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Petition for the Determination of Nonregulated Status for Increased Ear Biomass MON 87403 Maize

The undersigned submits this petition under 7 CFR § 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

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RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. Monsanto understands that the USDA complies with the provisions of the Freedom of Information Act (FOIA). In the event the USDA receives a FOIA request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of the information in this petition, Monsanto expects that, in advance of the release of the document(s), USDA will, pursuant to Executive Order 12600, the Privacy Act and other applicable authorities, provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, *e.g.*, responsiveness, confidentiality, and/or competitive concerns. Monsanto understands that a CBI-deleted copy of this information may be made available to the public in a reading room and upon individual request as part of a public comment period. Monsanto also understands that when deemed complete, a copy of the petition may be posted to the USDA-APHIS BRS website or other U.S. government websites (e.g., www.regulations.gov). Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information without Monsanto's prior notice and consent.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner that are unfavorable to the petition.

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EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived maize product, MON 87403, any progeny derived from crosses between MON 87403 and conventional maize, and any progeny derived from crosses of MON 87403 with biotechnology-derived maize that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed biotechnology-derived maize MON 87403 that has increased ear biomass at an early reproductive phase (R1) compared to conventional control maize. MON 87403 was produced through insertion of the coding region of the full-length *Arabidopsis thaliana ATHB17* gene through *Agrobacterium*-mediated transformation. ATHB17 is a member of the HD-Zip family of plant transcription factors, which are proteins that bind to specific DNA sequences and regulate gene expression. The HD-Zip family of proteins is found broadly across plant species and specific HD-Zip proteins have been shown to play an important role in the modulation of plant growth and development. The HD-Zip family consists of four subfamilies and ATHB17 is a member of the class II subfamily. HD-Zip II proteins form either homodimers or heterodimers with other HD-Zip II proteins within the same subfamily and function as repressors of gene expression. In MON 87403, maize-specific splicing of the *ATHB17* transcript results in a truncated protein, ATHB17Δ113, which is missing the first 113 N-terminal amino acids that are expressed in *Arabidopsis thaliana*. The ATHB17Δ113 protein retains the ability to form homo- and hetero-dimers and bind to target DNA sequences like the full-length protein. The ATHB17Δ113 protein is, however, unable to function as a transcriptional repressor because the protein lacks a functional repression domain. By a dominant-negative mechanism, ATHB17Δ113 can alter the activity of endogenous maize HD-Zip II proteins, which are predominantly expressed in ear tissue. Thus, the ATHB17Δ113 protein likely modulates HD-Zip II-regulated pathways in the ear, which leads to increased ear growth at the early reproductive phase. This increased ear growth is associated with increased partitioning of dry matter (photosynthate) from the source (vegetative) tissue to the sink (ear) tissue in MON 87403 compared to control plants.

Early reproductive stages in maize are a critical period of maize growth at which the maximum ear biomass (sink size) is determined by a combination of genetics and

environmental conditions. Dry matter (photosynthate) produced by the plant during reproductive stages is allocated to the ear for its growth after the sink size is determined. Thus, ear biomass, which is set during early reproductive stages, is considered an important determinant of reproductive success and a larger ear biomass at early reproductive stages is associated with increased grain yield at harvest. Consistent with this, multiple years of field testing showed that MON 87403 out-yielded its comparators at a majority of locations tested.

MON 87403 will be combined with other deregulated biotechnology-derived traits through traditional breeding methods to create commercial products with increased yield opportunity as well as protection against maize pests and tolerance to multiple herbicides. These next generation combined-trait maize products will continue to offer broader grower choice and continued pest control durability. Adoption of improved maize hybrids with increased yield opportunity that results in incremental increases in national average grain yield can positively impact production, exports and economic welfare.

Data and Information Presented Confirms the Lack of Plant Pest Potential and the Food and Feed Safety of MON 87403 Compared to Conventional Maize

The data and information presented in this petition demonstrate MON 87403 is agronomically, phenotypically, and compositionally comparable to commercially cultivated maize, with the exception of the introduced trait. Moreover, the data and information presented demonstrate MON 87403 is not expected to pose an increased plant pest risk, including weediness, compared to commercially cultivated maize. The food, feed, and environmental safety of MON 87403 was confirmed based on multiple, well-established lines of evidence:

- Maize does not possess any of the attributes commonly associated with weeds, has a history of safe consumption, and serves as an appropriate basis of comparison for MON 87403.

- A detailed molecular characterization of the inserted DNA demonstrates a single, intact copy of the T-DNA insert in a single locus within the maize genome.

- Extensive evaluation of the truncated ATHB17Δ113 protein expressed in MON 87403 confirms that it is unlikely to be a toxin or allergen.

- A compositional assessment supports the conclusion that MON 87403 grain and forage is compositionally equivalent to grain and forage of conventional maize.

- An extensive evaluation of MON 87403 phenotypic and agronomic characteristics and environmental interactions demonstrates MON 87403 has no increased plant pest risk potential compared to conventional maize.

