.22]	-0.0017 (0.0096) [-0.0082 - 0.010]	-0.022, 0.018	0.859	(0.20 - 0.24) [0.16, 0.27]
Glutamic Acid (% DW)	1.71 (0.099) [1.63 - 1.86]	1.80 (0.099) [1.76 - 1.85]	-0.085 (0.14) [-0.22 - 0.099]	-0.38, 0.21	0.540	(1.64 - 2.26) [1.09, 2.72]
Glycine (% DW)	0.33 (0.011) [0.32 - 0.35]	0.34 (0.011) [0.33 - 0.35]	-0.0086 (0.015) [-0.029 - 0.012]	-0.041, 0.024	0.579	(0.31 - 0.38) [0.26, 0.42]
Histidine (% DW)	0.28 (0.011) [0.26 - 0.29]	0.29 (0.011) [0.28 - 0.29]	-0.010 (0.015) [-0.031 - 0.011]	-0.042, 0.021	0.489	(0.24 - 0.30) [0.20, 0.34]
Isoleucine (% DW)	0.31 (0.018) [0.29 - 0.34]	0.33 (0.018) [0.32 - 0.34]	-0.022 (0.025) [-0.044 - 0.011]	-0.075, 0.032	0.401	(0.30 - 0.41) [0.19, 0.49]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.13 (0.072) [1.08 - 1.24]	1.18 (0.072) [1.16 - 1.20]	-0.047 (0.099) [-0.13 - 0.069]	-0.26, 0.16	0.643	(1.06 - 1.53) [0.66, 1.87]
Lysine (% DW)	0.27 (0.0077) [0.26 - 0.28]	0.28 (0.0077) [0.27 - 0.29]	-0.011 (0.010) [-0.027 - 0.013]	-0.033, 0.011	0.298	(0.25 - 0.31) [0.19, 0.35]
Methionine (% DW)	0.18 (0.0092) [0.17 - 0.18]	0.18 (0.0092) [0.17 - 0.19]	-0.0061 (0.013) [-0.020 - 0.011]	-0.033, 0.021	0.643	(0.18 - 0.23) [0.14, 0.26]
Phenylalanine (% DW)	0.45 (0.026) [0.43 - 0.49]	0.47 (0.026) [0.47 - 0.49]	-0.021 (0.036) [-0.056 - 0.024]	-0.097, 0.056	0.578	(0.44 - 0.60) [0.28, 0.72]
Proline (% DW)	0.81 (0.041) [0.77 - 0.88]	0.85 (0.041) [0.84 - 0.86]	-0.038 (0.055) [-0.092 - 0.045]	-0.15, 0.079	0.497	(0.72 - 0.99) [0.48, 1.18]
Serine (% DW)	0.44 (0.022) [0.43 - 0.47]	0.45 (0.022) [0.44 - 0.47]	-0.0099 (0.031) [-0.041 - 0.030]	-0.075, 0.055	0.749	(0.43 - 0.55) [0.32, 0.65]
Threonine (% DW)	0.30 (0.013) [0.28 - 0.33]	0.32 (0.013) [0.31 - 0.33]	-0.018 (0.019) [-0.033 - 0.011]	-0.057, 0.021	0.348	(0.30 - 0.37) [0.23, 0.42]
Tryptophan (% DW)	0.054 (0.0027) [0.051 - 0.057]	0.051 (0.0027) [0.046 - 0.053]	0.0031 (0.0038) [-0.00009 - 0.0057]	-0.0048, 0.011	0.424	(0.040 - 0.059) [0.022, 0.078]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.18 (0.034) [0.12 - 0.28]	0.18 (0.034) [0.13 - 0.21]	-0.0034 (0.046) [-0.083 - 0.079]	-0.10, 0.094	0.942	(0.14 - 0.32) [0, 0.53]
Valine (% DW)	0.44 (0.021) [0.42 - 0.47]	0.46 (0.021) [0.45 - 0.47]	-0.023 (0.029) [-0.054 - 0.017]	-0.084, 0.038	0.436	(0.41 - 0.54) [0.29, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	10.91 (0.24) [10.68 - 11.06]	11.03 (0.24) [10.96 - 11.08]	-0.12 (0.35) [-0.40 - 0.042]	-0.84, 0.60	0.733	(9.53 - 12.33) [7.43, 14.09]
18:0 Stearic (% Total FA)	1.80 (0.052) [1.78 - 1.83]	1.86 (0.052) [1.81 - 1.91]	-0.052 (0.066) [-0.11 - 0.023]	-0.19, 0.089	0.441	(1.28 - 2.13) [0.60, 2.58]
18:1 Oleic (% Total FA)	21.19 (0.33) [21.10 - 21.28]	21.34 (0.33) [20.75 - 21.93]	-0.15 (0.44) [-0.84 - 0.44]	-1.07, 0.77	0.739	(22.13 - 31.09) [12.40, 36.28]
18:2 Linoleic (% Total FA)	64.26 (0.50) [64.06 - 64.65]	63.89 (0.50) [63.22 - 64.48]	0.37 (0.71) [-0.42 - 1.43]	-1.11, 1.84	0.607	(55.17 - 64.97) [49.61, 73.18]
18:3 Linolenic (% Total FA)	1.17 (0.023) [1.15 - 1.20]	1.21 (0.023) [1.20 - 1.22]	-0.035 (0.032) [-0.049 - -0.021]	-0.10, 0.032	0.292	(1.00 - 1.32) [0.72, 1.66]
20:0 Arachidic (% Total FA)	0.33 (0.0086) [0.31 - 0.34]	0.34 (0.0086) [0.34 - 0.34]	-0.0073 (0.012) [-0.026 - 0.0090]	-0.033, 0.018	0.553	(0.29 - 0.42) [0.19, 0.52]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.19 (0.0058) [0.18 - 0.20]	0.19 (0.0058) [0.18 - 0.20]	-0.0015 (0.0078) [-0.0036 - 0.0015]	-0.018, 0.015	0.845	(0.20 - 0.31) [0.10, 0.36]
22:0 Behenic (% Total FA)	0.14 (0.019) [0.13 - 0.16]	0.15 (0.019) [0.14 - 0.16]	-0.0053 (0.027) [-0.011 - 0.0048]	-0.062, 0.051	0.845	(0.061 - 0.33) [0, 0.48]
Fiber						
Acid Detergent Fiber (% DW)	2.75 (0.38) [2.51 - 2.99]	2.99 (0.38) [2.26 - 4.41]	-0.25 (0.54) [-1.43 - 0.48]	-1.38, 0.88	0.650	(1.95 - 3.76) [0.29, 5.01]
Neutral Detergent Fiber (% DW)	8.55 (0.41) [8.29 - 8.83]	9.13 (0.41) [8.75 - 9.70]	-0.58 (0.58) [-1.41 - -0.078]	-1.80, 0.65	0.333	(7.15 - 9.41) [5.23, 10.90]
Total Dietary Fiber (% DW)	13.97 (0.52) [12.83 - 16.00]	13.29 (0.52) [12.84 - 13.98]	0.68 (0.73) [-1.15 - 2.97]	-0.85, 2.22	0.361	(10.24 - 13.51) [6.72, 16.07]
Mineral						
Calcium (% DW)	0.0042 (0.00035) [0.0035 - 0.0051]	0.0038 (0.00035) [0.0036 - 0.0040]	0.00041 (0.00049) [-0.00002 - 0.0012]	-0.00061, 0.0014	0.411	(0.0032 - 0.0057) [0.00076, 0.0080]
Copper (mg/kg DW)	1.80 (0.19) [1.66 - 1.92]	2.09 (0.19) [1.65 - 2.81]	-0.28 (0.27) [-0.89 - 0.031]	-0.86, 0.29	0.311	(1.29 - 4.16) [0, 5.74]
Iron (mg/kg DW)	14.92 (0.92) [14.53 - 15.44]	15.87 (0.92) [15.22 - 16.86]	-0.95 (1.29) [-2.07 - -0.096]	-3.68, 1.77	0.470	(14.37 - 19.48) [10.40, 23.42]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.12 (0.0049) [0.11 - 0.12]	0.11 (0.0049) [0.11 - 0.11]	0.012 (0.0070) [0.0083 - 0.014]	-0.0025, 0.027	0.099	(0.095 - 0.13) [0.064, 0.16]
Manganese (mg/kg DW)	6.59 (0.36) [6.06 - 7.08]	6.75 (0.36) [5.48 - 7.58]	-0.16 (0.51) [-1.51 - 1.61]	-1.28, 0.96	0.755	(4.55 - 9.02) [0.69, 10.70]
Phosphorus (% DW)	0.32 (0.014) [0.32 - 0.33]	0.30 (0.014) [0.28 - 0.32]	0.018 (0.020) [0.0020 - 0.041]	-0.024, 0.060	0.374	(0.27 - 0.36) [0.21, 0.40]
Potassium (% DW)	0.40 (0.014) [0.37 - 0.41]	0.43 (0.014) [0.38 - 0.45]	-0.028 (0.020) [-0.078 - 0.034]	-0.070, 0.013	0.165	(0.32 - 0.42) [0.25, 0.47]
Zinc (mg/kg DW)	23.55 (1.17) [22.70 - 25.09]	22.92 (1.17) [19.62 - 25.34]	0.63 (1.65) [-2.48 - 5.47]	-2.90, 4.16	0.709	(18.12 - 29.69) [7.39, 38.63]
Proximate						
Ash (% DW)	1.45 (0.068) [1.35 - 1.53]	1.48 (0.068) [1.26 - 1.60]	-0.030 (0.087) [-0.22 - 0.19]	-0.22, 0.15	0.731	(1.14 - 1.47) [0.90, 1.76]
Carbohydrates (% DW)	86.07 (0.55) [84.85 - 87.63]	85.45 (0.55) [85.07 - 85.73]	0.62 (0.74) [-0.88 - 2.56]	-0.95, 2.18	0.416	(83.60 - 86.65) [81.08, 89.71]
Moisture (% FW)	11.97 (0.33) [11.90 - 12.10]	11.97 (0.33) [11.30 - 12.80]	0 (0.46) [-0.70 - 0.60]	-0.96, 0.96	1.000	(11.00 - 12.20) [10.10, 13.35]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	8.75 (0.47) [7.57 - 9.78]	9.36 (0.47) [9.19 - 9.50]	-0.61 (0.65) [-1.93 - 0.40]	-1.98, 0.76	0.361	(8.69 - 11.33) [5.83, 13.57]
Total Fat (% DW)	3.73 (0.089) [3.45 - 3.92]	3.71 (0.089) [3.63 - 3.86]	0.022 (0.12) [-0.42 - 0.29]	-0.23, 0.28	0.854	(3.16 - 4.07) [2.47, 4.68]
Vitamin						
Folic Acid (mg/kg DW)	0.24 (0.020) [0.23 - 0.26]	0.28 (0.020) [0.23 - 0.33]	-0.036 (0.025) [-0.085 - 0.00026]	-0.089, 0.017	0.163	(0.26 - 0.41) [0.11, 0.55]
Niacin (mg/kg DW)	19.99 (0.93) [18.50 - 21.50]	19.50 (0.93) [18.81 - 20.63]	0.50 (1.23) [-0.65 - 2.69]	-2.11, 3.10	0.690	(14.92 - 26.80) [5.96, 38.50]
Thiamine HCl (mg/kg DW)	2.80 (0.093) [2.72 - 2.84]	2.88 (0.093) [2.75 - 3.06]	-0.075 (0.13) [-0.22 - 0.092]	-0.35, 0.20	0.574	(2.94 - 4.78) [1.01, 6.00]
Vitamin B2 (mg/kg DW)	1.97 (0.18) [1.91 - 2.10]	1.80 (0.18) [1.61 - 2.06]	0.17 (0.25) [-0.16 - 0.37]	-0.34, 0.69	0.490	(1.62 - 2.62) [0.87, 3.38]
Vitamin B6 (mg/kg DW)	6.14 (0.35) [5.63 - 6.61]	6.59 (0.35) [6.17 - 6.93]	-0.45 (0.50) [-1.30 - 0.0070]	-1.50, 0.59	0.374	(4.01 - 6.70) [1.86, 8.29]
Vitamin E (mg/kg DW)	12.46 (0.46) [12.37 - 12.51]	10.50 (0.46) [10.19 - 10.76]	1.96 (0.56) [1.73 - 2.32]	0.78, 3.14	0.002	(2.83 - 11.69) [0, 19.32]

Table 7 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.73 (0.047) [0.69 - 0.75]	0.78 (0.047) [0.71 - 0.85]	-0.052 (0.064) [-0.098 - -0.018]	-0.19, 0.082	0.425	(0.58 - 0.97) [0.28, 1.15]
Raffinose (% DW)	0.12 (0.0068) [0.11 - 0.12]	0.13 (0.0068) [0.13 - 0.14]	-0.016 (0.0079) [-0.022 - -0.0048]	-0.033, 0.0015	0.070	(0.028 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1810.07 (167.78) [1498.30 - 2002.28]	1915.81 (167.78) [1678.00 - 2096.96]	-105.75 (202.84) [-179.71 - 29.80]	-535.46, 323.96	0.609	(1504.52 - 2224.72) [1019.70, 2703.40]
p-Coumaric Acid (µg/g DW)	140.38 (18.23) [77.98 - 185.02]	174.57 (18.23) [126.98 - 204.06]	-34.19 (21.43) [-49.00 - -19.04]	-79.62, 11.23	0.130	(84.79 - 239.33) [0, 378.84]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

Table 8. Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.78 (0.038) [0.78 - 0.80]	0.76 (0.038) [0.68 - 0.89]	0.024 (0.051) [-0.11 - 0.098]	-0.085, 0.13	0.641	(0.77 - 0.96) [0.59, 1.09]
Arginine (% DW)	0.43 (0.019) [0.41 - 0.44]	0.40 (0.019) [0.34 - 0.47]	0.029 (0.026) [-0.058 - 0.10]	-0.026, 0.085	0.282	(0.41 - 0.50) [0.32, 0.56]
Aspartic Acid (% DW)	0.65 (0.027) [0.64 - 0.65]	0.64 (0.027) [0.59 - 0.73]	0.0045 (0.038) [-0.090 - 0.062]	-0.075, 0.084	0.905	(0.63 - 0.76) [0.52, 0.88]
Cystine (% DW)	0.22 (0.0068) [0.22 - 0.22]	0.22 (0.0068) [0.20 - 0.24]	0.00015 (0.0096) [-0.021 - 0.027]	-0.020, 0.020	0.987	(0.20 - 0.26) [0.15, 0.30]
Glutamic Acid (% DW)	2.03 (0.099) [2.02 - 2.06]	1.95 (0.099) [1.71 - 2.29]	0.083 (0.14) [-0.28 - 0.30]	-0.21, 0.37	0.551	(1.94 - 2.44) [1.51, 2.80]
Glycine (% DW)	0.37 (0.011) [0.36 - 0.37]	0.35 (0.011) [0.33 - 0.39]	0.011 (0.015) [-0.021 - 0.034]	-0.021, 0.044	0.465	(0.35 - 0.42) [0.30, 0.45]
Histidine (% DW)	0.31 (0.011) [0.31 - 0.31]	0.30 (0.011) [0.27 - 0.34]	0.0093 (0.015) [-0.024 - 0.034]	-0.022, 0.041	0.539	(0.27 - 0.33) [0.23, 0.36]
Isoleucine (% DW)	0.37 (0.018) [0.37 - 0.38]	0.36 (0.018) [0.32 - 0.41]	0.016 (0.025) [-0.043 - 0.048]	-0.037, 0.070	0.523	(0.34 - 0.44) [0.27, 0.50]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.37 (0.072) [1.35 - 1.41]	1.31 (0.072) [1.13 - 1.56]	0.067 (0.099) [-0.22 - 0.24]	-0.14, 0.28	0.508	(1.29 - 1.65) [0.98, 1.91]
Lysine (% DW)	0.29 (0.0077) [0.28 - 0.30]	0.29 (0.0077) [0.28 - 0.31]	-0.0015 (0.010) [-0.015 - 0.0092]	-0.023, 0.020	0.887	(0.28 - 0.31) [0.25, 0.34]
Methionine (% DW)	0.19 (0.0092) [0.18 - 0.21]	0.19 (0.0092) [0.16 - 0.22]	0.0061 (0.013) [-0.016 - 0.038]	-0.021, 0.033	0.642	(0.19 - 0.30) [0.095, 0.35]
Phenylalanine (% DW)	0.54 (0.026) [0.53 - 0.55]	0.52 (0.026) [0.45 - 0.61]	0.024 (0.036) [-0.078 - 0.086]	-0.053, 0.10	0.515	(0.51 - 0.63) [0.41, 0.72]
Proline (% DW)	0.94 (0.041) [0.93 - 0.95]	0.91 (0.041) [0.84 - 1.03]	0.025 (0.055) [-0.099 - 0.089]	-0.091, 0.14	0.650	(0.78 - 1.03) [0.64, 1.23]
Serine (% DW)	0.51 (0.022) [0.50 - 0.52]	0.50 (0.022) [0.43 - 0.59]	0.014 (0.031) [-0.087 - 0.089]	-0.051, 0.079	0.643	(0.48 - 0.60) [0.36, 0.71]
Threonine (% DW)	0.35 (0.013) [0.35 - 0.35]	0.34 (0.013) [0.31 - 0.39]	0.0087 (0.019) [-0.042 - 0.039]	-0.030, 0.048	0.645	(0.33 - 0.39) [0.28, 0.44]
Tryptophan (% DW)	0.053 (0.0027) [0.051 - 0.055]	0.052 (0.0027) [0.050 - 0.057]	0.00016 (0.0038) [-0.0014 - 0.00092]	-0.0077, 0.0080	0.967	(0.043 - 0.063) [0.031, 0.082]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.27 (0.034) [0.18 - 0.33]	0.21 (0.034) [0.12 - 0.30]	0.058 (0.046) [-0.11 - 0.16]	-0.039, 0.16	0.224	(0.25 - 0.41) [0.12, 0.52]
Valine (% DW)	0.51 (0.021) [0.50 - 0.51]	0.49 (0.021) [0.45 - 0.55]	0.019 (0.029) [-0.041 - 0.050]	-0.042, 0.081	0.508	(0.47 - 0.58) [0.39, 0.64]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.18 (0.24) [10.99 - 11.33]	11.28 (0.24) [11.20 - 11.38]	-0.095 (0.35) [-0.21 - 0.078]	-0.82, 0.63	0.787	(9.84 - 12.33) [7.71, 14.14]
18:0 Stearic (% Total FA)	1.94 (0.052) [1.93 - 1.95]	1.84 (0.052) [1.68 - 2.08]	0.093 (0.066) [-0.15 - 0.25]	-0.048, 0.23	0.182	(1.30 - 2.10) [0.71, 2.57]
18:1 Oleic (% Total FA)	21.31 (0.33) [20.93 - 21.51]	20.74 (0.33) [19.59 - 21.98]	0.56 (0.44) [-0.50 - 1.33]	-0.36, 1.48	0.215	(20.78 - 29.13) [12.15, 35.55]
18:2 Linoleic (% Total FA)	63.67 (0.50) [63.27 - 64.32]	64.30 (0.50) [62.75 - 65.65]	-0.63 (0.71) [-1.32 - 0.66]	-2.10, 0.84	0.383	(56.51 - 64.46) [50.63, 72.71]
18:3 Linolenic (% Total FA)	1.21 (0.023) [1.20 - 1.23]	1.22 (0.023) [1.17 - 1.26]	-0.0040 (0.032) [-0.057 - 0.048]	-0.071, 0.063	0.901	(1.03 - 1.38) [0.67, 1.76]
20:0 Arachidic (% Total FA)	0.33 (0.0086) [0.32 - 0.34]	0.32 (0.0086) [0.31 - 0.33]	0.012 (0.012) [0.0092 - 0.014]	-0.014, 0.037	0.349	(0.30 - 0.41) [0.18, 0.52]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.18 (0.0058) [0.18 - 0.19]	0.19 (0.0058) [0.19 - 0.19]	-0.0022 (0.0078) [-0.0076 - 0.0010]	-0.019, 0.014	0.784	(0.18 - 0.27) [0.11, 0.34]
22:0 Behenic (% Total FA)	0.17 (0.019) [0.13 - 0.20]	0.11 (0.019) [0.067 - 0.14]	0.064 (0.027) [-0.0059 - 0.13]	0.0071, 0.12	0.029	(0.062 - 0.18) [0, 0.32]
Fiber						
Acid Detergent Fiber (% DW)	2.57 (0.38) [1.85 - 3.05]	2.36 (0.38) [1.83 - 3.05]	0.20 (0.54) [0.0025 - 0.58]	-0.93, 1.33	0.711	(1.83 - 3.39) [0.88, 4.63]
Neutral Detergent Fiber (% DW)	8.57 (0.41) [7.33 - 10.00]	8.34 (0.41) [7.95 - 8.58]	0.23 (0.58) [-1.25 - 2.05]	-0.99, 1.46	0.692	(6.08 - 10.36) [2.87, 13.22]
Total Dietary Fiber (% DW)	12.31 (0.52) [11.89 - 12.87]	12.69 (0.52) [12.18 - 13.70]	-0.38 (0.73) [-1.53 - 0.68]	-1.91, 1.15	0.609	(10.57 - 14.56) [6.50, 17.54]
Mineral						
Calcium (% DW)	0.0051 (0.00035) [0.0048 - 0.0056]	0.0045 (0.00035) [0.0041 - 0.0052]	0.00057 (0.00049) [-0.00036 - 0.0015]	-0.00045, 0.0016	0.255	(0.0035 - 0.0070) [0, 0.010]
Copper (mg/kg DW)	2.32 (0.19) [2.23 - 2.49]	2.16 (0.23) [1.87 - 2.23]	0.17 (0.30) [0.011 - 0.62]	-0.47, 0.80	0.589	(1.39 - 2.76) [0.22, 3.82]
Iron (mg/kg DW)	17.40 (0.92) [16.38 - 18.68]	18.29 (0.92) [16.12 - 22.21]	-0.89 (1.29) [-3.53 - 1.02]	-3.61, 1.84	0.500	(15.90 - 24.66) [7.05, 30.38]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.13 (0.0049) [0.13 - 0.13]	0.12 (0.0049) [0.10 - 0.14]	0.0082 (0.0070) [-0.011 - 0.024]	-0.0063, 0.023	0.252	(0.11 - 0.14) [0.083, 0.16]
Manganese (mg/kg DW)	8.00 (0.36) [7.22 - 8.66]	7.29 (0.36) [6.38 - 7.77]	0.72 (0.51) [-0.54 - 2.28]	-0.40, 1.83	0.187	(4.78 - 9.35) [0.72, 11.82]
Phosphorus (% DW)	0.34 (0.014) [0.32 - 0.34]	0.32 (0.014) [0.27 - 0.38]	0.015 (0.020) [-0.034 - 0.075]	-0.027, 0.057	0.458	(0.30 - 0.38) [0.25, 0.42]
Potassium (% DW)	0.41 (0.014) [0.39 - 0.42]	0.40 (0.014) [0.37 - 0.43]	0.0032 (0.020) [-0.038 - 0.038]	-0.038, 0.045	0.873	(0.36 - 0.43) [0.29, 0.49]
Zinc (mg/kg DW)	24.35 (1.17) [22.51 - 26.77]	24.44 (1.17) [21.29 - 27.79]	-0.088 (1.65) [-1.72 - 2.49]	-3.62, 3.44	0.958	(18.25 - 30.44) [6.01, 42.60]
Proximate						
Ash (% DW)	1.53 (0.068) [1.47 - 1.58]	1.50 (0.068) [1.39 - 1.63]	0.024 (0.087) [-0.11 - 0.091]	-0.16, 0.21	0.785	(1.27 - 1.63) [1.06, 1.93]
Carbohydrates (% DW)	84.18 (0.55) [83.87 - 84.45]	84.66 (0.55) [83.04 - 85.98]	-0.48 (0.74) [-1.76 - 1.41]	-2.04, 1.08	0.525	(82.10 - 85.17) [80.40, 87.76]
Moisture (% FW)	12.27 (0.33) [12.10 - 12.50]	11.73 (0.33) [11.30 - 12.20]	0.53 (0.46) [0 - 1.20]	-0.43, 1.49	0.260	(11.70 - 13.20) [10.50, 14.11]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	10.36 (0.47) [10.19 - 10.60]	10.07 (0.47) [9.17 - 11.50]	0.29 (0.65) [-1.31 - 1.13]	-1.08, 1.66	0.657	(9.99 - 12.19) [8.12, 13.56]
Total Fat (% DW)	3.93 (0.089) [3.77 - 4.01]	3.77 (0.089) [3.47 - 3.96]	0.16 (0.12) [-0.19 - 0.54]	-0.091, 0.42	0.187	(3.18 - 4.22) [2.07, 5.10]
Vitamin						
Folic Acid (mg/kg DW)	0.26 (0.020) [0.25 - 0.27]	0.26 (0.020) [0.26 - 0.27]	-0.0060 (0.025) [-0.0080 - -0.0033]	-0.059, 0.047	0.811	(0.26 - 0.42) [0.098, 0.58]
Niacin (mg/kg DW)	17.32 (0.93) [16.23 - 18.34]	19.33 (0.93) [17.08 - 21.20]	-2.00 (1.23) [-4.97 - 1.25]	-4.61, 0.60	0.122	(13.64 - 27.42) [2.23, 41.53]
Thiamine HCl (mg/kg DW)	3.00 (0.093) [2.84 - 3.09]	2.91 (0.093) [2.71 - 3.19]	0.093 (0.13) [-0.11 - 0.38]	-0.18, 0.37	0.489	(2.87 - 4.33) [1.55, 5.85]
Vitamin B2 (mg/kg DW)	2.08 (0.18) [1.51 - 2.63]	2.44 (0.18) [2.23 - 2.81]	-0.37 (0.25) [-1.30 - 0.35]	-0.88, 0.15	0.156	(1.81 - 2.78) [0.88, 3.61]
Vitamin B6 (mg/kg DW)	6.44 (0.35) [6.31 - 6.57]	6.95 (0.35) [6.08 - 8.27]	-0.51 (0.50) [-1.96 - 0.36]	-1.55, 0.53	0.321	(5.30 - 8.22) [2.06, 9.98]
Vitamin E (mg/kg DW)	13.34 (0.46) [12.57 - 14.24]	11.16 (0.46) [10.15 - 12.19]	2.18 (0.56) [2.05 - 2.42]	1.00, 3.36	0.001	(2.84 - 15.53) [0, 22.61]

Table 8 (cont). Statistical Summary of Site CL Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.87 (0.047) [0.84 - 0.89]	0.69 (0.047) [0.60 - 0.86]	0.18 (0.064) [0.023 - 0.27]	0.043, 0.31	0.012	(0.67 - 0.94) [0.40, 1.12]
Raffinose (% DW)	0.13 (0.0068) [0.12 - 0.14]	0.14 (0.0068) [0.14 - 0.15]	-0.011 (0.0079) [-0.018 - 0]	-0.029, 0.0060	0.179	(0.061 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2025.21 (167.78) [1877.13 - 2107.06]	1658.51 (167.78) [1088.34 - 1993.17]	366.70 (202.84) [113.90 - 788.80]	-63.01, 796.41	0.089	(1011.40 - 2539.86) [0, 4071.51]
p-Coumaric Acid (µg/g DW)	140.97 (18.23) [131.97 - 147.43]	118.88 (18.23) [66.48 - 151.48]	22.09 (21.43) [-7.97 - 65.49]	-23.33, 67.52	0.317	(84.15 - 259.68) [0, 378.67]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