- An assessment of potential impact to non-target organisms (NTOs) including organisms beneficial to agriculture and endangered species indicates that

MON 87403 is not expected to have an effect on other organisms compared to conventional maize under normal agricultural practices.

- Evaluation of the agronomic and phenotypic characteristics of MON 87403, using current maize cultivation and management practices, leads to the conclusion that deregulation of MON 87403 would not have an effect on maize agronomic practices.

Maize is a Familiar Crop Lacking Weedy Characteristics

Maize is grown extensively throughout the world, and is the largest cultivated crop followed by wheat (*Triticum* sp.) and rice (*Oryza sativa* L.) in total global production. In the U.S., maize is grown in almost all states and is the largest crop grown in terms of acreage planted and net value. Maize has been studied extensively, and the domestication of maize can be traced back to approximately 10,000 years ago in southern Mexico.

Because of the importance of maize in agriculture, plant breeders continuously strive to improve commercial maize yields. Initial improvements in maize yield were due to the domestication of varieties with desirable traits like larger ear biomass. Maize ears became larger over time during this domestication. During the hybrid era (1939 to present), commercial maize yield in the U.S. increased nearly six-fold with an average 99 kg ha^{-1} increase every year. The major factor that contributed to yield increase during this era is a favorable response of hybrids to increased plant population density which resulted in an increase in the number of ears per hectare. Whereas increased planting densities resulted in a net increase in grain yields, they resulted in a decrease in ear size at the individual plant level. Commercial varieties with MON 87403 can provide increased yield opportunity as this trait increases ear biomass during early reproductive stages at production planting densities.

Maize is not listed as a weed in the major literature references on weeds, nor is it present on the lists of noxious weed species published by the federal government (7 CFR part 360). In addition, maize has been grown throughout the world without any report that it is a serious weed. Maize is poorly suited to survive without human assistance and is not capable of surviving as a weed due to past selection in the domestication of maize. During domestication of maize, traits often associated with weediness, such as seed dormancy, a dispersal mechanism, or the ability to establish reproducing populations outside of cultivation, have not been selected. Similarly, the history of hybrid breeding in the U.S. does not indicate there are any changes in the characteristics of maize that would change the weediness profile of the crop. Although maize seed can overwinter in a rotation with soybeans or other crops, mechanical and chemical measures are routinely used to control maize volunteers. Some populations of wild annual and perennial species that could hybridize with maize, such as hybrids containing MON 87403, are known to exist in the U.S., however key differences in several factors such as flowering time, geographical separation, and development timings make natural crosses in the U.S. highly unlikely.

Conventional Maize MPA640B and EXP257 are Appropriate Comparators to MON 87403

Conventional control materials developed for use as comparators in safety assessment studies were based on the appropriate fit for various studies and seed availability. The conventional control materials included the original transformation line (LH244) and LH244 crossed to two conventional lines (LH287 and LH295) to create F_1 starting control materials. Both MPA640B (LH244 × LH287) and EXP257 (LH244 × LH295) were used as controls in molecular characterization studies. MPA640B was used as the conventional control in compositional analysis studies and in phenotypic, agronomic and environmental interactions assessments. Where appropriate, commercial hybrid maize materials (reference hybrids) were also used to establish a range of variability or responses representative of commercial maize in the U.S.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA in MON 87403

MON 87403 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from inbred line LH244 utilizing plasmid PV-ZMAP5714. PV-ZMAP5714 is approximately 11.7 kb in size and contains three cassettes: one T-DNA, delineated by Left and Right Border regions, contains the *ATHB17* expression cassette, the plasmid backbone contains the *cp4 epsps* selectable marker cassette, and the *aadA* expression cassette. PV-ZMAP5714 employs a tandem T-DNA approach to generate marker-free plants. In this tandem T-DNA approach, a single right border and a single left border were used to achieve separate, unlinked insertion of the T-DNA as well as the *cp4 epsps* selectable marker gene located in the plasmid backbone. After initial selection of transformants for glyphosate tolerance, the *cp4 epsps* cassette was segregated away by conventional breeding and molecular analysis was used to identify plants containing only the intended T-DNA (and not the *cp4 epsps*).

Characterization of the DNA insert in MON 87403 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87403 contains one copy of the intended transfer DNA (T-DNA) containing the *ATHB17* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 87403 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87403 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87403 to determine the presence and identity of sequences derived from PV-ZMAP5714 and demonstrated that MON 87403 contained a single T-DNA insert with no detectable backbone sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87403 was used to determine the complete sequence of the

single DNA insert from PV-ZMAP5714, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the inserted DNA is identical to the corresponding region in the PV-ZMAP5714 T-DNA. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87403 to the sequence of the insertion site in conventional maize. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 87403 upon DNA integration.

- Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMAP5714 T-DNA insert in MON 87403 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87403.

- Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the nature of the T-DNA as a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 87403 demonstrates that a single copy of the intended T-DNA was stably integrated at a single locus of the maize genome and that no plasmid backbone sequences are present in MON 87403.

Data Confirms ATHB17Δ113 Protein Safety

Characterization of the introduced protein(s) in a biotechnology-derived crop is important to establishing food, feed, and environmental safety and a multistep approach was used to characterize and assess the safety of the ATHB17Δ113 protein expressed in MON 87403 resulting from the genetic modification. The expression level of the ATHB17Δ113 protein in selected tissues of MON 87403 was determined and exposure to humans and animals through diet was evaluated. In addition, the donor organism for the ATHB17Δ113 protein coding sequence, *Arabidopsis thaliana*, is ubiquitous in the environment and is not commonly known for human or animal pathogenicity or allergenicity.

Assessing the safety of the ATHB17Δ113 protein requires a consideration of both the hazard associated with the protein and the potential exposure to it. Data presented demonstrate that the expression of this protein in maize grain is below the limit of detection (LOD) and is extremely low in other tissues tested, hence exposure to ATHB17Δ113 protein is negligible. Bioinformatic searches using the ATHB17Δ113 amino acid sequence as the query identified homologous sequences from several different food plants, including soybean, rice, maize, tomato, potato, orange, papaya, grape, and cruciferous vegetables. Overall the protein sequence identity of ATHB17Δ113 to homologs in these species ranges from ~58-83%, with the highest identity to the homologs in the Brassica species *Brassica rapa* (a species including crops such as turnip and napa cabbage) and *Brassica oleracea* (a species including crops such as cabbage and Brussels sprouts). The amino acid sequence alignment between ATHB17Δ113 and its

food crop homologs spans the length of the ATHB17Δ113 protein. Thus ATHB17Δ113 shares sequence identity and structural similarity with proteins present in plants currently consumed, establishing that humans and animals are exposed to this class of proteins and that no adverse effects have been attributed to this class of proteins.

Bioinformatics analysis also determined that the ATHB17Δ113 protein lacks structural similarity to known allergens, gliadins, glutenins, or protein toxins. Testing also showed that the ATHB17Δ113 protein is rapidly digested in pepsin and pancreatin suggesting that the negligible amount of protein expressed is further reduced by proteolysis during ingestion thereby reducing the already negligible exposure. Additionally, a mouse gavage study demonstrated no acute oral toxicity with a No Observable Adverse Effect Level (NOAEL) for ATHB17Δ113 of 1335 mg/kg, the highest dose tested. Undetectably low expression levels in grain coupled with rapid digestibility and no evidence of any toxic or allergenic concerns supports the conclusion that consumption of the ATHB17Δ113 protein from MON 87403 or its progeny poses no meaningful risk to human and animal health or an increased plant pest risk.

MON 87403 is Compositionally Equivalent to Conventional Maize

Compositional analysis was conducted on grain and forage of MON 87403 grown at eight sites representative of typical agricultural regions for maize production in the U.S. in 2012. The evaluation of MON 87403 followed considerations relevant to the compositional quality of maize as defined by a 2002 OECD consensus document. Grain samples were analyzed for levels of nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals, and vitamins. The anti-nutrients analyzed in grain included phytic acid and raffinose. Secondary metabolites analyzed in grain included furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber, and minerals. In total, 78 different components were assayed (nine in forage and 69 in grain).

Of those 78 components, 14 fatty acids, sodium, and furfural had more than 50% of observations below the assay limit of quantitation (LOQ) and were excluded from statistical analysis. Moisture in grain and forage was measured for conversion of components to dry weight, but was not statistically analyzed. Therefore, 60 components were statistically analyzed. The statistical comparisons were based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). The compositional data from the reference hybrids were combined across all field sites to calculate a 99% tolerance interval for each component to estimate the natural variability of each component in maize.

Of the 60 components statistically assessed for MON 87403, none of the components showed a significant difference between MON 87403 and the conventional control. These results support the overall conclusion that MON 87403 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87403 to the conventional control in levels of these components. These results support the overall food and feed safety and lack of plant pest risk of MON 87403.

MON 87403 Does Not Change Maize Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 87403 included genetically similiar conventional controls as comparators. This evaluation used a weight of evidence approach and considered statistical differences between MON 87403 and the conventional control with respect to reproducibility, magnitude, and directionality. The observations were taken on plants not treated with herbicides in order to evaluate only the impact of the introduced trait in MON 87403. Comparison to a range of commercial references grown concurrently established the range of natural variability for maize, and provided a context from which to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, plant phenotypic observations and environmental interaction evaluations. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 87403 is comparable to the conventional control. Thus, MON 87403 is not expected to have increased weediness or plant pest potential compared to conventional maize.

Seed dormancy and germination characterization indicated that MON 87403 seed had dormancy and germination characteristics similar to seed of the conventional control. In particular, the lack of hard seed, a well recognized seed characteristic associated with weediness, supports a conclusion of no increased weediness of MON 87403 compared to the conventional control. For pollen characteristic assessments, there were no statistically significant differences (α=0.05) detected between MON 87403 and the conventional control for pollen viability and diameter, and no visual differences in general pollen morphology were observed.