Table 9. Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.72 (0.032) [0.71 - 0.73]	0.71 (0.032) [0.63 - 0.76]	0.0045 (0.040) [-0.046 - 0.076]	-0.081, 0.090	0.911	(0.66 - 0.89) [0.44, 1.06]
Arginine (% DW)	0.39 (0.022) [0.35 - 0.43]	0.41 (0.022) [0.38 - 0.43]	-0.012 (0.024) [-0.030 - -0.00098]	-0.063, 0.039	0.615	(0.34 - 0.46) [0.23, 0.55]
Aspartic Acid (% DW)	0.61 (0.021) [0.60 - 0.62]	0.61 (0.021) [0.56 - 0.63]	0.0028 (0.025) [-0.021 - 0.033]	-0.050, 0.055	0.912	(0.58 - 0.77) [0.39, 0.88]
Cystine (% DW)	0.21 (0.0049) [0.21 - 0.21]	0.21 (0.0049) [0.20 - 0.22]	0.0067 (0.0070) [-0.0068 - 0.017]	-0.0079, 0.021	0.349	(0.20 - 0.24) [0.16, 0.27]
Glutamic Acid (% DW)	1.84 (0.081) [1.82 - 1.88]	1.82 (0.081) [1.62 - 1.97]	0.019 (0.10) [-0.15 - 0.22]	-0.20, 0.24	0.856	(1.64 - 2.26) [1.09, 2.72]
Glycine (% DW)	0.34 (0.010) [0.34 - 0.34]	0.33 (0.010) [0.31 - 0.35]	0.0045 (0.013) [-0.0067 - 0.026]	-0.022, 0.031	0.727	(0.31 - 0.38) [0.26, 0.42]
Histidine (% DW)	0.29 (0.010) [0.29 - 0.29]	0.29 (0.010) [0.26 - 0.30]	0.0039 (0.012) [-0.0099 - 0.028]	-0.022, 0.030	0.758	(0.24 - 0.30) [0.20, 0.34]
Isoleucine (% DW)	0.34 (0.016) [0.33 - 0.34]	0.33 (0.016) [0.30 - 0.36]	0.0016 (0.020) [-0.020 - 0.037]	-0.040, 0.043	0.937	(0.30 - 0.41) [0.19, 0.49]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.23 (0.059) [1.21 - 1.27]	1.22 (0.059) [1.08 - 1.30]	0.012 (0.073) [-0.088 - 0.14]	-0.14, 0.17	0.871	(1.06 - 1.53) [0.66, 1.87]
Lysine (% DW)	0.28 (0.0080) [0.27 - 0.28]	0.28 (0.0080) [0.26 - 0.29]	0.0024 (0.0093) [-0.0063 - 0.017]	-0.017, 0.022	0.799	(0.25 - 0.31) [0.19, 0.35]
Methionine (% DW)	0.17 (0.0059) [0.17 - 0.18]	0.17 (0.0059) [0.16 - 0.19]	0.0031 (0.0084) [-0.021 - 0.016]	-0.014, 0.021	0.714	(0.18 - 0.23) [0.14, 0.26]
Phenylalanine (% DW)	0.49 (0.022) [0.48 - 0.50]	0.49 (0.022) [0.43 - 0.52]	0.0011 (0.027) [-0.034 - 0.051]	-0.055, 0.057	0.968	(0.44 - 0.60) [0.28, 0.72]
Proline (% DW)	0.92 (0.043) [0.91 - 0.93]	0.91 (0.043) [0.83 - 0.95]	0.011 (0.055) [-0.040 - 0.086]	-0.11, 0.13	0.849	(0.72 - 0.99) [0.48, 1.18]
Serine (% DW)	0.47 (0.018) [0.47 - 0.48]	0.46 (0.018) [0.42 - 0.50]	0.0090 (0.024) [-0.032 - 0.050]	-0.042, 0.060	0.713	(0.43 - 0.55) [0.32, 0.65]
Threonine (% DW)	0.32 (0.010) [0.31 - 0.33]	0.32 (0.010) [0.31 - 0.33]	-0.00091 (0.012) [-0.015 - 0.0079]	-0.027, 0.025	0.942	(0.30 - 0.37) [0.23, 0.42]
Tryptophan (% DW)	0.049 (0.0030) [0.047 - 0.050]	0.049 (0.0030) [0.046 - 0.054]	-0.00029 (0.0042) [-0.0037 - 0.0029]	-0.0090, 0.0084	0.945	(0.040 - 0.059) [0.022, 0.078]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.22 (0.037) [0.16 - 0.30]	0.30 (0.037) [0.27 - 0.32]	-0.079 (0.046) [-0.11 - -0.027]	-0.18, 0.019	0.105	(0.14 - 0.32) [0, 0.53]
Valine (% DW)	0.46 (0.019) [0.46 - 0.47]	0.46 (0.019) [0.41 - 0.49]	0.0025 (0.023) [-0.021 - 0.046]	-0.047, 0.052	0.915	(0.41 - 0.54) [0.29, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	10.84 (0.11) [10.74 - 11.06]	11.15 (0.11) [11.00 - 11.27]	-0.31 (0.15) [-0.44 - -0.22]	-0.63, 0.018	0.062	(9.53 - 12.33) [7.43, 14.09]
18:0 Stearic (% Total FA)	1.78 (0.026) [1.76 - 1.79]	1.78 (0.026) [1.75 - 1.82]	-0.0047 (0.037) [-0.030 - 0.028]	-0.083, 0.073	0.900	(1.28 - 2.13) [0.60, 2.58]
18:1 Oleic (% Total FA)	20.97 (0.20) [20.70 - 21.21]	21.48 (0.20) [21.13 - 21.90]	-0.50 (0.25) [-1.20 - -0.13]	-1.04, 0.028	0.061	(22.13 - 31.09) [12.40, 36.28]
18:2 Linoleic (% Total FA)	64.64 (0.28) [64.11 - 65.10]	63.82 (0.28) [63.51 - 64.23]	0.82 (0.39) [0.38 - 1.59]	0.0079, 1.63	0.048	(55.17 - 64.97) [49.61, 73.18]
18:3 Linolenic (% Total FA)	1.18 (0.018) [1.18 - 1.20]	1.20 (0.018) [1.19 - 1.22]	-0.018 (0.022) [-0.021 - -0.013]	-0.064, 0.028	0.415	(1.00 - 1.32) [0.72, 1.66]
20:0 Arachidic (% Total FA)	0.30 (0.0045) [0.29 - 0.31]	0.30 (0.0045) [0.29 - 0.31]	-0.0021 (0.0063) [-0.015 - 0.0077]	-0.015, 0.011	0.745	(0.29 - 0.42) [0.19, 0.52]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.18 (0.0047) [0.17 - 0.19]	0.18 (0.0047) [0.17 - 0.19]	-0.0011 (0.0063) [-0.016 - 0.0089]	-0.014, 0.012	0.867	(0.20 - 0.31) [0.10, 0.36]
22:0 Behenic (% Total FA)	0.10 (0.035) [0.062 - 0.14]	0.088 (0.035) [0.063 - 0.14]	0.016 (0.050) [-0.075 - 0.072]	-0.088, 0.12	0.751	(0.061 - 0.33) [0, 0.48]
Fiber						
Acid Detergent Fiber (% DW)	2.39 (0.35) [2.30 - 2.47]	1.89 (0.35) [1.41 - 2.41]	0.50 (0.50) [0.065 - 0.89]	-0.54, 1.54	0.330	(1.95 - 3.76) [0.29, 5.01]
Neutral Detergent Fiber (% DW)	8.60 (0.48) [8.19 - 9.37]	8.37 (0.48) [7.74 - 9.18]	0.23 (0.67) [-0.98 - 1.62]	-1.18, 1.64	0.735	(7.15 - 9.41) [5.23, 10.90]
Total Dietary Fiber (% DW)	12.24 (0.59) [11.68 - 12.95]	11.87 (0.59) [11.20 - 12.22]	0.37 (0.84) [-0.52 - 1.76]	-1.38, 2.12	0.663	(10.24 - 13.51) [6.72, 16.07]
Mineral						
Calcium (% DW)	0.0044 (0.00022) [0.0044 - 0.0045]	0.0043 (0.00022) [0.0039 - 0.0050]	0.00015 (0.00029) [-0.00054 - 0.00052]	-0.00047, 0.00076	0.614	(0.0032 - 0.0057) [0.00076, 0.0080]
Copper (mg/kg DW)	1.73 (0.10) [1.58 - 1.97]	1.60 (0.10) [1.47 - 1.72]	0.13 (0.13) [-0.15 - 0.37]	-0.13, 0.40	0.303	(1.29 - 4.16) [0, 5.74]
Iron (mg/kg DW)	16.86 (0.47) [16.76 - 16.93]	17.37 (0.47) [16.53 - 18.49]	-0.51 (0.55) [-1.56 - 0.36]	-1.67, 0.66	0.372	(14.37 - 19.48) [10.40, 23.42]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.11 (0.0042) [0.10 - 0.11]	0.11 (0.0042) [0.11 - 0.11]	-0.00079 (0.0057) [-0.0076 - 0.0031]	-0.013, 0.011	0.891	(0.095 - 0.13) [0.064, 0.16]
Manganese (mg/kg DW)	6.36 (0.24) [5.91 - 6.89]	6.03 (0.24) [5.86 - 6.19]	0.33 (0.30) [0.046 - 0.70]	-0.31, 0.97	0.297	(4.55 - 9.02) [0.69, 10.70]
Phosphorus (% DW)	0.31 (0.0089) [0.30 - 0.32]	0.31 (0.0089) [0.29 - 0.33]	0.0015 (0.012) [-0.034 - 0.027]	-0.024, 0.027	0.899	(0.27 - 0.36) [0.21, 0.40]
Potassium (% DW)	0.40 (0.0058) [0.39 - 0.41]	0.40 (0.0058) [0.39 - 0.42]	-0.00047 (0.0082) [-0.032 - 0.017]	-0.018, 0.017	0.955	(0.32 - 0.42) [0.25, 0.47]
Zinc (mg/kg DW)	20.46 (0.69) [19.20 - 21.66]	21.07 (0.69) [19.38 - 23.11]	-0.60 (0.97) [-3.91 - 2.27]	-2.65, 1.45	0.541	(18.12 - 29.69) [7.39, 38.63]
Proximate						
Ash (% DW)	1.44 (0.050) [1.35 - 1.53]	1.43 (0.050) [1.36 - 1.48]	0.0089 (0.070) [-0.11 - 0.17]	-0.14, 0.16	0.900	(1.14 - 1.47) [0.90, 1.76]
Carbohydrates (% DW)	85.04 (0.37) [84.90 - 85.17]	85.46 (0.37) [84.91 - 86.31]	-0.42 (0.53) [-1.25 - -0.0043]	-1.52, 0.68	0.437	(83.60 - 86.65) [81.08, 89.71]
Moisture (% FW)	12.03 (0.19) [11.80 - 12.30]	11.73 (0.19) [11.30 - 12.30]	0.30 (0.27) [-0.50 - 0.70]	-0.27, 0.87	0.283	(11.00 - 12.20) [10.10, 13.35]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	9.53 (0.31) [9.36 - 9.77]	9.26 (0.31) [8.55 - 9.77]	0.27 (0.40) [-0.095 - 0.92]	-0.58, 1.13	0.509	(8.69 - 11.33) [5.83, 13.57]
Total Fat (% DW)	3.98 (0.086) [3.88 - 4.11]	3.84 (0.086) [3.79 - 3.90]	0.14 (0.12) [0.042 - 0.21]	-0.12, 0.39	0.268	(3.16 - 4.07) [2.47, 4.68]
Vitamin						
Folic Acid (mg/kg DW)	0.26 (0.017) [0.25 - 0.27]	0.27 (0.017) [0.22 - 0.31]	-0.0045 (0.022) [-0.040 - 0.029]	-0.052, 0.043	0.842	(0.26 - 0.41) [0.11, 0.55]
Niacin (mg/kg DW)	17.96 (0.78) [16.42 - 19.09]	18.02 (0.78) [17.42 - 19.16]	-0.058 (1.10) [-1.00 - 1.62]	-2.35, 2.23	0.958	(14.92 - 26.80) [5.96, 38.50]
Thiamine HCl (mg/kg DW)	2.84 (0.13) [2.73 - 2.96]	2.91 (0.13) [2.85 - 2.94]	-0.066 (0.15) [-0.20 - 0.023]	-0.40, 0.26	0.677	(2.94 - 4.78) [1.01, 6.00]
Vitamin B2 (mg/kg DW)	1.87 (0.20) [1.61 - 2.02]	2.14 (0.20) [1.74 - 2.63]	-0.27 (0.27) [-1.02 - 0.24]	-0.85, 0.32	0.346	(1.62 - 2.62) [0.87, 3.38]
Vitamin B6 (mg/kg DW)	6.24 (0.35) [6.15 - 6.39]	6.95 (0.35) [6.71 - 7.08]	-0.71 (0.49) [-0.92 - -0.53]	-1.74, 0.32	0.163	(4.01 - 6.70) [1.86, 8.29]
Vitamin E (mg/kg DW)	11.29 (0.61) [10.64 - 11.97]	11.85 (0.61) [11.25 - 12.78]	-0.56 (0.80) [-0.81 - -0.27]	-2.30, 1.17	0.493	(2.83 - 11.69) [0, 19.32]

Table 9 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.81 (0.040) [0.74 - 0.88]	0.78 (0.040) [0.68 - 0.90]	0.025 (0.057) [-0.037 - 0.12]	-0.094, 0.14	0.669	(0.58 - 0.97) [0.28, 1.15]
Raffinose (% DW)	0.082 (0.0053) [0.075 - 0.088]	0.080 (0.0053) [0.077 - 0.082]	0.0013 (0.0068) [-0.0076 - 0.0062]	-0.013, 0.016	0.847	(0.028 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1860.65 (227.11) [1511.36 - 2052.45]	1566.49 (227.11) [820.14 - 2098.06]	294.16 (316.33) [-269.92 - 1232.32]	-378.07, 966.38	0.366	(1504.52 - 2224.72) [1019.70, 2703.40]
p-Coumaric Acid (µg/g DW)	121.39 (20.72) [68.64 - 161.00]	123.37 (20.72) [64.03 - 156.71]	-1.97 (25.74) [-88.07 - 70.52]	-56.53, 52.58	0.939	(84.79 - 239.33) [0, 378.84]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

Table 10. Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.78 (0.032) [0.77 - 0.81]	0.83 (0.032) [0.79 - 0.87]	-0.046 (0.040) [-0.10 - -0.015]	-0.13, 0.039	0.270	(0.77 - 0.96) [0.59, 1.09]
Arginine (% DW)	0.43 (0.022) [0.42 - 0.44]	0.43 (0.022) [0.40 - 0.47]	-0.0046 (0.024) [-0.039 - 0.040]	-0.055, 0.046	0.851	(0.41 - 0.50) [0.32, 0.56]
Aspartic Acid (% DW)	0.64 (0.021) [0.64 - 0.66]	0.67 (0.021) [0.65 - 0.71]	-0.031 (0.025) [-0.070 - -0.0085]	-0.084, 0.022	0.228	(0.63 - 0.76) [0.52, 0.88]
Cystine (% DW)	0.23 (0.0049) [0.22 - 0.23]	0.23 (0.0049) [0.23 - 0.23]	-0.0026 (0.0070) [-0.0043 - 0.00047]	-0.017, 0.012	0.712	(0.20 - 0.26) [0.15, 0.30]
Glutamic Acid (% DW)	2.02 (0.081) [1.99 - 2.07]	2.14 (0.081) [2.03 - 2.24]	-0.12 (0.10) [-0.25 - -0.016]	-0.34, 0.098	0.260	(1.94 - 2.44) [1.51, 2.80]
Glycine (% DW)	0.36 (0.010) [0.36 - 0.37]	0.37 (0.010) [0.36 - 0.37]	-0.0045 (0.013) [-0.013 - 0.0016]	-0.031, 0.022	0.725	(0.35 - 0.42) [0.30, 0.45]
Histidine (% DW)	0.31 (0.010) [0.31 - 0.31]	0.32 (0.010) [0.31 - 0.33]	-0.0087 (0.012) [-0.019 - -0.00018]	-0.035, 0.017	0.491	(0.27 - 0.33) [0.23, 0.36]
Isoleucine (% DW)	0.37 (0.016) [0.37 - 0.38]	0.39 (0.016) [0.36 - 0.41]	-0.015 (0.020) [-0.038 - 0.0073]	-0.057, 0.026	0.446	(0.34 - 0.44) [0.27, 0.50]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.38 (0.059) [1.36 - 1.41]	1.46 (0.059) [1.38 - 1.54]	-0.085 (0.073) [-0.19 - -0.022]	-0.24, 0.069	0.261	(1.29 - 1.65) [0.98, 1.91]
Lysine (% DW)	0.29 (0.0080) [0.28 - 0.29]	0.29 (0.0080) [0.29 - 0.30]	-0.0071 (0.0093) [-0.011 - -0.00080]	-0.027, 0.013	0.460	(0.28 - 0.31) [0.25, 0.34]
Methionine (% DW)	0.19 (0.0059) [0.19 - 0.20]	0.19 (0.0059) [0.19 - 0.20]	-0.0044 (0.0084) [-0.0050 - -0.0032]	-0.022, 0.013	0.610	(0.19 - 0.30) [0.095, 0.35]
Phenylalanine (% DW)	0.54 (0.022) [0.53 - 0.55]	0.57 (0.022) [0.53 - 0.60]	-0.028 (0.027) [-0.067 - -0.0020]	-0.084, 0.028	0.310	(0.51 - 0.63) [0.41, 0.72]
Proline (% DW)	0.98 (0.043) [0.96 - 1.00]	1.03 (0.043) [0.98 - 1.11]	-0.056 (0.055) [-0.14 - -0.012]	-0.17, 0.060	0.319	(0.78 - 1.03) [0.64, 1.23]
Serine (% DW)	0.50 (0.018) [0.48 - 0.52]	0.53 (0.018) [0.52 - 0.55]	-0.033 (0.024) [-0.075 - 0.0017]	-0.084, 0.017	0.183	(0.48 - 0.60) [0.36, 0.71]
Threonine (% DW)	0.34 (0.010) [0.34 - 0.34]	0.36 (0.010) [0.35 - 0.37]	-0.018 (0.012) [-0.036 - -0.0087]	-0.044, 0.0083	0.164	(0.33 - 0.39) [0.28, 0.44]
Tryptophan (% DW)	0.054 (0.0030) [0.047 - 0.059]	0.048 (0.0030) [0.042 - 0.052]	0.0060 (0.0042) [0.0037 - 0.0095]	-0.0027, 0.015	0.166	(0.043 - 0.063) [0.031, 0.082]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.31 (0.037) [0.29 - 0.33]	0.28 (0.037) [0.19 - 0.35]	0.024 (0.046) [-0.057 - 0.14]	-0.074, 0.12	0.610	(0.25 - 0.41) [0.12, 0.52]
Valine (% DW)	0.51 (0.019) [0.50 - 0.51]	0.52 (0.019) [0.49 - 0.55]	-0.018 (0.023) [-0.040 - 0.0051]	-0.067, 0.032	0.461	(0.47 - 0.58) [0.39, 0.64]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	10.78 (0.11) [10.54 - 10.91]	11.08 (0.11) [10.75 - 11.28]	-0.30 (0.15) [-0.39 - -0.21]	-0.62, 0.021	0.065	(9.84 - 12.33) [7.71, 14.14]
18:0 Stearic (% Total FA)	1.84 (0.026) [1.84 - 1.85]	1.86 (0.026) [1.81 - 1.93]	-0.016 (0.037) [-0.082 - 0.030]	-0.094, 0.062	0.672	(1.30 - 2.10) [0.71, 2.57]
18:1 Oleic (% Total FA)	21.29 (0.20) [20.93 - 21.60]	20.87 (0.20) [20.20 - 21.32]	0.43 (0.25) [0.022 - 0.74]	-0.11, 0.96	0.109	(20.78 - 29.13) [12.15, 35.55]
18:2 Linoleic (% Total FA)	64.39 (0.28) [64.21 - 64.57]	64.41 (0.28) [64.00 - 64.73]	-0.022 (0.39) [-0.16 - 0.21]	-0.83, 0.79	0.954	(56.51 - 64.46) [50.63, 72.71]
18:3 Linolenic (% Total FA)	1.16 (0.018) [1.13 - 1.17]	1.18 (0.018) [1.12 - 1.22]	-0.025 (0.022) [-0.079 - 0.049]	-0.071, 0.021	0.261	(1.03 - 1.38) [0.67, 1.76]
20:0 Arachidic (% Total FA)	0.30 (0.0045) [0.30 - 0.30]	0.31 (0.0045) [0.30 - 0.32]	-0.0093 (0.0063) [-0.024 - 0.00089]	-0.022, 0.0038	0.154	(0.30 - 0.41) [0.18, 0.52]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.17 (0.0047) [0.16 - 0.17]	0.18 (0.0047) [0.17 - 0.20]	-0.017 (0.0063) [-0.025 - -0.0089]	-0.030, -0.0035	0.016	(0.18 - 0.27) [0.11, 0.34]
22:0 Behenic (% Total FA)	0.079 (0.035) [0.058 - 0.12]	0.11 (0.035) [0.059 - 0.15]	-0.034 (0.050) [-0.092 - -0.00034]	-0.14, 0.070	0.502	(0.062 - 0.18) [0, 0.32]
Fiber						
Acid Detergent Fiber (% DW)	2.47 (0.35) [2.08 - 2.96]	2.31 (0.35) [1.84 - 2.61]	0.17 (0.50) [-0.53 - 0.54]	-0.87, 1.21	0.739	(1.83 - 3.39) [0.88, 4.63]
Neutral Detergent Fiber (% DW)	9.17 (0.48) [7.54 - 11.31]	8.12 (0.48) [7.91 - 8.24]	1.06 (0.67) [-0.37 - 3.07]	-0.35, 2.47	0.132	(6.08 - 10.36) [2.87, 13.22]
Total Dietary Fiber (% DW)	12.36 (0.59) [10.78 - 13.34]	11.87 (0.59) [11.06 - 12.77]	0.50 (0.84) [-1.99 - 1.91]	-1.25, 2.25	0.558	(10.57 - 14.56) [6.50, 17.54]
Mineral						
Calcium (% DW)	0.0048 (0.00022) [0.0046 - 0.0051]	0.0050 (0.00022) [0.0048 - 0.0052]	-0.00014 (0.00029) [-0.00038 - 0.00032]	-0.00076, 0.00047	0.626	(0.0035 - 0.0070) [0, 0.010]
Copper (mg/kg DW)	2.07 (0.10) [1.88 - 2.17]	2.09 (0.10) [2.01 - 2.14]	-0.021 (0.13) [-0.27 - 0.16]	-0.29, 0.25	0.868	(1.39 - 2.76) [0.22, 3.82]
Iron (mg/kg DW)	17.61 (0.47) [17.06 - 18.24]	18.81 (0.47) [18.70 - 18.97]	-1.19 (0.55) [-1.69 - -0.73]	-2.36, -0.024	0.046	(15.90 - 24.66) [7.05, 30.38]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.12 (0.0042) [0.12 - 0.12]	0.13 (0.0042) [0.12 - 0.13]	-0.0068 (0.0057) [-0.016 - 0.0013]	-0.019, 0.0053	0.250	(0.11 - 0.14) [0.083, 0.16]
Manganese (mg/kg DW)	6.56 (0.24) [6.40 - 6.84]	6.88 (0.24) [6.55 - 7.53]	-0.32 (0.30) [-1.13 - 0.29]	-0.96, 0.32	0.303	(4.78 - 9.35) [0.72, 11.82]
Phosphorus (% DW)	0.32 (0.0089) [0.32 - 0.32]	0.35 (0.0089) [0.32 - 0.37]	-0.029 (0.012) [-0.054 - -0.0026]	-0.055, -0.0036	0.027	(0.30 - 0.38) [0.25, 0.42]
Potassium (% DW)	0.37 (0.0058) [0.37 - 0.38]	0.39 (0.0058) [0.38 - 0.40]	-0.013 (0.0082) [-0.028 - -0.0041]	-0.030, 0.0039	0.122	(0.36 - 0.43) [0.29, 0.49]
Zinc (mg/kg DW)	23.87 (0.69) [22.74 - 25.20]	25.27 (0.69) [25.14 - 25.43]	-1.40 (0.97) [-2.69 - 0.057]	-3.45, 0.65	0.167	(18.25 - 30.44) [6.01, 42.60]
Proximate						
Ash (% DW)	1.42 (0.050) [1.35 - 1.47]	1.50 (0.050) [1.41 - 1.56]	-0.080 (0.070) [-0.20 - 0.045]	-0.23, 0.066	0.266	(1.27 - 1.63) [1.06, 1.93]
Carbohydrates (% DW)	84.30 (0.37) [83.67 - 84.79]	83.51 (0.37) [82.95 - 84.10]	0.78 (0.53) [0.19 - 1.84]	-0.32, 1.89	0.155	(82.10 - 85.17) [80.40, 87.76]
Moisture (% FW)	12.00 (0.19) [11.60 - 12.30]	12.27 (0.19) [12.00 - 12.50]	-0.27 (0.27) [-0.70 - 0.10]	-0.84, 0.30	0.339	(11.70 - 13.20) [10.50, 14.11]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	10.12 (0.31) [9.73 - 10.58]	10.81 (0.31) [10.36 - 11.34]	-0.69 (0.40) [-1.61 - -0.15]	-1.54, 0.17	0.107	(9.99 - 12.19) [8.12, 13.56]
Total Fat (% DW)	4.16 (0.086) [4.06 - 4.28]	4.17 (0.086) [4.12 - 4.23]	-0.012 (0.12) [-0.055 - 0.050]	-0.27, 0.24	0.919	(3.18 - 4.22) [2.07, 5.10]
Vitamin						
Folic Acid (mg/kg DW)	0.30 (0.017) [0.25 - 0.37]	0.25 (0.017) [0.23 - 0.26]	0.048 (0.022) [-0.0054 - 0.13]	0.00078, 0.095	0.046	(0.26 - 0.42) [0.098, 0.58]
Niacin (mg/kg DW)	17.73 (0.78) [16.86 - 19.27]	17.33 (0.78) [16.36 - 18.93]	0.40 (1.10) [-2.07 - 2.58]	-1.88, 2.69	0.716	(13.64 - 27.42) [2.23, 41.53]
Thiamine HCl (mg/kg DW)	3.22 (0.13) [3.07 - 3.42]	3.08 (0.13) [3.07 - 3.09]	0.14 (0.15) [0.0035 - 0.34]	-0.19, 0.47	0.372	(2.87 - 4.33) [1.55, 5.85]
Vitamin B2 (mg/kg DW)	1.96 (0.20) [1.43 - 2.32]	1.96 (0.20) [1.64 - 2.21]	0 (0.27) [-0.78 - 0.50]	-0.58, 0.58	0.999	(1.81 - 2.78) [0.88, 3.61]
Vitamin B6 (mg/kg DW)	6.09 (0.35) [5.90 - 6.28]	5.73 (0.35) [5.42 - 6.23]	0.36 (0.49) [0.054 - 0.53]	-0.67, 1.39	0.475	(5.30 - 8.22) [2.06, 9.98]
Vitamin E (mg/kg DW)	13.18 (0.61) [13.00 - 13.42]	12.92 (0.61) [11.97 - 13.64]	0.26 (0.80) [-0.21 - 1.15]	-1.47, 1.99	0.746	(2.84 - 15.53) [0, 22.61]