The field evaluation of phenotypic, agronomic, and environmental characteristics also supports the conclusion that MON 87403 is not likely to have increased weediness or plant pest potential compared to conventional maize. Data were collected at 13 sites in the U.S. during 2012 to evaluate phenotypic and agronomic characteristics of MON 87403 compared to the conventional control and four reference hybrids. These 13 sites provided a diverse range of environmental and agronomic conditions representative of commercial maize production areas in North America. In the combined-site analysis, no statistically significant differences were detected between MON 87403 and the conventional control for 12 of 13 characteristics including early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, plant height, dropped ear count, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and yield. One significant difference was detected for ear height in the combined-site analysis.

MON 87403 had increased ear height (111.1 vs. 107.5 cm) compared to the conventional control. However, the mean value of MON 87403 for ear height was within the reference range for this characteristic Ear height is not a reported weediness characteristic and whereas large increases in ear height might be expected to increase stalk lodging, there was no increase in stalk lodging in these trials. In addition, a small change in ear height would not be expected to change agronomic practices. Thus, the measured phenotypic characteristics of MON 87403 were not altered in terms of pest/weed potential compared to conventional maize.

In an assessment of abiotic stress, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 143 comparisons of plant response to abiotic stressors. In an assessment of disease responses, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 176 comparisons. Finally, in an assessment of arthropod damage, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 150 comparisons for plant damage caused by arthropods.

In summary, the phenotypic, agronomic, and environmental interaction data were evaluated to characterize MON 87403, and to assess whether the trait introduced in MON 87403 alter the plant pest potential compared to conventional maize. The evaluation, using a weight of evidence approach, considered the reproducibility, magnitude, and direction of detected differences between MON 87403 and the conventional control, and comparison to the range of the commercial reference hybrids. Results from the phenotypic, agronomic, and environmental interactions assessment indicated that MON 87403 does not possess enhanced weediness characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk compared to conventional maize.

MON 87403 Will Not Negatively Affect NTOs Including Those Beneficial to Agriculture

An evaluation of the impacts of MON 87403 on non-target organisms (NTOs) is a component of the plant pest risk assessment. Because MON 87403 does not possess pesticidal activity, all organisms that interact with MON 87403 are considered to be NTOs. Data from 2012 U.S. phenotypic and agronomic studies and observational data on environmental interactions such as plant-disease interactions, arthropod damage and arthropod abundance, were collected at select sites for MON 87403 and conventional controls. Results from these extensive studies support conclusions of no adverse impacts to non-target arthropod populations and no changes to plant-disease interactions.

The biochemical information and experimental data for evaluation of MON 87403 included molecular characterization, ATHB17Δ113 protein safety assessment, data from the environmental interactions assessment, demonstration of compositional equivalence to conventional maize, and demonstration of agronomic and phenotypic equivalence to conventional maize (with the exception of presence of ATHB17Δ113 protein in MON 87403). Taken together, these data support the conclusion that MON 87403 has no

reasonable mechanism to harm NTOs, nor does it pose an additional risk to threatened and endangered species compared to the cultivation of conventional maize.

Deregulation of MON 87403 is Not Expected to Have Effects on Maize Agronomic Practices

An assessment of current maize agronomic practices was conducted to determine whether the cultivation of MON 87403 has the potential to impact current maize agronomic practices. Maize fields are typically highly managed areas that are dedicated to grain and/or forage production.

MON 87403 was developed to provide increased ear biomass at an early reproductive stage in maize hybrids through expression of the ATHB17Δ113 protein. As such, there are no additional phenotypes associated with expression of the intended T-DNA in this product. As phenotypic evaluations, evaluations of stress responses, and pest/disease susceptibility showed no differences between MON 87403 and reference hybrids no changes are anticipated in crop rotations, tillage practices, planting practices, fertility management, weed and disease management, and volunteer management from the introduction of MON 87403.

MON 87403 is similar to conventional maize in its agronomic, phenotypic, environmental, and compositional characteristics and has naturally occurring levels of protection against pests and diseases comparable to and typical of conventional commercial maize hybrids. Based on this assessment, the introduction of MON 87403 is not expected to result in changes or impacts to current maize agronomic practices.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87403 is not expected to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87403 and any progeny derived from crosses between MON 87403 and conventional maize or deregulated biotechnology-derived maize be granted nonregulated status under 7 CFR Part 340.

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ABBREVIATIONS AND DEFINITIONS[1]

~	approximately
AA	amino acid
ADF	acid detergent fiber
ANOVA	analysis of variance
APHIS	Animal and Plant Health Inspection Service
ATHB17	a member of the class II family of homeodomain-leucine zipper (HD-Zip) transcription factors
ATHB17Δ113	the protein expressed from the *ATHB17* gene in MON 87403
BLASTX	Basic Local Alignment Search Tool
B-Left Border Region	DNA region from *Agrobacterium tumefaciens* containing the left border sequence
B-Right Border Region	DNA region from *Agrobacterium tumefaciens* containing the right border sequence
BSA	bovine serum albumin
CaMV	cauliflower mosaic virus
CEW	corn earworm
CFR	Code of Federal Regulations
CTAB	cetyltrimethylammonium bromide
DAS	days after sowing
DEEM-FCID	Dietary Exposure Evaluation Model - Food Commodity Intake Database
DTT	dithiothreitol
DWCF	dry weight conversion factor
dwt	dry weight of tissue
E. coli	*Escherichia coli*
ECB	European corn borer
EDTA	ethylenediaminetetraacetic
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FA	fatty acid
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FMOC	fluorenylmethyl chloroformate
Gb	gigabase
GC	gas chromatography
GE	genetically engineered
GLP	Good Laboratory Practice
GRIN	Germplasm Resources Information Network
HD	homeodomain
HD-Zip	homeodomain leucine zipper class family of transcription factors
HPLC	high-performance liquid chromatography

[1] Alred, G.J., C.T. Brusaw, and W.E. Oliu. 2003. Handbook of Technical Writing, 7th edn., pp. 2-7. Bedford/St. Martin's, Boston, MA.

HRP	horseradish peroxidase
ICP	inductively coupled plasma
IgG	immunoglobulin G
ILSI-CCDB	International Life Sciences Institute Crop Composition Database
JSA	junction sequence analysis
JSC	junction sequence class
LB	loading buffer
LOD	limit of detection
LOQ	limit of quantitation
LZ	leucine zipper
MALDI TOF MS	matrix assisted laser desorption /ionization - time of flight mass spectrometry
MIXED	a statistical tool developed by the SAS Institute that uses a mixed-model ANOVA to analyze data
MMT	million metric tons
MOE	margin of exposure
MW	molecular weight
n	number of samples
NDF	neutral detergent fiber
NFDM	non-fat dry milk
NGS	next generation sequencing
NHANES	National Health and Nutrition Examination Survey
NOAEL	no observable adverse effect level
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator (Australia)
OPA	o-phthalaldehyde
OSL	over season leaf
OSR	over season root
PBST	phosphate-buffered saline with Tween- 20
PCR	polymerase chain reaction
PMSF	phenylmethylsulfonyl fluoride
ppm	parts per million
PRESS	predicted residual sum of squares
PVP	polyvinylpyrrolidone
QC	quality control
RT-PCR	reverse transcription polymersae chain reaction
SD	standard deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	standard error
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
SOP	standard operating procedure
TDF	total dietary fiber
TFA	trifluoroacetic acid
UTR	untranslated region
v/v	volume to volume ratio
UV	ultraviolet
w/v	weight to volume ratio

I. RATIONALE FOR THE DEVELOPMENT OF MON 87403

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR § 340.6

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived maize product, MON 87403, any progeny derived from crosses between MON 87403 and conventional maize, and any progeny derived from crosses of MON 87403 with biotechnology-derived maize that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of MON 87403

Monsanto Company has developed biotechnology-derived maize MON 87403 that has increased ear biomass at an early reproductive stage (R1) compared to conventional control maize. MON 87403 was produced through insertion of the coding region of the full-length *Arabidopsis thaliana ATHB17* gene through *Agrobacterium*-mediated transformation. The ATHB17 protein is a member of the HD-Zip family of plant transcription factors, which are proteins that bind to specific DNA sequences and regulate gene expression. The HD-Zip family of proteins is found broadly across plant species and specific HD-Zip proteins have been shown to play an important role in the modulation of plant growth and development. The HD-Zip family consists of four subfamilies and ATHB17 is a member of the class II subfamily. HD-Zip II proteins form either homodimers or heterodimers with other HD-Zip II proteins and function as repressors of gene expression. In MON 87403, maize-specific splicing of the *ATHB17* transcript results in a truncated protein, ATHB17Δ113, which is missing the first 113 N-terminal amino acids that are expressed in Arabidopsis. The ATHB17Δ113 protein retains the ability to form homo- and hetero-dimers and bind to target DNA sequences like the full-length protein. ATHB17Δ113 is, however, unable to function as a transcriptional repressor because the protein lacks a functional repression domain. By a dominant-negative mechanism, ATHB17Δ113 can alter the activity of endogenous maize HD-Zip II proteins, which are predominantly expressed in ear tissue. Thus, the ATHB17Δ113 protein likely modulates HD-Zip II-regulated pathways in the ear, which leads to increased ear growth at an early reproductive stage (R1). This increased ear growth is associated with increased partitioning of dry matter (photosynthate) from the source (vegetative) tissue to the sink (ear) tissue in MON 87403 compared to conventional control plants. Supplemental information on the function of the

ATHB17Δ113 protein in MON 87403 is described in Appendix B: and is also published in Rice et al. (2014).