Table 10 (cont). Statistical Summary of Site CT Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.84 (0.040) [0.81 - 0.86]	0.84 (0.040) [0.79 - 0.89]	-0.0021 (0.057) [-0.031 - 0.015]	-0.12, 0.12	0.970	(0.67 - 0.94) [0.40, 1.12]
Raffinose (% DW)	0.089 (0.0053) [0.087 - 0.090]	0.099 (0.0053) [0.097 - 0.10]	-0.010 (0.0068) [-0.014 - -0.0069]	-0.025, 0.0040	0.145	(0.061 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1867.57 (227.11) [1797.50 - 1972.63]	1857.67 (227.11) [1771.43 - 2000.00]	9.90 (316.33) [-202.50 - 201.21]	-662.33, 682.12	0.975	(1011.40 - 2539.86) [0, 4071.51]
p-Coumaric Acid (µg/g DW)	133.41 (20.72) [113.23 - 144.80]	146.62 (20.72) [120.00 - 164.77]	-13.21 (25.74) [-22.57 - -6.77]	-67.76, 41.35	0.614	(84.15 - 259.68) [0, 378.67]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

Table 11. Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.76 (0.029) [0.69 - 0.86]	0.69 (0.029) [0.64 - 0.72]	0.075 (0.041) [0.032 - 0.14]	-0.011, 0.16	0.083	(0.66 - 0.89) [0.44, 1.06]
Arginine (% DW)	0.43 (0.022) [0.39 - 0.47]	0.38 (0.022) [0.33 - 0.42]	0.054 (0.028) [-0.00018 - 0.14]	-0.0053, 0.11	0.071	(0.34 - 0.46) [0.23, 0.55]
Aspartic Acid (% DW)	0.65 (0.019) [0.61 - 0.70]	0.60 (0.019) [0.57 - 0.63]	0.045 (0.026) [0.024 - 0.073]	-0.0099, 0.10	0.103	(0.58 - 0.77) [0.39, 0.88]
Cystine (% DW)	0.22 (0.0063) [0.20 - 0.23]	0.22 (0.0063) [0.20 - 0.22]	0.0040 (0.0087) [-0.0018 - 0.0078]	-0.014, 0.022	0.652	(0.20 - 0.24) [0.16, 0.27]
Glutamic Acid (% DW)	1.97 (0.074) [1.78 - 2.21]	1.77 (0.074) [1.64 - 1.86]	0.20 (0.10) [0.090 - 0.35]	-0.022, 0.41	0.076	(1.64 - 2.26) [1.09, 2.72]
Glycine (% DW)	0.36 (0.0090) [0.34 - 0.39]	0.34 (0.0090) [0.33 - 0.35]	0.023 (0.013) [0.013 - 0.041]	-0.0033, 0.050	0.083	(0.31 - 0.38) [0.26, 0.42]
Histidine (% DW)	0.31 (0.0084) [0.28 - 0.34]	0.29 (0.0084) [0.28 - 0.30]	0.017 (0.012) [0.0047 - 0.037]	-0.0080, 0.042	0.171	(0.24 - 0.30) [0.20, 0.34]
Isoleucine (% DW)	0.36 (0.016) [0.31 - 0.42]	0.34 (0.016) [0.32 - 0.35]	0.025 (0.022) [-0.0090 - 0.074]	-0.022, 0.071	0.275	(0.30 - 0.41) [0.19, 0.49]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.32 (0.053) [1.18 - 1.52]	1.18 (0.053) [1.09 - 1.24]	0.14 (0.076) [0.060 - 0.28]	-0.016, 0.30	0.074	(1.06 - 1.53) [0.66, 1.87]
Lysine (% DW)	0.29 (0.0070) [0.28 - 0.31]	0.28 (0.0070) [0.27 - 0.29]	0.011 (0.0099) [0.0070 - 0.018]	-0.0098, 0.032	0.283	(0.25 - 0.31) [0.19, 0.35]
Methionine (% DW)	0.19 (0.0094) [0.16 - 0.21]	0.18 (0.0094) [0.16 - 0.19]	0.013 (0.013) [-0.00054 - 0.027]	-0.015, 0.040	0.358	(0.18 - 0.23) [0.14, 0.26]
Phenylalanine (% DW)	0.52 (0.020) [0.47 - 0.60]	0.47 (0.020) [0.44 - 0.50]	0.051 (0.028) [0.022 - 0.10]	-0.0072, 0.11	0.082	(0.44 - 0.60) [0.28, 0.72]
Proline (% DW)	0.92 (0.039) [0.81 - 1.07]	0.86 (0.039) [0.82 - 0.91]	0.062 (0.055) [-0.013 - 0.16]	-0.052, 0.18	0.268	(0.72 - 0.99) [0.48, 1.18]
Serine (% DW)	0.50 (0.017) [0.47 - 0.54]	0.44 (0.017) [0.40 - 0.47]	0.059 (0.024) [0.038 - 0.071]	0.0085, 0.11	0.024	(0.43 - 0.55) [0.32, 0.65]
Threonine (% DW)	0.35 (0.011) [0.33 - 0.37]	0.32 (0.011) [0.29 - 0.33]	0.032 (0.015) [0.016 - 0.040]	0.00033, 0.063	0.047	(0.30 - 0.37) [0.23, 0.42]
Tryptophan (% DW)	0.052 (0.0040) [0.039 - 0.063]	0.052 (0.0040) [0.051 - 0.053]	0.00005 (0.0057) [-0.013 - 0.012]	-0.012, 0.012	0.993	(0.040 - 0.059) [0.022, 0.078]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.29 (0.039) [0.20 - 0.35]	0.28 (0.039) [0.23 - 0.30]	0.016 (0.055) [-0.034 - 0.053]	-0.10, 0.13	0.775	(0.14 - 0.32) [0, 0.53]
Valine (% DW)	0.49 (0.018) [0.43 - 0.56]	0.47 (0.018) [0.44 - 0.49]	0.025 (0.024) [-0.011 - 0.077]	-0.026, 0.077	0.313	(0.41 - 0.54) [0.29, 0.62]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.03 (0.13) [10.63 - 11.60]	11.26 (0.13) [11.21 - 11.32]	-0.23 (0.18) [-0.69 - 0.33]	-0.62, 0.15	0.215	(9.53 - 12.33) [7.43, 14.09]
18:0 Stearic (% Total FA)	1.83 (0.036) [1.74 - 1.97]	1.71 (0.036) [1.66 - 1.75]	0.12 (0.046) [0.051 - 0.22]	0.018, 0.22	0.024	(1.28 - 2.13) [0.60, 2.58]
18:1 Oleic (% Total FA)	20.63 (0.15) [20.29 - 20.86]	20.21 (0.15) [19.78 - 20.51]	0.42 (0.22) [0.35 - 0.51]	-0.045, 0.88	0.073	(22.13 - 31.09) [12.40, 36.28]
18:2 Linoleic (% Total FA)	64.62 (0.25) [63.92 - 65.27]	64.91 (0.25) [64.52 - 65.48]	-0.29 (0.36) [-0.81 - 0.16]	-1.04, 0.46	0.432	(55.17 - 64.97) [49.61, 73.18]
18:3 Linolenic (% Total FA)	1.20 (0.013) [1.17 - 1.23]	1.23 (0.013) [1.21 - 1.25]	-0.030 (0.013) [-0.037 - -0.021]	-0.058, -0.0011	0.042	(1.00 - 1.32) [0.72, 1.66]
20:0 Arachidic (% Total FA)	0.31 (0.0059) [0.30 - 0.33]	0.32 (0.0059) [0.30 - 0.34]	-0.0067 (0.0084) [-0.0092 - -0.0029]	-0.024, 0.011	0.430	(0.29 - 0.42) [0.19, 0.52]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.19 (0.0042) [0.18 - 0.19]	0.19 (0.0042) [0.19 - 0.20]	-0.0097 (0.0059) [-0.013 - -0.0055]	-0.022, 0.0027	0.117	(0.20 - 0.31) [0.10, 0.36]
22:0 Behenic (% Total FA)	0.19 (0.024) [0.13 - 0.26]	0.16 (0.024) [0.14 - 0.17]	0.035 (0.034) [-0.0076 - 0.097]	-0.035, 0.11	0.305	(0.061 - 0.33) [0, 0.48]
Fiber						
Acid Detergent Fiber (% DW)	2.59 (0.31) [2.08 - 3.18]	2.52 (0.31) [2.22 - 2.86]	0.071 (0.44) [-0.39 - 0.96]	-0.85, 1.00	0.872	(1.95 - 3.76) [0.29, 5.01]
Neutral Detergent Fiber (% DW)	8.83 (0.55) [8.33 - 9.45]	8.30 (0.55) [7.91 - 8.69]	0.53 (0.73) [-0.36 - 1.15]	-1.01, 2.08	0.474	(7.15 - 9.41) [5.23, 10.90]
Total Dietary Fiber (% DW)	11.89 (0.48) [11.59 - 12.20]	12.43 (0.48) [11.74 - 12.84]	-0.54 (0.58) [-0.81 - -0.15]	-1.76, 0.69	0.368	(10.24 - 13.51) [6.72, 16.07]
Mineral						
Calcium (% DW)	0.0053 (0.00050) [0.0047 - 0.0058]	0.0054 (0.00050) [0.0051 - 0.0059]	-0.00014 (0.00070) [-0.00060 - 0.00071]	-0.0016, 0.0013	0.848	(0.0032 - 0.0057) [0.00076, 0.0080]
Copper (mg/kg DW)	2.04 (0.31) [1.81 - 2.17]	1.93 (0.31) [1.89 - 1.95]	0.12 (0.43) [-0.086 - 0.22]	-0.82, 1.05	0.794	(1.29 - 4.16) [0, 5.74]
Iron (mg/kg DW)	18.01 (1.06) [15.80 - 20.30]	17.62 (1.06) [16.36 - 19.95]	0.40 (1.36) [-0.74 - 1.58]	-2.50, 3.29	0.775	(14.37 - 19.48) [10.40, 23.42]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.13 (0.0048) [0.11 - 0.14]	0.11 (0.0048) [0.10 - 0.11]	0.017 (0.0069) [0.0094 - 0.028]	0.0026, 0.031	0.022	(0.095 - 0.13) [0.064, 0.16]
Manganese (mg/kg DW)	5.87 (0.26) [5.25 - 6.51]	5.31 (0.26) [4.64 - 5.84]	0.56 (0.30) [0.0048 - 1.06]	-0.079, 1.20	0.081	(4.55 - 9.02) [0.69, 10.70]
Phosphorus (% DW)	0.31 (0.014) [0.29 - 0.34]	0.27 (0.014) [0.26 - 0.28]	0.037 (0.020) [0.010 - 0.086]	-0.0045, 0.079	0.077	(0.27 - 0.36) [0.21, 0.40]
Potassium (% DW)	0.40 (0.0084) [0.39 - 0.41]	0.38 (0.0084) [0.36 - 0.41]	0.017 (0.012) [-0.0085 - 0.047]	-0.0074, 0.042	0.159	(0.32 - 0.42) [0.25, 0.47]
Zinc (mg/kg DW)	22.00 (1.09) [19.20 - 24.52]	19.06 (1.09) [18.36 - 19.61]	2.94 (1.54) [0.85 - 4.91]	-0.28, 6.16	0.071	(18.12 - 29.69) [7.39, 38.63]
Proximate						
Ash (% DW)	1.42 (0.065) [1.39 - 1.47]	1.36 (0.065) [1.27 - 1.46]	0.068 (0.092) [-0.047 - 0.20]	-0.12, 0.26	0.470	(1.14 - 1.47) [0.90, 1.76]
Carbohydrates (% DW)	84.39 (0.45) [82.98 - 85.36]	85.68 (0.45) [85.37 - 86.28]	-1.29 (0.64) [-2.41 - -0.54]	-2.62, 0.040	0.056	(83.60 - 86.65) [81.08, 89.71]
Moisture (% FW)	12.27 (0.15) [12.00 - 12.50]	12.57 (0.15) [12.30 - 12.80]	-0.30 (0.16) [-0.50 - -0.10]	-0.64, 0.035	0.076	(11.00 - 12.20) [10.10, 13.35]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	10.23 (0.35) [9.45 - 11.32]	9.35 (0.35) [8.77 - 9.71]	0.88 (0.49) [0.34 - 1.61]	-0.15, 1.90	0.088	(8.69 - 11.33) [5.83, 13.57]
Total Fat (% DW)	3.96 (0.087) [3.80 - 4.23]	3.61 (0.087) [3.60 - 3.62]	0.34 (0.12) [0.18 - 0.61]	0.088, 0.60	0.010	(3.16 - 4.07) [2.47, 4.68]
Vitamin						
Folic Acid (mg/kg DW)	0.28 (0.019) [0.28 - 0.29]	0.27 (0.019) [0.25 - 0.28]	0.013 (0.023) [0.0030 - 0.028]	-0.035, 0.061	0.583	(0.26 - 0.41) [0.11, 0.55]
Niacin (mg/kg DW)	19.57 (3.66) [18.36 - 20.80]	20.17 (3.66) [19.61 - 21.17]	-0.60 (5.18) [-1.62 - 1.07]	-11.40, 10.19	0.908	(14.92 - 26.80) [5.96, 38.50]
Thiamine HCl (mg/kg DW)	2.93 (0.093) [2.61 - 3.19]	2.78 (0.093) [2.74 - 2.86]	0.14 (0.13) [-0.12 - 0.44]	-0.13, 0.41	0.277	(2.94 - 4.78) [1.01, 6.00]
Vitamin B2 (mg/kg DW)	2.17 (0.21) [1.92 - 2.54]	1.96 (0.21) [1.46 - 2.45]	0.21 (0.24) [-0.064 - 0.61]	-0.30, 0.72	0.395	(1.62 - 2.62) [0.87, 3.38]
Vitamin B6 (mg/kg DW)	6.57 (0.44) [5.49 - 7.39]	6.94 (0.44) [6.62 - 7.37]	-0.36 (0.62) [-1.32 - 0.21]	-1.67, 0.94	0.564	(4.01 - 6.70) [1.86, 8.29]
Vitamin E (mg/kg DW)	11.96 (0.57) [10.83 - 13.57]	10.63 (0.57) [9.30 - 11.31]	1.33 (0.80) [0.17 - 2.30]	-0.34, 3.01	0.112	(2.83 - 11.69) [0, 19.32]