Maize is one of the largest U.S. crops based on acreage and quantity harvested each year. In 2013 maize was planted on 35.48 million ha in the United States (USDA-FAS 2014). Because of its importance, plant breeders continuously strive to improve commercial maize yield. Initial improvements in maize yield were due to the domestication of varieties with desirable traits like larger ear biomass. Maize ears became larger over time during the domestication era (University of Utah 2014). During the hybrid era (1939 to present), commercial maize yield in the U.S. increased nearly six-fold with an average 99 kg ha^{-1} increase every year (Lee and Tollenaar 2007). The major factor that contributed to yield increase during the hybrid era is a favorable response of hybrids to increased plant population density which resulted in an increase in the number of ears per hectare (Bruns and Abbas 2003). However, at the individual plant level, yield increase was associated with a decrease in ear size. Commercial varieties with MON 87403 can provide increased yield opportunity as this trait increases ear biomass during early reproductive stages.

Because of the expected increase in human population growth over time, increased agricultural output will be required to come from increased productivity (*i.e.*, yield per unit area) as opposed to an increase in area under production (OECD-FAO 2008). Agricultural biotechnology provides tools to help address the increasing demand for food and feed due to this population growth. Augmenting gains in yield opportunity from biotechnology as well as with continual gains from breeding would have a positive impact on the U.S. economy. For example, increasing the average rate of yield gain by just 1 bu/ac/year, over and above historical average yield gains for the next ten years, would have a net economic impact of $16B USD (Leibman et al. 2014).

Early reproductive stages in maize have been identified as a crucial phase during maize growth when a determination of maximum ear size is set based on plant genetics and environmental conditions (Borrás and Westgate 2006; Jones et al. 1996). Dry matter produced by the plant during reproductive stages is allocated to the ear for its growth (Ritchie et al. 1997) after the sink size is determined. Thus increase in ear size that is set during early reproductive stages is considered an important determinant of reproductive success (Borrás et al. 2004; Zaidi et al. 2003). Published literature suggests that a larger ear size at early reproductive stages can result in increased kernels per hectare (Fisher and Palmer 1983; Severini et al. 2011) thus potentially increasing agronomic benefit to farmers.

Efficacy of MON 87403 was demonstrated by directly comparing its R1 (silking stage) ear weight to a conventional control with the same genetic background. Ear weight is the total weight of the primary ear including the husk, shank, cob, silk and ovules at the R1 stage. MON 87403 and the control were grown at 13 field locations within U.S. maize production regions in 2012. MON 87403 and the control were planted at each site in randomized complete block designs with four replications. Measurements were collected from all of the plants at the R1 stage in a 1 m length of the designated row. Statistical comparisons were made between MON 87403 and the control across all 13 sites

(combined-site analyses). The level of statistical significance for all statistical comparisons was predetermined to be 5% (α=0.05).

There was a statistically significant increase in R1 ear weight between MON 87403 and the control in the combined site analysis (Table I-1). The R1 ear weight in MON 87403 was 11.7% higher than the control (Table I-1).

Table I-1. Differences in R1 Ear Weight between MON 87403 and the Conventional Control in 2012 U.S. Field Trials[1]

Characteristic (units)	MON 87403 (Mean ± SE)	Control (Mean ± SE)	Change (%)	p-value
R1 ear weight[2] (g)	144.50 (±8.47)	129.30 (±8.13)	11.7	0.004*

[1] Locations included in the combined-site analysis: Jackson, Arkansas; Vermilion, Illinois; Warren, Illinois; Boone, Indiana; Greene, Iowa; Jefferson, Iowa; Pawnee, Kansas; (2 sites) Polk, Nebraska; York, Nebraska; Perquimans, North Carolina; Berks, Pennsylvania; Lehigh, Pennsylvania.

[2] R1 ear weight is the dry weight of the entire primary ear at silking stage from all plants in the 1 m sampling.

*Denotes statistical difference between MON 87403 and the control (α = 0.05). N = 51.

In summary, MON 87403 provides a potential benefit to growers and the food and feed industry by conferring an increase in yield opportunity for maize.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology (CFR) (USDA-APHIS 1986), the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of plant incorporated protectants (PIPs), the Environmental Protection Agency (EPA). Deregulation of MON 87403 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87403 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions. As MON 87403 does not contain a PIP, no submission will be made to the EPA.

I.C.1. Submission to FDA

MON 87403 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA 1992). In accordance with this policy, Monsanto will be submitting a food/feed safety and nutritional assessment summary document to FDA in the near future.

I.C.2. Submissions to Foreign Government Agencies

Consistent with our commitments to the Excellence Through Stewardship® (ETS) Program[2], Monsanto intends to obtain import approvals from all key maize import markets with functioning regulatory systems prior to commercial release of hybrids containing MON 87403.

® Excellence Through Stewardship is a registered trademark of Excellence Through Stewardship, Washington, DC.

[2] http://www.excellencethroughstewardship.org/.

II. THE BIOLOGY OF MAIZE

The Organisation for Economic Co-operation and Development (OECD) Consensus Document on the biology of maize (OECD 2003) provides key information on:

- general description of maize biology, including taxonomy and morphology and use of maize as a crop plant
- agronomic practices in maize cultivation
- geographic centers of origin
- reproductive biology
- cultivated maize as a volunteer weed
- inter-species/genus introgression into relatives and interactions with other organisms
- a summary of the ecology of maize

Additional information on the biology and uses of maize can also be found on the Australian Government Department of Health and Ageing (Office of the Gene Technology Regulator) web site (OGTR 2008), and in the USDA-ARS GRIN database (USDA-ARS 2013).

To support the evaluation of the plant pest potential of MON 87403 relative to conventional maize, additional information regarding several aspects of maize biology can be found elsewhere in this petition. This includes: agronomic practices for maize in Section VIII; volunteer management of maize in Section VIII.H; and inter-species/genus introgression potential in Section IX.G.

II.A. Maize as a Crop

Maize is grown in nearly all areas of the world and is the largest cultivated crop in the world followed by wheat (*Triticum* sp.) and rice (*Oryza sativa* L.) in total global metric ton production. In 2012, maize was planted globally on ~174 million hectares (ha) with a total grain production of an estimated 854 million metric tons (MMT) (USDA-FAS 2013). The top five production regions in 2012 were: USA (274 MMT), China (208 MMT), Brazil (73 MMT), EU-27 (55 MMT), and Argentina (27 MMT) (USDA-FAS 2013). In the U.S., maize is grown in almost every state and in 2012, its production value of over $77 billion was the highest of any crop (USDA-NASS 2013a).

In industrialized countries maize has two major uses: (1) as animal feed in the form of grain, forage or silage; and (2) as a raw material for wet- or dry-milled processed products such as high fructose maize syrup, oil, starch, glucose, dextrose and ethanol. By-products of the wet- and dry- mill processes are also used as animal feed. These processed products are used as ingredients in many industrial applications and in human food products. Most maize produced in industrialized countries is used as animal feed or for industrial purposes, but maize remains an important food staple in many developing regions, especially sub-Saharan Africa and Central America, where it is frequently the mainstay of human diets (Morris 1998).

Maize is a very familiar plant that has been rigorously studied due to its use as a staple food/feed and the economic opportunity it brings to growers. The domestication of maize likely occurred in southern Mexico between 7,000 and 10,000 years ago (Goodman 1988). While the putative progenitor species of maize have not been recovered, it is likely that teosinte played an important role in contributing to the genetic background of maize. Although grown extensively throughout the world, maize is not considered a persistent weed or a plant that is difficult to control. Maize, as we know it today, cannot survive in the wild because the female inflorescence (the ear) is covered by a husk thereby restricting seed dispersal, it has no seed dormancy, and is a poor competitor in an unmanaged ecosystem. The transformation from a wild, weedy species to one dependent on humans for its survival most likely evolved over a long period of time through plant breeding by the indigenous inhabitants of the Western Hemisphere. Today, virtually all maize varieties grown in the U.S. are hybrids, a production practice that started in the 1930's (Wych 1988). Maize hybrids are developed and used based on the positive yield increases and plant vigor associated with heterosis, also known as hybrid vigor (Duvick 1999).

Conventional plant breeding results in desirable characteristics in a plant through the unique combination of genes already present in the plant. However, there is a limit to genetic diversity with conventional plant breeding. Biotechnology, as an additional tool to conventional breeding, offers access to greater genetic diversity than conventional breeding alone, resulting in expression of highly desirable traits that are profitable to growers.

II.B. Characteristics of the Recipient Plant

The transformation for MON 87403 was conducted with inbred maize line LH244, a patented maize line assigned to Holden's Foundation Seeds LLC in 2001 (U.S. Patent #6,252,148). LH244 is a medium season yellow dent maize line with a Stiff Stalk background that is best adapted to the central regions of the U.S. corn belt. LH244 was initiated from a single cross of LH197 × LH199 followed by a backcross to LH197. The F_2 combination ((LH197 × LH197) × LH199) was then selfed and used in the development of LH244.

Following transformation of immature LH244 embryos, a single transformed plant was selected and self-crossed to increase seed supplies. A homozygous inbred line was developed though further self-crossing and selection and was then used to produce other lines which were used for product testing, safety assessment studies, and commercial hybrid development.