Table 11 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Well-Watered Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.75 (0.052) [0.58 - 0.93]	0.71 (0.052) [0.63 - 0.80]	0.048 (0.074) [-0.21 - 0.31]	-0.11, 0.20	0.523	(0.58 - 0.97) [0.28, 1.15]
Raffinose (% DW)	0.12 (0.0047) [0.12 - 0.12]	0.11 (0.0047) [0.11 - 0.11]	0.0099 (0.0067) [0.0076 - 0.013]	-0.0040, 0.024	0.152	(0.028 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1876.89 (226.31) [1265.68 - 2240.00]	1777.28 (226.31) [1277.08 - 2128.15]	99.62 (320.05) [-660.93 - 847.92]	-568.00, 767.23	0.758	(1504.52 - 2224.72) [1019.70, 2703.40]
p-Coumaric Acid (µg/g DW)	150.39 (24.39) [97.95 - 188.64]	150.17 (24.39) [101.14 - 176.61]	0.21 (34.49) [-78.66 - 87.50]	-71.73, 72.16	0.995	(84.79 - 239.33) [0, 378.84]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

Table 12. Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	0.76 (0.029) [0.67 - 0.85]	0.77 (0.029) [0.73 - 0.80]	-0.0090 (0.041) [-0.12 - 0.12]	-0.094, 0.076	0.827	(0.77 - 0.96) [0.59, 1.09]
Arginine (% DW)	0.43 (0.022) [0.42 - 0.44]	0.41 (0.022) [0.37 - 0.46]	0.013 (0.028) [-0.044 - 0.072]	-0.047, 0.072	0.655	(0.41 - 0.50) [0.32, 0.56]
Aspartic Acid (% DW)	0.65 (0.019) [0.59 - 0.71]	0.65 (0.019) [0.61 - 0.67]	0.0050 (0.026) [-0.064 - 0.098]	-0.050, 0.060	0.850	(0.63 - 0.76) [0.52, 0.88]
Cystine (% DW)	0.23 (0.0063) [0.22 - 0.25]	0.23 (0.0063) [0.23 - 0.24]	-0.00024 (0.0087) [-0.014 - 0.018]	-0.019, 0.018	0.978	(0.20 - 0.26) [0.15, 0.30]
Glutamic Acid (% DW)	1.98 (0.074) [1.74 - 2.21]	2.00 (0.074) [1.88 - 2.07]	-0.019 (0.10) [-0.31 - 0.32]	-0.24, 0.20	0.859	(1.94 - 2.44) [1.51, 2.80]
Glycine (% DW)	0.37 (0.0090) [0.34 - 0.39]	0.37 (0.0090) [0.35 - 0.38]	-0.0016 (0.013) [-0.033 - 0.037]	-0.028, 0.025	0.900	(0.35 - 0.42) [0.30, 0.45]
Histidine (% DW)	0.30 (0.0084) [0.28 - 0.32]	0.31 (0.0084) [0.29 - 0.32]	-0.0072 (0.012) [-0.031 - 0.029]	-0.032, 0.018	0.552	(0.27 - 0.33) [0.23, 0.36]
Isoleucine (% DW)	0.35 (0.016) [0.32 - 0.37]	0.37 (0.016) [0.35 - 0.39]	-0.021 (0.022) [-0.052 - 0.015]	-0.067, 0.026	0.358	(0.34 - 0.44) [0.27, 0.50]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	1.33 (0.053) [1.16 - 1.47]	1.34 (0.053) [1.26 - 1.39]	-0.016 (0.076) [-0.22 - 0.22]	-0.17, 0.14	0.829	(1.29 - 1.65) [0.98, 1.91]
Lysine (% DW)	0.29 (0.0070) [0.27 - 0.31]	0.29 (0.0070) [0.28 - 0.30]	-0.0047 (0.0099) [-0.023 - 0.029]	-0.025, 0.016	0.640	(0.28 - 0.31) [0.25, 0.34]
Methionine (% DW)	0.21 (0.0094) [0.18 - 0.22]	0.21 (0.0094) [0.20 - 0.22]	0.0013 (0.013) [-0.023 - 0.025]	-0.026, 0.029	0.921	(0.19 - 0.30) [0.095, 0.35]
Phenylalanine (% DW)	0.52 (0.020) [0.46 - 0.58]	0.53 (0.020) [0.50 - 0.55]	-0.0093 (0.028) [-0.079 - 0.077]	-0.067, 0.049	0.742	(0.51 - 0.63) [0.41, 0.72]
Proline (% DW)	0.96 (0.039) [0.85 - 1.04]	0.96 (0.039) [0.88 - 1.00]	-0.0016 (0.055) [-0.15 - 0.16]	-0.12, 0.11	0.977	(0.78 - 1.03) [0.64, 1.23]
Serine (% DW)	0.51 (0.017) [0.45 - 0.58]	0.50 (0.017) [0.47 - 0.52]	0.012 (0.024) [-0.059 - 0.11]	-0.039, 0.063	0.622	(0.48 - 0.60) [0.36, 0.71]
Threonine (% DW)	0.36 (0.011) [0.32 - 0.39]	0.35 (0.011) [0.32 - 0.36]	0.014 (0.015) [-0.033 - 0.067]	-0.018, 0.045	0.367	(0.33 - 0.39) [0.28, 0.44]
Tryptophan (% DW)	0.053 (0.0040) [0.046 - 0.059]	0.056 (0.0040) [0.047 - 0.063]	-0.0027 (0.0057) [-0.0044 - -0.00045]	-0.015, 0.0091	0.639	(0.043 - 0.063) [0.031, 0.082]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	0.30 (0.039) [0.28 - 0.32]	0.23 (0.039) [0.17 - 0.33]	0.067 (0.055) [-0.0046 - 0.13]	-0.052, 0.19	0.245	(0.25 - 0.41) [0.12, 0.52]
Valine (% DW)	0.48 (0.018) [0.44 - 0.51]	0.51 (0.018) [0.48 - 0.53]	-0.025 (0.024) [-0.068 - 0.029]	-0.077, 0.026	0.315	(0.47 - 0.58) [0.39, 0.64]
Fatty Acid (% Total FA)						
16:0 Palmitic (% Total FA)	11.21 (0.13) [11.14 - 11.29]	11.18 (0.13) [10.82 - 11.45]	0.035 (0.18) [-0.16 - 0.32]	-0.35, 0.42	0.852	(9.84 - 12.33) [7.71, 14.14]
18:0 Stearic (% Total FA)	1.79 (0.036) [1.73 - 1.83]	1.89 (0.036) [1.83 - 1.93]	-0.095 (0.046) [-0.12 - -0.069]	-0.19, 0.0039	0.058	(1.30 - 2.10) [0.71, 2.57]
18:1 Oleic (% Total FA)	20.38 (0.15) [20.20 - 20.48]	20.89 (0.15) [20.66 - 21.23]	-0.51 (0.22) [-1.03 - -0.18]	-0.97, -0.054	0.030	(20.78 - 29.13) [12.15, 35.55]
18:2 Linoleic (% Total FA)	64.80 (0.25) [64.65 - 65.10]	64.17 (0.25) [64.14 - 64.22]	0.63 (0.36) [0.43 - 0.94]	-0.12, 1.37	0.096	(56.51 - 64.46) [50.63, 72.71]
18:3 Linolenic (% Total FA)	1.21 (0.013) [1.18 - 1.25]	1.22 (0.013) [1.20 - 1.26]	-0.010 (0.013) [-0.030 - 0.0092]	-0.039, 0.018	0.451	(1.03 - 1.38) [0.67, 1.76]
20:0 Arachidic (% Total FA)	0.31 (0.0059) [0.30 - 0.32]	0.33 (0.0059) [0.32 - 0.33]	-0.013 (0.0084) [-0.025 - 0.0062]	-0.030, 0.0048	0.146	(0.30 - 0.41) [0.18, 0.52]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Fatty Acid (% Total FA)						
20:1 Eicosenoic (% Total FA)	0.18 (0.0042) [0.17 - 0.19]	0.18 (0.0042) [0.18 - 0.19]	-0.0056 (0.0059) [-0.016 - 0.014]	-0.018, 0.0068	0.353	(0.18 - 0.27) [0.11, 0.34]
22:0 Behenic (% Total FA)	0.12 (0.024) [0.061 - 0.16]	0.14 (0.024) [0.13 - 0.15]	-0.023 (0.034) [-0.083 - 0.029]	-0.093, 0.047	0.500	(0.062 - 0.18) [0, 0.32]
Fiber						
Acid Detergent Fiber (% DW)	2.73 (0.31) [2.05 - 3.58]	2.32 (0.31) [2.13 - 2.59]	0.41 (0.44) [-0.079 - 0.99]	-0.52, 1.33	0.366	(1.83 - 3.39) [0.88, 4.63]
Neutral Detergent Fiber (% DW)	8.85 (0.55) [7.77 - 9.66]	8.22 (0.55) [7.93 - 8.66]	0.64 (0.73) [-0.89 - 1.73]	-0.91, 2.19	0.396	(6.08 - 10.36) [2.87, 13.22]
Total Dietary Fiber (% DW)	12.77 (0.48) [11.34 - 14.43]	11.89 (0.48) [11.45 - 12.24]	0.89 (0.58) [-0.11 - 2.19]	-0.34, 2.11	0.145	(10.57 - 14.56) [6.50, 17.54]
Mineral						
Calcium (% DW)	0.0057 (0.00050) [0.0049 - 0.0065]	0.0056 (0.00050) [0.0049 - 0.0063]	0.00008 (0.00070) [-0.0014 - 0.00084]	-0.0014, 0.0015	0.914	(0.0035 - 0.0070) [0, 0.010]
Copper (mg/kg DW)	2.19 (0.31) [1.90 - 2.43]	2.17 (0.31) [1.96 - 2.30]	0.021 (0.43) [-0.40 - 0.26]	-0.91, 0.95	0.962	(1.39 - 2.76) [0.22, 3.82]
Iron (mg/kg DW)	18.00 (1.06) [16.65 - 19.27]	18.70 (1.06) [18.48 - 19.05]	-0.70 (1.36) [-1.92 - 0.79]	-3.59, 2.19	0.613	(15.90 - 24.66) [7.05, 30.38]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Mineral						
Magnesium (% DW)	0.13 (0.0048) [0.11 - 0.14]	0.13 (0.0048) [0.12 - 0.13]	-0.00008 (0.0069) [-0.022 - 0.020]	-0.014, 0.014	0.990	(0.11 - 0.14) [0.083, 0.16]
Manganese (mg/kg DW)	5.58 (0.26) [5.28 - 6.07]	5.44 (0.26) [5.25 - 5.81]	0.13 (0.30) [-0.53 - 0.82]	-0.50, 0.77	0.662	(4.78 - 9.35) [0.72, 11.82]
Phosphorus (% DW)	0.31 (0.014) [0.25 - 0.36]	0.32 (0.014) [0.31 - 0.33]	-0.015 (0.020) [-0.074 - 0.049]	-0.056, 0.027	0.474	(0.30 - 0.38) [0.25, 0.42]
Potassium (% DW)	0.41 (0.0084) [0.39 - 0.43]	0.40 (0.0084) [0.39 - 0.41]	0.0031 (0.012) [-0.014 - 0.037]	-0.022, 0.028	0.798	(0.36 - 0.43) [0.29, 0.49]
Zinc (mg/kg DW)	21.67 (1.09) [18.36 - 23.70]	23.39 (1.09) [21.98 - 24.60]	-1.72 (1.54) [-3.62 - 0.11]	-4.94, 1.50	0.278	(18.25 - 30.44) [6.01, 42.60]
Proximate						
Ash (% DW)	1.45 (0.065) [1.24 - 1.75]	1.49 (0.065) [1.39 - 1.55]	-0.041 (0.092) [-0.31 - 0.35]	-0.23, 0.15	0.663	(1.27 - 1.63) [1.06, 1.93]
Carbohydrates (% DW)	84.16 (0.45) [82.64 - 85.64]	84.12 (0.45) [83.79 - 84.71]	0.034 (0.64) [-2.06 - 1.77]	-1.30, 1.37	0.957	(82.10 - 85.17) [80.40, 87.76]
Moisture (% FW)	12.03 (0.15) [11.80 - 12.30]	11.93 (0.15) [11.80 - 12.20]	0.10 (0.16) [0 - 0.20]	-0.24, 0.44	0.538	(11.70 - 13.20) [10.50, 14.11]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Proximate						
Protein (% DW)	10.43 (0.35) [9.41 - 11.45]	10.43 (0.35) [10.05 - 10.69]	-0.0056 (0.49) [-1.29 - 1.41]	-1.03, 1.02	0.990	(9.99 - 12.19) [8.12, 13.56]
Total Fat (% DW)	3.97 (0.087) [3.71 - 4.16]	3.96 (0.087) [3.85 - 4.13]	0.012 (0.12) [-0.18 - 0.31]	-0.24, 0.27	0.924	(3.18 - 4.22) [2.07, 5.10]
Vitamin						
Folic Acid (mg/kg DW)	0.32 (0.019) [0.29 - 0.35]	0.32 (0.019) [0.29 - 0.35]	-0.0084 (0.023) [-0.062 - 0.028]	-0.057, 0.040	0.716	(0.26 - 0.42) [0.098, 0.58]
Niacin (mg/kg DW)	20.58 (3.66) [17.80 - 25.00]	28.53 (3.66) [21.30 - 42.06]	-7.95 (5.18) [-24.26 - 2.78]	-18.75, 2.84	0.140	(13.64 - 27.42) [2.23, 41.53]
Thiamine HCl (mg/kg DW)	3.07 (0.093) [2.85 - 3.40]	2.95 (0.093) [2.85 - 3.06]	0.12 (0.13) [-0.11 - 0.45]	-0.15, 0.39	0.372	(2.87 - 4.33) [1.55, 5.85]
Vitamin B2 (mg/kg DW)	2.33 (0.21) [1.66 - 2.89]	2.46 (0.21) [2.35 - 2.57]	-0.12 (0.24) [-0.91 - 0.44]	-0.64, 0.39	0.616	(1.81 - 2.78) [0.88, 3.61]
Vitamin B6 (mg/kg DW)	5.97 (0.44) [5.43 - 6.32]	5.78 (0.44) [4.97 - 6.30]	0.19 (0.62) [-0.87 - 1.20]	-1.12, 1.50	0.763	(5.30 - 8.22) [2.06, 9.98]
Vitamin E (mg/kg DW)	12.50 (0.57) [12.16 - 12.93]	12.41 (0.57) [11.62 - 13.38]	0.091 (0.80) [-0.45 - 0.81]	-1.58, 1.77	0.911	(2.84 - 15.53) [0, 22.61]

Table 12 (cont). Statistical Summary of Site LUM Corn Grain Amino Acid, Fatty Acid, Fiber, Mineral, Proximate, Vitamin, Antinutrient and Secondary Metabolite Content for MON 87460 vs. Conventional Control Grown Under Water-Limited Conditions

Analytical Component (Units)[1]	Test Mean (S.E.) [Range]	Control Mean (S.E.) [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Int.[2]]
Antinutrient						
Phytic Acid (% DW)	0.67 (0.052) [0.63 - 0.70]	0.78 (0.052) [0.77 - 0.79]	-0.11 (0.074) [-0.16 - -0.082]	-0.26, 0.046	0.157	(0.67 - 0.94) [0.40, 1.12]
Raffinose (% DW)	0.12 (0.0047) [0.12 - 0.13]	0.13 (0.0047) [0.12 - 0.14]	-0.0044 (0.0067) [-0.010 - 0.0025]	-0.018, 0.0095	0.515	(0.061 - 0.15) [0, 0.21]
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1878.59 (226.31) [1208.67 - 2352.27]	2040.15 (226.31) [1757.37 - 2301.59]	-161.56 (320.05) [-852.84 - 317.46]	-829.18, 506.05	0.619	(1011.40 - 2539.86) [0, 4071.51]
p-Coumaric Acid (µg/g DW)	137.48 (24.39) [85.52 - 168.18]	182.85 (24.39) [162.13 - 208.43]	-45.38 (34.49) [-122.91 - -3.40]	-117.32, 26.56	0.203	(84.15 - 259.68) [0, 378.67]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial materials. Negative limits were set to zero.

APPENDIX K. Supplementary Compositional Analysis Data

1. Compositional Analysis Study from Chile 2006/2007 (Non-Combined Site)

This appendix reports compositional and metabolite data on forage and grain collected from MON 87460 grown at an individual site (QUI) of the 2006/2007 Chile field production. As noted in Part VII, Section 3.2, this site was excluded from the combined-site data analysis because it did not meet the requirements specified by the intended water-limited conditions. Table 1 presents data on applied water and temperatures from the production period for the QUI site.

Evaluation of the overall data set confirmed analyte results were as expected from the respective assays and were similar to reference and published ranges for conventional corn. No unexpected compositional values for any components were observed.

Mean values, ranges, and statistical analyses for the compositional data are presented in Tables 2 and 3 for forage and Tables 4 to 15 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table 16.

Mean values, ranges, and statistical analyses for the additional secondary metabolites are presented in Tables 17 and 19 for forage and Tables 18 and 20 for grain. A summary of significant differences ($p<0.05$) between test and control is presented in Table 21.

The QUI site results reported below do not impact the conclusion of compositional equivalence of MON 87460 to conventional corn as established in Part VII.

Table 1. Monthly Temperature and Monthly Accumulated Water Data for the QUI Site from the 2006/2007 Chile Field Production

Site[1]	Measurement	December	January	February	March	April	May
QUI	Accumulated water (in.), well-watered	1.9	10.3	8.5	9.4	1.9	0.0
	Accumulated water (in.), water-limited[1, 2]	1.9	10.3	1.9	5.6	1.9	0.0
	Avg Max temp (°F)	NA[4]	83	79	77	74	68
	Avg Min temp (°F)	NA[4]	51	51	49	44	37
	Range[3] (°F)	NA[4]	48 - 92	44 - 90	41 - 93	34 - 92	29 – 77

[1] Water limitation began at the V10 growth stage which occurred at approximately February 7.
[2] Water limitation ended at the R2 growth stage which occurred at approximately March 13.
[3] The range is the absolute maximum and minimum temperature in each month.
[4] Temperature data are available from January 6 through May 25; planting occurred in late December and early January. Rainfall did not occur during the production period.

Table 2. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Fiber						
Acid Detergent Fiber (% DW)	34.70 (1.57) [31.50 - 39.52]	32.92 (1.57) [31.44 - 34.55]	1.78 (2.21) [-3.05 - 8.08]	-2.91, 6.47	0.433	(32.07 - 39.64)
Neutral Detergent Fiber (% DW)	46.04 (1.90) [42.13 - 50.79]	48.39 (1.90) [43.78 - 52.67]	-2.35 (2.45) [-7.46 - 2.06]	-7.57, 2.87	0.353	(46.84 - 50.22)
Mineral						
Calcium (% DW)	0.31 (0.021) [0.29 - 0.34]	0.33 (0.021) [0.31 - 0.35]	-0.020 (0.26) [-0.033 - - 0.0051]	-0.075, 0.034	0.443	(0.26 - 0.33)
Phosphorus (% DW)	0.16 (0.010) [0.14 - 0.17]	0.16 (0.010) [0.13 - 0.17]	0.0075 (0.014) [-0.021 - 0.041]	-0.022, 0.037	0.594	(0.13 - 0.17)
Proximate						
Ash (% DW)	4.13 (0.18) [4.02 - 4.33]	4.55 (0.18) [4.51 - 4.62]	-0.42 (0.25) [-0.50 - -0.29]	-0.96, 0.13	0.123	(4.73 - 6.65)
Carbohydrates (% DW)	88.78 (0.34) [88.57 - 89.20]	88.57 (0.34) [88.12 - 88.91]	0.21 (0.47) [-0.34 – 0.52]	-0.78, 1.20	0.665	(86.88 - 88.82)
Moisture (% FW)	74.43 (0.96) [73.90 - 74.80]	76.27 (0.96) [75.70 - 76.70]	-1.83 (1.17) [-2.10 - -1.60]	-4.30, 0.64	0.135	(75.10 - 77.90)
Protein (% DW)	5.89 (0.24) [5.63 - 6.10]	6.12 (0.24) [5.92 - 6.30]	-0.23 (0.34) [-0.51 – 0.18]	-0.93, 0.47	0.504	(5.78 - 6.47)
Total Fat (% DW)	1.20 (0.22) [0.84 - 1.47]	0.76 (0.22) [0.56 - 1.06]	0.44 (0.29) [0.29 - 0.61]	-0.18, 1.06	0.154	(0.23 - 0.86)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 3. Comparison of Proximates, Fiber, and Mineral Content in Forage from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Fiber						
Acid Detergent Fiber (% DW)	29.22 (1.57) [26.90 - 32.72]	30.72 (1.57) [28.45 - 33.32]	-1.50 (2.21) [-3.49 - -0.40]	-6.19, 3.20	0.508	(28.33 - 32.37)
Neutral Detergent Fiber (% DW)	41.55 (1.90) [38.01 - 44.93]	45.54 (1.90) [42.49 - 48.44]	-4.00 (2.45) [-10.43 - -0.77]	-9.22, 1.22	0.123	(37.80 - 47.14)
Mineral						
Calcium (% DW)	0.27 (0.021) [0.24 - 0.31]	0.30 (0.021) [0.29 - 0.30]	-0.028 (0.027) [-0.051 - 0.013]	-0.085, 0.030	0.316	(0.23 - 0.32)
Phosphorus (% DW)	0.15 (0.010) [0.13 - 0.16]	0.14 (0.010) [0.13 - 0.15]	0.0082 (0.014) [-0.0045 - 0.025]	-0.021, 0.037	0.561	(0.14 - 0.17)
Proximate						
Ash (% DW)	3.82 (0.18) [3.61 - 4.09]	4.10 (0.18) [3.86 - 4.53]	-0.28 (0.25) [-0.92 - 0.18]	-0.82, 0.27	0.291	(4.80 - 5.14)
Carbohydrates (% DW)	88.97 (0.34) [88.28 - 89.83]	88.38 (0.34) [87.74 - 89.09]	0.58 (0.47) [-0.81 - 2.08]	-0.41, 1.57	0.232	(87.43 - 89.00)
Moisture (% FW)	73.53 (0.96) [72.40 - 75.30]	76.57 (0.96) [74.40 - 78.60]	-3.03 (1.17) [-6.20 - -1.40]	-5.50, -0.56	0.019	(74.30 - 77.30)
Protein (% DW)	5.78 (0.24) [5.25 - 6.57]	6.69 (0.24) [6.25 - 7.47]	-0.91 (0.34) [-1.96 - 0.32]	-1.62, -0.21	0.013	(5.44 - 6.38)
Total Fat (% DW)	1.44 (0.22) [0.58 - 2.34]	0.82 (0.22) [0.77 - 0.88]	0.62 (0.29) [-0.31 - 1.58]	0.00006, 1.24	0.049	(0.58 - 1.42)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 4. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Proximates						
Ash (% DW)	1.48 (0.033) [1.39 - 1.54]	1.44 (0.033) [1.34 - 1.57]	0.036 (0.046) [-0.18 - 0.20]	-0.061, 0.13	0.448	(1.30 - 1.36)
Carbohydrates (% DW)	85.22 (0.24) [85.00 - 85.65]	85.41 (0.24) [84.63 - 86.01]	-0.19 (0.33) [-1.01 - 1.02]	-0.89, 0.51	0.571	(86.05 - 86.57)
Moisture (% FW)	13.63 (0.24) [13.10 - 14.20]	8.48 (0.24) [8.15 - 8.82]	5.16 (0.34) [4.95 - 5.38]	4.42, 5.89	<0.001	(9.41 - 13.70)
Protein (% DW)	9.23 (0.19) [9.16 - 9.30]	9.20 (0.19) [8.87 - 9.45]	0.029 (0.25) [-0.29 - 0.37]	-0.50, 0.56	0.909	(8.35 - 9.23)
Total Fat (% DW)	4.07 (0.14) [3.81 - 4.25]	3.94 (0.14) [3.71 - 4.35]	0.13 (0.19) [-0.54 - 0.55]	-0.28, 0.54	0.518	(3.15 - 4.16)
Fiber						
Acid Detergent Fiber (% DW)	3.58 (0.37) [2.45 - 4.15]	3.34 (0.37) [2.48 - 3.78]	0.24 (0.52) [-1.33 - 1.67]	-0.84, 1.32	0.652	(2.83 - 4.02)
Neutral Detergent Fiber (% DW)	10.25 (0.40) [10.01 - 10.61]	9.12 (0.40) [8.63 - 9.54]	1.13 (0.57) [0.94 - 1.38]	-0.057, 2.32	0.060	(8.91 - 10.10)
Total Dietary Fiber (% DW)	14.32 (0.58) [13.89 - 14.92]	13.55 (0.58) [12.96 - 14.26]	0.77 (0.70) [0.45 - 1.20]	-0.71, 2.25	0.286	(11.76 - 13.56)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 5. Comparison of the Proximates and Fiber Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Proximates						
Ash (% DW)	1.39 (0.033) [1.38 - 1.40]	1.43 (0.033) [1.39 - 1.48]	-0.039 (0.046) [-0.085 - -0.0024]	-0.14, 0.058	0.409	(1.21 - 1.50)
Carbohydrates (% DW)	85.95 (0.24) [85.69 - 86.33]	85.21 (0.24) [84.92 - 85.65]	0.74 (0.33) [0.18 - 1.41]	0.042, 1.44	0.038	(85.53 - 86.50)
Moisture (% FW)	13.60 (0.24) [12.70 - 14.10]	8.72 (0.24) [8.53 - 8.89]	4.88 (0.34) [4.17 - 5.26]	4.15, 5.61	<0.001	(9.55 - 13.40)
Protein (% DW)	8.69 (0.19) [8.24 - 8.99]	9.24 (0.19) [9.20 - 9.28]	-0.54 (0.25) [-1.05 - -0.24]	-1.07, -0.018	0.043	(8.32 - 9.67)
Total Fat (% DW)	3.97 (0.14) [3.80 - 4.06]	4.13 (0.14) [3.71 - 4.41]	-0.16 (0.19) [-0.36 - 0.088]	-0.56, 0.25	0.429	(3.46 - 3.97)
Fiber						
Acid Detergent Fiber (% DW)	3.14 (0.37) [2.91 - 3.57]	3.58 (0.37) [3.10 - 3.93]	-0.43 (0.52) [-0.98 - 0.47]	-1.51, 0.65	0.412	(2.85 - 4.42)
Neutral Detergent Fiber (% DW)	9.65 (0.40) [8.85 - 10.54]	10.89 (0.40) [10.32 - 11.70]	-1.24 (0.57) [-1.47 - -1.08]	-2.42, -0.049	0.042	(7.75 - 10.73)
Total Dietary Fiber (% DW)	13.81 (0.58) [13.02 - 14.43]	14.17 (0.58) [13.26 - 15.15]	-0.36 (0.70) [-1.18 - 0.33]	-1.84, 1.12	0.612	(11.74 - 14.40)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 6. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Calcium (% DW)	0.0047 (0.00021) [0.0044 - 0.0051]	0.0047 (0.00021) [0.0046 - 0.0051]	0 (0.00022) [-0.00068 - 0.00059]	-0.00049, 0.00047	0.966	(0.0041 - 0.0054)
Copper (mg/kg DW)	1.70 (0.80) [1.54 - 1.79]	3.78 (0.80) [1.93 - 7.38]	-2.08 (1.14) [-5.61 - -0.25]	-4.46, 0.29	0.082	(1.33 - 1.72)
Iron (mg/kg DW)	16.87 (0.37) [16.32 - 17.59]	16.20 (0.37) [14.81 - 17.26]	0.66 (0.50) [0.14 - 1.51]	-0.39, 1.71	0.202	(13.79 - 18.40)
Magnesium (% DW)	0.12 (0.0030) [0.12 - 0.13]	0.11 (0.0030) [0.11 - 0.12]	0.0090 (0.0042) [-0.0088 - 0.020]	0.00028, 0.018	0.043	(0.10 - 0.12)
Manganese (mg/kg DW)	6.46 (0.19) [6.01 - 7.10]	6.68 (0.19) [5.91 - 7.08]	-0.22 (0.26) [-1.07 - 1.19]	-0.76, 0.33	0.421	(4.92 - 5.28)
Phosphorus (% DW)	0.32 (0.0076) [0.31 - 0.34]	0.30 (0.0076) [0.28 - 0.33]	0.023 (0.011) [-0.013 - 0.046]	0.00032, 0.046	0.047	(0.27 - 0.33)
Potassium (% DW)	0.39 (0.0084) [0.38 - 0.40]	0.38 (0.0084) [0.36 - 0.39]	0.014 (0.011) [-0.015 - 0.033]	-0.011, 0.038	0.256	(0.32 - 0.40)
Zinc (mg/kg DW)	18.18 (0.58) [17.01 - 19.35]	19.09 (0.58) [17.96 - 19.96]	-0.91 (0.78) [-2.32 - 0.22]	-2.54, 0.73	0.260	(16.12 - 18.18)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 7. Comparison of the Mineral Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Calcium (% DW)	0.0045 (0.00021) [0.0041 - 0.0049]	0.0044 (0.00021) [0.0042 - 0.0046]	0.00012 (0.00022) [-0.00022 - 0.00030]	-0.00036, 0.00060	0.595	(0.0038 - 0.0055)
Copper (mg/kg DW)	1.61 (0.80) [1.53 - 1.65]	4.22 (0.80) [2.17 - 7.76]	-2.61 (1.14) [-6.23 - -0.52]	-4.99, -0.24	0.032	(1.37 - 1.87)
Iron (mg/kg DW)	14.35 (0.37) [13.75 - 14.77]	15.48 (0.37) [15.20 - 15.70]	-1.13 (0.50) [-1.45 - -0.79]	-2.18, -0.075	0.037	(14.04 - 19.16)
Magnesium (% DW)	0.11 (0.0030) [0.11 - 0.11]	0.11 (0.0030) [0.11 - 0.12]	-0.0013 (0.0042) [-0.010 - 0.0052]	-0.010, 0.0075	0.767	(0.11 - 0.11)
Manganese (mg/kg DW)	5.84 (0.19) [5.66 - 6.07]	6.07 (0.19) [5.94 - 6.17]	-0.23 (0.26) [-0.51 - -0.037]	-0.77, 0.32	0.397	(4.59 - 5.18)
Phosphorus (% DW)	0.30 (0.0076) [0.29 - 0.30]	0.29 (0.0076) [0.28 - 0.32]	0.00040 (0.011) [-0.025 - 0.019]	-0.022, 0.023	0.970	(0.26 - 0.31)
Potassium (% DW)	0.38 (0.0084) [0.38 - 0.39]	0.38 (0.0084) [0.38 - 0.38]	0.0033 (0.011) [-0.0023 - 0.0089]	-0.021, 0.028	0.774	(0.31 - 0.38)
Zinc (mg/kg DW)	18.17 (0.58) [17.33 - 18.67]	19.13 (0.58) [17.86 - 20.77]	-0.96 (0.78) [-2.10 - -0.26]	-2.60, 0.67	0.231	(15.65 - 19.27)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 8. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range)**
Alanine (% DW)	0.67 (0.018) [0.67 - 0.68]	0.68 (0.018) [0.64 - 0.71]	-0.0098 (0.025) [-0.032 - 0.032]	-0.062, 0.043	0.697	(0.62 - 0.72)
Arginine (% DW)	0.43 (0.011) [0.42 - 0.43]	0.40 (0.011) [0.39 - 0.42]	0.023 (0.015) [0.016 - 0.034]	-0.0096, 0.055	0.158	(0.38 - 0.42)
Aspartic Acid (% DW)	0.60 (0.012) [0.59 - 0.60]	0.58 (0.012) [0.55 - 0.61]	0.011 (0.017) [-0.014 - 0.042]	-0.025, 0.047	0.530	(0.52 - 0.63)
Cystine (% DW)	0.21 (0.0050) [0.21 - 0.22]	0.21 (0.0050) [0.20 - 0.21]	0.0028 (0.0058) [-0.0043 - 0.0089]	-0.0096, 0.015	0.639	(0.19 - 0.21)
Glutamic Acid (% DW)	1.73 (0.045) [1.71 - 1.74]	1.71 (0.045) [1.61 - 1.76]	0.014 (0.063) [-0.034 - 0.10]	-0.12, 0.14	0.830	(1.52 - 1.81)
Glycine (% DW)	0.34 (0.0050) [0.34 - 0.34]	0.33 (0.0050) [0.31 - 0.34]	0.0097 (0.0068) [0.00028 - 0.026]	-0.0048, 0.024	0.175	(0.32 - 0.35)
Histidine (% DW)	0.29 (0.0053) [0.29 - 0.29]	0.28 (0.0053) [0.26 - 0.29]	0.0078 (0.0072) [-0.0014 - 0.022]	-0.0073, 0.023	0.292	(0.24 - 0.28)
Isoleucine (% DW)	0.32 (0.0079) [0.32 - 0.33]	0.32 (0.0079) [0.31 - 0.33]	0.0027 (0.011) [-0.0077 - 0.020]	-0.021, 0.026	0.813	(0.28 - 0.32)

Table 8 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Leucine (% DW)	1.15 (0.033) [1.14 - 1.15]	1.14 (0.033) [1.07 - 1.18]	0.0086 (0.045) [-0.025 - 0.068]	-0.087, 0.10	0.851	(0.99 - 1.17)
Lysine (% DW)	0.29 (0.0050) [0.28 - 0.29]	0.28 (0.0050) [0.27 - 0.29]	0.0059 (0.0071) [-0.0043 - 0.020]	-0.0089, 0.021	0.413	(0.27 - 0.30)
Methionine (% DW)	0.17 (0.0045) [0.16 - 0.17]	0.16 (0.0045) [0.16 - 0.17]	0.0047 (0.0060) [-0.0072 - 0.011]	-0.0081, 0.017	0.447	(0.16 - 0.18)
Phenylalanine (% DW)	0.46 (0.012) [0.46 - 0.46]	0.46 (0.012) [0.43 - 0.48]	0.0050 (0.017) [-0.012 - 0.028]	-0.030, 0.040	0.767	(0.41 - 0.48)
Proline (% DW)	0.88 (0.022) [0.86 - 0.90]	0.86 (0.022) [0.80 - 0.91]	0.025 (0.032) [-0.048 - 0.084]	-0.041, 0.090	0.445	(0.74 - 0.85)
Serine (% DW)	0.43 (0.013) [0.42 - 0.44]	0.43 (0.013) [0.41 - 0.46]	-0.0020 (0.018) [-0.019 - 0.010]	-0.039, 0.035	0.912	(0.39 - 0.46)
Threonine (% DW)	0.31 (0.0071) [0.31 - 0.32]	0.30 (0.0071) [0.29 - 0.32]	0.0074 (0.010) [-0.0015 - 0.015]	-0.013, 0.028	0.468	(0.28 - 0.33)
Tryptophan (% DW)	0.051 (0.0013) [0.049 - 0.052]	0.049 (0.0013) [0.048 - 0.050]	0.0019 (0.0018) [0.00056 - 0.0031]	-0.0020, 0.0058	0.318	(0.045 - 0.054)

Table 8 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range)**
Tyrosine (% DW)	0.29 (0.021) [0.27 - 0.30]	0.28 (0.021) [0.27 - 0.32]	0.0038 (0.030) [-0.026 - 0.029]	-0.060, 0.067	0.901	(0.24 - 0.30)
Valine (% DW)	0.46 (0.0095) [0.45 - 0.46]	0.45 (0.0095) [0.42 - 0.47]	0.0049 (0.013) [-0.014 - 0.035]	-0.023, 0.033	0.716	(0.39 - 0.46)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 9. Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E. [1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range)**
Alanine (% DW)	0.62 (0.018) [0.59 - 0.65]	0.70 (0.018) [0.68 - 0.72]	-0.077 (0.025) [-0.093 - -0.049]	-0.13, -0.025	0.006	(0.62 - 0.70)
Arginine (% DW)	0.40 (0.011) [0.38 - 0.40]	0.41 (0.011) [0.38 - 0.45]	-0.015 (0.015) [-0.063 - 0.027]	-0.047, 0.018	0.352	(0.34 - 0.42)
Aspartic Acid (% DW)	0.56 (0.012) [0.54 - 0.58]	0.60 (0.012) [0.59 - 0.61]	-0.045 (0.017) [-0.058 - -0.027]	-0.081, -0.0086	0.018	(0.53 - 0.60)
Cystine (% DW)	0.20 (0.0050) [0.19 - 0.21]	0.21 (0.0050) [0.20 - 0.22]	-0.0097 (0.0058) [-0.029 - 0.0015]	-0.022, 0.0026	0.112	(0.19 - 0.22)
Glutamic Acid (% DW)	1.60 (0.045) [1.50 - 1.66]	1.76 (0.045) [1.72 - 1.79]	-0.16 (0.063) [-0.22 - -0.10]	-0.29, -0.027	0.020	(1.53 - 1.76)
Glycine (% DW)	0.33 (0.0050) [0.33 - 0.34]	0.34 (0.0050) [0.33 - 0.35]	-0.0088 (0.0068) [-0.018 - -0.0014]	-0.023, 0.0057	0.218	(0.31 - 0.33)
Histidine (% DW)	0.27 (0.0053) [0.26 - 0.28]	0.29 (0.0053) [0.28 - 0.29]	-0.013 (0.0072) [-0.018 - -0.0080]	-0.028, 0.0020	0.084	(0.23 - 0.27)
Isoleucine (% DW)	0.29 (0.0079) [0.28 - 0.31]	0.33 (0.0079) [0.33 - 0.33]	-0.033 (0.011) [-0.047 - -0.020]	-0.056, -0.0099	0.007	(0.29 - 0.32)

Table 9 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Leucine (% DW)	1.04 (0.033) [0.97 - 1.09]	1.16 (0.033) [1.15 - 1.17]	-0.12 (0.045) [-0.18 - -0.074]	-0.22, -0.026	0.015	(0.97 - 1.14)
Lysine (% DW)	0.27 (0.0050) [0.27 - 0.27]	0.29 (0.0050) [0.28 - 0.30]	-0.016 (0.0071) [-0.033 - -0.0028]	-0.030, -0.00096	0.037	(0.26 - 0.29)
Methionine (% DW)	0.17 (0.0045) [0.16 - 0.17]	0.17 (0.0045) [0.16 - 0.18]	-0.0027 (0.0060) [-0.022 - 0.016]	-0.016, 0.010	0.658	(0.17 - 0.19)
Phenylalanine (% DW)	0.43 (0.012) [0.40 - 0.44]	0.47 (0.012) [0.46 - 0.48]	-0.041 (0.017) [-0.057 - -0.022]	-0.076, -0.0063	0.022	(0.40 - 0.46)
Proline (% DW)	0.81 (0.022) [0.76 - 0.84]	0.87 (0.022) [0.84 - 0.91]	-0.062 (0.032) [-0.11 - -0.0054]	-0.13, 0.0039	0.063	(0.71 - 0.86)
Serine (% DW)	0.41 (0.013) [0.39 - 0.43]	0.44 (0.013) [0.42 - 0.46]	-0.030 (0.018) [-0.044 - -0.020]	-0.067, 0.0074	0.109	(0.37 - 0.43)
Threonine (% DW)	0.30 (0.0071) [0.29 - 0.30]	0.31 (0.0071) [0.30 - 0.33]	-0.017 (0.010) [-0.028 - -0.011]	-0.038, 0.0035	0.098	(0.27 - 0.31)
Tryptophan (% DW)	0.052 (0.0013) [0.048 - 0.055]	0.050 (0.0013) [0.048 - 0.051]	0.0019 (0.0018) [-0.00035 - 0.0038]	-0.0019, 0.0058	0.304	(0.048 - 0.052)

Table 9 (cont). Comparison of the Amino Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Tyrosine (% DW)	0.26 (0.021) [0.22 - 0.30]	0.27 (0.021) [0.20 - 0.32]	-0.0032 (0.030) [-0.10 - 0.097]	-0.067, 0.060	0.915	(0.13- 0.28)
Valine (% DW)	0.43 (0.0095) [0.41 - 0.44]	0.46 (0.0095) [0.46 - 0.47]	-0.034 (0.013) [-0.049 - -0.020]	-0.062, -0.0065	0.018	(0.40 - 0.45)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 10. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
16:0 Palmitic (% Total FA)	11.06 (0.099) [10.92 - 11.29]	11.38 (0.099) [11.21 - 11.53]	-0.31 (0.12) [-0.41 - -0.24]	-0.57, -0.063	0.017	(9.56 - 12.93)
18:0 Stearic (% Total FA)	1.83 (0.038) [1.74 - 1.89]	1.84 (0.038) [1.75 - 1.90]	-0.0084 (0.053) [-0.13 - 0.12]	-0.12, 0.11	0.877	(1.32 - 2.11)
18:1 Oleic (% Total FA)	20.91 (0.22) [20.27 - 21.55]	20.88 (0.22) [20.41 - 21.29]	0.030 (0.27) [-0.67 - 0.50]	-0.56, 0.62	0.913	(20.59 - 31.56)
18:2 Linoleic (% Total FA)	64.43 (0.26) [63.86 - 64.94]	64.06 (0.26) [63.58 - 64.86]	0.36 (0.31) [-0.39 - 1.20]	-0.29, 1.02	0.257	(55.08 - 64.79)
18:3 Linolenic (% Total FA)	1.21 (0.017) [1.19 - 1.23]	1.26 (0.017) [1.22 - 1.28]	-0.043 (0.023) [-0.055 - -0.030]	-0.091, 0.0058	0.080	(1.10 - 1.61)
20:0 Arachidic (% Total FA)	0.30 (0.0063) [0.29 - 0.30]	0.30 (0.0063) [0.28 - 0.31]	-0.0010 (0.0081) [-0.010 - 0.017]	-0.018, 0.016	0.901	(0.32 - 0.36)
20:1 Eicosenoic (% Total FA)	0.18 (0.0024) [0.17 - 0.18]	0.18 (0.0024) [0.17 - 0.18]	-0.0020 (0.0033) [-0.0097 - 0.0047]	-0.0089, 0.0050	0.560	(0.20 - 0.26)
22:0 Behenic (% Total FA)	0.087 (0.021) [0.060 - 0.14]	0.11 (0.021) [0.058 - 0.15]	-0.026 (0.029) [-0.086 - 0.081]	-0.086, 0.035	0.386	(0.060 - 0.17)

[1]FA = fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 11. Comparison of the Fatty Acid Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
16:0 Palmitic (% Total FA)	11.35 (0.099) [11.21 - 11.45]	11.60 (0.099) [11.48 - 11.72]	-0.25 (0.12) [-0.51 - -0.080]	-0.50, 0.0021	0.051	(9.61 - 12.95)
18:0 Stearic (% Total FA)	1.93 (0.038) [1.84 - 2.01]	1.89 (0.038) [1.84 - 1.92]	0.035 (0.053) [-0.0075 - 0.10]	-0.079, 0.15	0.527	(1.39 - 2.21)
18:1 Oleic (% Total FA)	21.26 (0.22) [20.92 - 21.89]	21.03 (0.22) [20.83 - 21.29]	0.23 (0.27) [-0.33 - 1.06]	-0.36, 0.82	0.412	(21.04 - 31.63)
18:2 Linoleic (% Total FA)	63.60 (0.26) [62.77 - 64.18]	63.64 (0.26) [63.46 - 63.85]	-0.043 (0.31) [-1.07 - 0.57]	-0.70, 0.61	0.890	(54.81 - 65.11)
18:3 Linolenic (% Total FA)	1.23 (0.017) [1.22 - 1.24]	1.21 (0.017) [1.18 - 1.23]	0.019 (0.023) [0.0040 - 0.041]	-0.029, 0.067	0.414	(1.14 - 1.60)
20:0 Arachidic (% Total FA)	0.32 (0.0063) [0.30 - 0.33]	0.31 (0.0063) [0.29 - 0.31]	0.012 (0.0081) [-0.0081 - 0.034]	-0.0054, 0.029	0.168	(0.32 - 0.35)
20:1 Eicosenoic (% Total FA)	0.18 (0.0024) [0.18 - 0.18]	0.19 (0.0024) [0.18 - 0.19]	-0.0057 (0.0033) [-0.015 - 0.0041]	-0.013, 0.0013	0.103	(0.20 - 0.25)
22:0 Behenic (% Total FA)	0.14 (0.021) [0.13 - 0.15]	0.14 (0.021) [0.12 - 0.18]	0.0034 (0.029) [-0.035 - 0.032]	-0.057, 0.064	0.906	(0.063 - 0.16)

[1]FA – fatty acid; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 12. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range)**
Folic Acid (mg/kg DW)	0.29 (0.024) [0.26 - 0.35]	0.24 (0.024) [0.23 - 0.25]	0.055 (0.026) [0.027 - 0.099]	-0.0020, 0.11	0.057	(0.28 - 0.30)
Niacin (mg/kg DW)	21.92 (1.34) [20.49 - 23.01]	23.52 (1.34) [22.48 - 24.71]	-1.60 (1.73) [-2.89 - -0.22]	-5.31, 2.10	0.369	(24.16 - 29.08)
Thiamine HCl (mg/kg DW)	2.89 (0.12) [2.80 - 3.01]	2.88 (0.12) [2.74 - 2.95]	0.017 (0.17) [-0.063 - 0.060]	-0.33, 0.37	0.917	(2.19 - 3.59)
Vitamin B2 (mg/kg DW)	2.17 (0.22) [1.77 - 2.87]	2.14 (0.22) [1.86 - 2.57]	0.034 (0.31) [-0.69 - 1.01]	-0.62, 0.69	0.913	(1.96 - 2.40)
Vitamin B6 (mg/kg DW)	5.64 (0.21) [5.32 - 6.05]	5.40 (0.21) [5.36 - 5.44]	0.24 (0.30) [-0.073 - 0.61]	-0.39, 0.87	0.430	(5.37 - 5.80)
Vitamin E (mg/kg DW)	11.63 (0.53) [11.06 - 12.47]	9.85 (0.53) [8.30 - 10.97]	1.78 (0.71) [1.08 - 2.76]	0.27, 3.29	0.023	(2.88 - 8.47)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 13. Comparison of the Vitamin Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	**Test Mean ± S.E.[1] [Range]**	**Control Mean ± S.E. [Range]**	**Mean ± S.E. [Range]**	**95% CI[1] (Lower,Upper)**	**p-Value**	**Commercial (Range)**
Folic Acid (mg/kg DW)	0.27 (0.024) [0.23 - 0.31]	0.31 (0.024) [0.24 - 0.39]	-0.032 (0.026) [-0.11 - 0.025]	-0.089, 0.026	0.251	(0.27 - 0.36)
Niacin (mg/kg DW)	24.28 (1.34) [20.00 - 28.64]	23.37 (1.34) [21.92 - 24.71]	0.91 (1.73) [-1.92 - 3.93]	-2.79, 4.62	0.605	(24.60 - 28.88)
Thiamine HCl (mg/kg DW)	2.97 (0.12) [2.86 - 3.14]	2.85 (0.12) [2.74 - 2.95]	0.12 (0.17) [-0.088 - 0.29]	-0.23, 0.47	0.468	(2.88 - 3.66)
Vitamin B2 (mg/kg DW)	2.15 (0.22) [1.79 - 2.42]	2.11 (0.22) [1.96 - 2.27]	0.036 (0.31) [-0.49 - 0.46]	-0.62, 0.69	0.908	(1.91 - 2.75)
Vitamin B6 (mg/kg DW)	5.43 (0.21) [5.41 - 5.46]	5.51 (0.21) [5.25 - 5.71]	-0.075 (0.30) [-0.29 - 0.22]	-0.71, 0.56	0.804	(4.19 - 6.07)
Vitamin E (mg/kg DW)	11.52 (0.53) [10.34 - 12.37]	10.57 (0.53) [10.05 - 10.94]	0.95 (0.71) [-0.40 - 2.32]	-0.56, 2.46	0.201	(5.20 - 9.95)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 14. Comparison of the Antinutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E. [1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Antinutrient						
Phytic Acid (% DW)	0.76 (0.042) [0.65 - 0.83]	0.71 (0.042) [0.68 - 0.73]	0.046 (0.060) [-0.034 - 0.11]	-0.079, 0.17	0.450	(0.57 - 0.71)
Raffinose (% DW)	0.096 (0.0064) [0.089 - 0.11]	0.093 (0.0064) [0.082 - 0.10]	0.0031 (0.0090) [-0.010 - 0.024]	-0.016, 0.022	0.738	(0.029 - 0.095)
Secondary Metabolite						
Ferulic Acid (µg/g DW)	1988.05 (116.42) [1910.24 - 2097.90]	1904.98 (116.42) [1704.17 - 2072.82]	83.07 (158.06) [-27.70 - 251.85]	-250.94, 417.09	0.606	(1263.58 - 2704.49)
p-Coumaric Acid (µg/g DW)	201.09 (10.65) [180.56 - 213.29]	176.29 (10.65) [159.49 - 188.64]	24.81 (14.34) [21.06 - 28.71]	-5.32, 54.93	0.100	(119.71 - 286.21)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 15. Comparison of the Antinutrient and Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Antinutrient						
Phytic Acid (% DW)	0.78 (0.042) [0.75 - 0.80]	0.69 (0.042) [0.63 - 0.74]	0.093 (0.060) [0.052 - 0.17]	-0.032, 0.22	0.136	(0.55 - 0.73)
Raffinose (% DW)	0.10 (0.0064) [0.092 - 0.11]	0.091 (0.0064) [0.081 - 0.11]	0.0094 (0.0090) [-0.010 - 0.028]	-0.0094, 0.028	0.308	(0.029 - 0.069)
Secondary Metabolite						
Ferulic Acid (µg/g DW)	2079.28 (116.42) [2034.88 - 2119.13]	1986.45 (116.42) [1898.80 - 2066.25]	92.82 (158.06) [40.58 - 185.01]	-241.19, 426.84	0.564	(1503.59 - 2078.52)
p-Coumaric Acid (µg/g DW)	192.51 (10.65) [181.40 - 201.40]	189.88 (10.65) [175.61 - 202.72]	2.62 (14.34) [-21.32 - 25.79]	-27.50, 32.75	0.856	(127.14 - 277.33)

[1]DW = dry weight; S.E. = standard error; CI = confidence interval.
[2]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial lines. Negative limits were set to zero.

Table 16. Summary of Significant Differences (p<0.05) Comparing MON 87460 to the Conventional Control from the 2006/2007 Chilean QUI Site

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	Commercial (Range)
Forage						
Water-Limited						
Moisture (% FW)	73.53	76.57	-3.96	0.019	(72.40 - 75.30)	(74.30 - 77.30)
Protein (% DW)	5.78	6.69	-13.68	0.013	(5.25 - 6.57)	(5.44 - 6.38)
Total Fat (% DW)	1.44	0.82	75.70	0.049	(0.58 - 2.34)	(0.58 - 1.42)
Grain						
Well-watered						
Moisture (% FW)	13.63	8.48	60.83	<0.001	(13.10 - 14.2)	(9.41 - 13.70)
Magnesium (% DW)	0.12	0.11	7.86	0.043	(0.12 - 0.13)	(0.10 - 0.12)
Phosphorus (% DW)	0.32	0.30	7.63	0.047	(0.31 - 0.34)	(0.27 - 0.33)
16:0 Palmitic (% Total FA)	11.06	11.38	-2.77	0.017	(10.92 - 11.29)	(9.56 - 12.93)
Vitamin E (mg/kg DW)	11.63	9.85	18.09	0.023	(11.06 - 12.47)	(2.88 - 8.47)
Water-Limited						
Carbohydrates (% DW)	85.95	85.21	0.87	0.038	(85.69 - 86.33)	(85.53 - 86.50)
Moisture (% FW)	13.60	8.72	55.96	<0.001	(12.70 - 14.10)	(9.55 - 13.40)
Protein (% DW)	8.69	9.24	-5.90	0.043	(8.24 - 8.99)	(8.32 - 9.67)
Neutral Detergent Fiber (% DW	9.65	10.89	-11.36	0.042	(8.85 - 10.54)	(7.75 - 10.73)
Copper (mg/kg DW)	1.61	4.22	-61.82	0.032	(1.53 - 1.65)	(1.37 - 1.87)
Iron (mg/kg DW)	14.35	15.48	-7.29	0.037	(13.75 - 14.77)	(14.04 - 19.16)
Alanine (% DW)	0.62	0.70	-11.03	0.006	(0.59 - 0.65)	(0.62 - 0.70)
Aspartic Acid (% DW)	0.56	0.60	-7.42	0.018	(0.54 - 0.58)	(0.53 - 0.60)
Glutamic Acid (% DW)	1.60	1.76	-9.04	0.020	(1.50 - 1.66)	(1.53 - 1.76)
Isoleucine (% DW)	0.29	0.33	-10.09	0.007	(0.28 - 0.31)	(0.29 - 0.32)
Leucine (% DW)	1.04	1.16	-10.50	0.015	(0.97 - 1.09)	(0.97 - 1.14)
Lysine (% DW)	0.27	0.29	-5.44	0.037	(0.27 - 0.27)	(0.26 - 0.29)
Phenylalanine (% DW)	0.43	0.47	-8.79	0.022	(0.40 - 0.44)	(0.40 - 0.46)
Valine (% DW)	0.43	0.46	-7.50	0.018	(0.41 - 0.44)	(0.40 - 0.45)

[1]DW= dry weight; FW=fresh weight, FA= fatty acid.

Table 17. Comparison of the Additional Secondary Metabolite Content in Forage from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Free Proline (% DW)	0.020 (0.0017) [0.017 - 0.022]	0.018 (0.0017) [0.017 - 0.019]	0.0019 (0.0023) [-0.0015 - 0.0042]	-0.0030, 0.0069	0.422	(0.014 - 0.015)
Abscisic Acid (ppb FW)	15.70 (4.35) [14.60 - 16.90]	12.53 (4.35) [10.50 - 14.00]	3.17 (5.90) [1.50 - 6.40]	-9.39, 15.72	0.599	(13.00 - 19.80)
Choline (ppm FW)	127.67 (6.53) [121.00 - 135.00]	120.00 (6.53) [111.00 - 133.00]	7.67 (8.71) [-6.00 - 24.00]	-10.78, 26.11	0.391	(111.00 - 115.00)
Glycerol (% DW)	0.14 (0.0084) [0.14 - 0.14]	0.14 (0.0084) [0.12 - 0.16]	0.0019 (0.011) [-0.026 - 0.028]	-0.021, 0.025	0.864	(0.12 - 0.18)
Glycine Betaine (ppm FW)	63.53 (5.24) [58.40 - 69.80]	57.83 (5.24) [51.60 - 62.80]	5.70 (6.24) [-4.40 - 10.80]	-7.73, 19.13	0.376	(49.80 - 64.20)
Salicylic Acid (ppm FW)	0.098 (0.040) [0.088 - 0.11]	0.16 (0.040) [0.091 - 0.21]	-0.058 (0.057) [-0.12 - 0.020]	-0.18, 0.061	0.321	(0.075 - 0.15)
Fructose (% DW)	5.30 (0.46) [4.40 - 6.31]	6.95 (0.46) [6.22 - 7.44]	-1.65 (0.51) [-2.26 - -0.88]	-2.74, -0.57	0.005	(6.50 - 8.09)
Glucose (% DW)	6.09 (0.52) [5.22 - 6.96]	7.73 (0.52) [6.99 - 8.15]	-1.64 (0.50) [-2.05 - -1.10]	-2.71, -0.57	0.005	(7.93 - 8.97)
Sucrose (% DW)	3.80 (1.31) [2.32 - 4.74]	2.53 (1.31) [2.08 - 2.93]	1.27 (1.74) [-0.25 - 2.25]	-2.41, 4.95	0.475	(0.11 - 3.77)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

Table 18. Comparison of the Additional Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Well-Watered Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Free Proline (% DW)	0.072 (0.0030) [0.068 - 0.076]	0.076 (0.0030) [0.075 - 0.078]	-0.0041 (0.0039) [-0.0091 - 0.0016]	-0.012, 0.0042	0.313	(0.048 - 0.059)
Abscisic Acid (ppb FW)	10.80 (1.71) [9.41 - 12.60]	18.93 (1.71) [17.60 - 20.30]	-8.13 (2.05) [-8.50 - -7.70]	-12.48, -3.78	0.001	(23.00 - 35.80)
Choline (ppm FW)	210.67 (12.86) [206.00 - 219.00]	227.33 (12.86) [213.00 - 248.00]	-16.67 (17.84) [-29.00 - -6.00]	-54.25, 20.92	0.363	(194.00 - 265.00)
Glycerol (% DW)	0.019 (0.0019) [0.018 - 0.022]	0.019 (0.0019) [0.016 - 0.024]	0.00018 (0.0025) [-0.0057 - 0.0061]	-0.0051, 0.0054	0.944	(0.018 - 0.025)
Glycine Betaine (ppm FW)	1.89 (0.17) [1.69 - 2.08]	1.75 (0.17) [1.61 - 1.82]	0.14 (0.23) [0.080 - 0.26]	-0.35, 0.64	0.548	(0.50 - 3.34)
Salicylic Acid (ppm FW)	0.092 (0.026) [0.076 - 0.12]	0.12 (0.026) [0.095 - 0.15]	-0.032 (0.030) [-0.043 - -0.019]	-0.099, 0.035	0.317	(0.069 - 0.32)
Fructose (% DW)	0.46 (0.031) [0.44 - 0.48]	0.54 (0.031) [0.54 - 0.54]	-0.081 (0.040) [-0.10 - -0.067]	-0.17, 0.0034	0.058	(0.41 - 0.65)
Glucose (% DW)	0.47 (0.031) [0.46 - 0.48]	0.57 (0.031) [0.53 - 0.60]	-0.10 (0.039) [-0.12 - -0.071]	-0.18, -0.017	0.021	(0.41 - 0.64)
Sucrose (% DW)	1.81 (0.083) [1.76 - 1.85]	2.22 (0.083) [2.10 - 2.38]	-0.41 (0.12) [-0.53 - -0.30]	-0.66, -0.17	0.002	(2.07 - 2.74)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

Table 19. Comparison of the Additional Secondary Metabolite Content in Forage from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Difference (Test minus Control) Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Free Proline (% DW)	0.024 (0.0017) [0.019 - 0.027]	0.020 (0.0017) [0.018 - 0.021]	0.0040 (0.0023) [-0.0020 - 0.0076]	-0.00095, 0.0089	0.106	(0.012 - 0.027)
Abscisic Acid (ppb FW)	17.10 (4.35) [13.80 - 21.40]	11.56 (4.35) [9.98 - 14.70]	5.54 (5.90) [3.82 - 6.70]	-7.01, 18.09	0.362	(14.10 - 28.70)
Choline (ppm FW)	127.67 (6.53) [116.00 - 139.00]	117.00 (6.53) [101.00 - 134.00]	10.67 (8.71) [0 - 27.00]	-7.78, 29.11	0.238	(115.00 - 119.00)
Glycerol (% DW)	0.17 (0.0084) [0.15 - 0.20]	0.15 (0.0084) [0.14 - 0.16]	0.022 (0.011) [0.0014 - 0.052]	-0.0017, 0.045	0.067	(0.14 - 0.17)
Glycine Betaine (ppm FW)	51.17 (5.24) [42.20 - 61.10]	52.97 (5.24) [41.90 - 66.60]	-1.80 (6.24) [-5.50 - 0.30]	-15.23, 11.63	0.777	(46.60 - 64.00)
Salicylic Acid (ppm FW)	0.098 (0.040) [0.073 - 0.15]	0.11 (0.040) [0.078 - 0.18]	-0.017 (0.057) [-0.035 - -0.0042]	-0.14, 0.10	0.769	(0.077 - 0.41)
Fructose (% DW)	5.48 (0.46) [5.00 - 5.97]	6.53 (0.46) [6.33 - 6.68]	-1.05 (0.51) [-1.68 - -0.61]	-2.14, 0.030	0.055	(5.68 - 6.42)
Glucose (% DW)	6.10 (0.52) [5.55 - 6.81]	7.53 (0.52) [7.51 - 7.56]	-1.43 (0.50) [-1.96 - -0.70]	-2.49, -0.36	0.012	(6.72 - 7.58)
Sucrose (% DW)	2.33 (1.31) [1.14 - 4.26]	3.06 (1.31) [1.49 - 5.56]	-0.72 (1.74) [-3.96 - 2.14]	-4.40, 2.96	0.682	(0.095 - 4.24)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

Table 20. Comparison of the Additional Secondary Metabolite Content in Grain from MON 87460 and Conventional Control from the 2006/2007 Chilean QUI Site Conducted under Water-Limited Conditions

			Difference (Test minus Control)			
Analytical Component[1]	Test Mean ± S.E.[1] [Range]	Control Mean ± S.E. [Range]	Mean ± S.E. [Range]	95% CI[1] (Lower,Upper)	p-Value	Commercial (Range)
Free Proline (% DW)	0.068 (0.0030) [0.064 - 0.075]	0.073 (0.0030) [0.067 - 0.078]	-0.0045 (0.0039) [-0.012 - 0.0076]	-0.013, 0.0038	0.268	(0.044 - 0.067)
Abscisic Acid (ppb FW)	8.04 (1.71) [7.02 - 8.66]	23.13 (1.71) [22.50 - 23.90]	-15.09 (2.05) [-16.88 - -13.84]	-19.44, -10.74	<0.001	(19.30 - 30.50)
Choline (ppm FW)	231.00 (12.86) [209.00 - 265.00]	234.00 (12.86) [212.00 - 262.00]	-3.00 (17.84) [-53.00 - 53.00]	-40.59, 34.59	0.868	(175.00 - 235.00)
Glycerol (% DW)	0.019 (0.0019) [0.018 - 0.020]	0.018 (0.0019) [0.014 - 0.024]	0.0016 (0.0025) [-0.0032 - 0.0042]	-0.0036, 0.0069	0.520	(0.017 - 0.021)
Glycine Betaine (ppm FW)	2.09 (0.17) [1.99 - 2.18]	2.10 (0.17) [1.63 - 2.44]	-0.010 (0.23) [-0.34 - 0.36]	-0.50, 0.48	0.966	(0.50 - 2.91)
Salicylic Acid (ppm FW)	0.094 (0.026) [0.087 - 0.10]	0.14 (0.026) [0.11 - 0.18]	-0.048 (0.030) [-0.080 - -0.019]	-0.11, 0.019	0.143	(0.055 - 0.38)
Fructose (% DW)	0.50 (0.031) [0.41 - 0.57]	0.48 (0.031) [0.45 - 0.52]	0.022 (0.040) [-0.038 - 0.054]	-0.063, 0.11	0.586	(0.38 - 0.66)
Glucose (% DW)	0.52 (0.031) [0.45 - 0.57]	0.48 (0.031) [0.47 - 0.52]	0.031 (0.039) [-0.021 - 0.063]	-0.053, 0.11	0.449	(0.38 - 0.66)
Sucrose (% DW)	1.84 (0.083) [1.67 - 2.08]	2.10 (0.083) [1.96 - 2.18]	-0.26 (0.12) [-0.51 - -0.086]	-0.50, -0.013	0.040	(2.12 - 2.59)

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = confidence interval.

Table 21. Summary of Significant Differences in Additional Secondary Metabolite Composition (p<0.05) Comparing MON 87460 to the Conventional Control from the 2006/2007 Chilean QUI Site

Tissue/Site/ Components (Units)[1]	Mean MON 87460	Mean Control	Mean Diff (% of Control)	Signif. (p-value)	MON 87460 (Range)	Commercial (Range)
Forage						
Well-watered						
Fructose (% DW)	5.30	6.95	-23.77	0.005	(4.40 - 6.31)	(6.50 - 8.09)
Glucose (% DW)	6.09	7.73	-21.18	0.005	(5.22 - 6.96)	(7.93 - 8.97)
Water-Limited						
Glucose (% DW)	6.10	7.53	-18.94	0.012	(5.55 - 6.81)	(6.72 - 7.58)
Grain						
Well-watered						
Abscisic Acid (ppb FW)	10.80	18.93	-42.94	0.001	(9.41 - 12.60)	(23.00 - 35.80)
Glucose (% DW)	0.47	0.57	-17.65	0.021	(0.46 - 0.48)	(0.41 - 0.64)
Sucrose (% DW)	1.81	2.22	-18.67	0.002	(1.76 - 1.85)	(2.07 - 2.74)
Water-Limited						
Abscisic Acid (ppb FW)	8.04	23.13	-65.24	<0.001	(7.02 - 8.66)	[(9.30 - 30.500
Sucrose (% DW)	1.84	2.10	-12.24	0.040	(1.67 - 2.08)	(2.12 - 2.59)

[1]DW= dry weight; FW=fresh weight.