II.C. Maize as a Test System in Product Safety Assessment

Hybrid maize MPA640B (LH244 × LH287) and EXP257 (LH244 × LH295) were used as near isogenic, conventional controls for this submission (hereafter referred to as conventional controls), based on seed availability. As noted, one parent of each of these control maize lines is LH244, the inbred from which MON 87403 is derived, while the other parents (LH287 and LH295) are other maize inbreds. As such, both of these maize

hybrids constitute relevant comparators for MON 87403. In addition, other commercial maize hybrids (hereafter referred to as reference hybrids) were used to establish ranges of natural variability representative of commercial maize hybrids. Reference hybrids used at each field trial location were selected based on their availability and agronomic fit for the respective geographic regions. Both MPA640B and EXP257 were used in molecular characterization studies, while MPA640B was used as the conventional control in compositional analysis and in phenotypic, agronomic and environmental interactions assessments. Where appropriate, reference hybrids were used to establish a range of variability or responses representative of commercial maize in the U.S.

In developing the data to support this petition, appropriate MON 87403 test materials were generated for the molecular characterization (Sections III and IV), protein characterization and expression analysis (Section V), compositional analysis (Section VI), and phenotypic, agronomic and environmental interactions assessment (Section VII). The full molecular characterization studies (NGS/JSA) were conducted with the R_3 generation (Figure IV-4). Initiation of commercial breeding efforts was conducted with the R_4 generation (Figure IV-4). Protein characterization and expression analysis, composition analysis, and phenotypic, agronomic and environmental interactions assessment were conducted with various MON 87403 breeding generations as noted in the Breeding Tree (Figure IV-4).

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 87403 was developed through *Agrobacterium tumefaciens*-mediated transformation of maize immature embryos from line LH244 utilizing plasmid PV-ZMAP5714. This section describes the plasmid vector, the donor genes, and the regulatory elements used in the development of MON 87403. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation and retained through the development of MON 87403. An expression cassette is comprised of sequences to be transcribed and the genetic regulatory elements necessary for the expression of those sequences.

III.A. The Plasmid Vector PV-ZMAP5714

Plasmid vector PV-ZMAP5714 was used for the transformation of maize to produce MON 87403 and its plasmid map is shown in Figure III-1. The elements included in this plasmid vector are described in Table III-1. PV-ZMAP5714 is approximately 11.7 kb in length and contains three cassettes: one T-DNA element, delineated by Left and Right Border regions, contains the *ATHB17* expression cassette, and the plasmid backbone contains the *cp4 epsps* selectable marker cassette and the *aadA* expression cassette. PV-ZMAP5714 employs a tandem T-DNA approach to generate marker-free plants (Huang et al. 2004). In this tandem T-DNA approach, a single right border and a single left border were used to achieve separate, unlinked insertion of the T-DNA as well as the *cp4 epsps* selectable marker gene located in the plasmid backbone. After initial selection of transformants for glyphosate tolerance, the *cp4 epsps* cassette was segregated away by conventional breeding and molecular analysis was used to identify plants containing only the intended T-DNA (and not the *cp4 epsps*).

The T-DNA contains the *ATHB17* expression cassette. The *ATHB17* expression cassette is regulated by the *e35s/Ract1* chimeric promoter from the 35S RNA of cauliflower mosaic virus (CaMV) and the *act1* gene from *Oryza sativa* , the 5´ untranslated leader sequence of the *Cab* gene from *Triticum aestivum*, the *act1* intron from *Oryza sativa*, and the 3´ untranslated region of heat shock protein 17 (*Hsp17*) of *Triticum aestivum*. The plasmid backbone contains the *cp4 epsps* and *aadA* expression cassettes. The *cp4 epsps* expression cassette is regulated by the *act1* promoter from *Oryza sativa*, the *act1* intron from *Oryza sativa*, the *CTP2* targeting sequence from *Arabidopsis thaliana*, and the *nos* 3′ untranslated region from *Agrobacterium tumefaciens*. The *aadA* expression cassette is regulated by the bacterial promoter, and 3' untranslated region of an aminoglycoside-modifying enzyme, 3''(9)-*O*-nucleotidyltransferase from the transposon Tn7. During transformation, both the T-DNA and the *cp4 epsps* expression cassette were inserted into the maize genome (Section III.B). Subsequently, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *ATHB17* expression cassette (T-DNA) and do not contain the *cp4 epsps* and *aadA* expression cassettes (backbone).

The backbone region of PV-ZMAP5714, located outside of the T-DNA, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori-V*, *ori-pUC*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer

(ROP) protein for the maintenance of the plasmid vector copy number in *Escherichia coli* (*E. coli*). The backbone also contains the *cp4 epsps* expression cassette. A description of the genetic elements and their prefixes (e.g., B-, P-, L-, I-, CS-, T-, and OR-) in PV-ZMAP5714 is provided in Table III-1.

III.B. Description of the Transformation System

MON 87403 was developed through *Agrobacterium tumefaciens*-mediated transformation of immature maize embryos based on the method described by Sidorov and Duncan (2009), utilizing PV-ZMAP5714. Immature embryos were excised from a post-pollinated maize ear of LH244. After co-culturing the excised immature embryos with *Agrobacterium* carrying the plasmid vector, the immature embryos were placed on selection medium containing glyphosate and carbenicillin disodium salt in order to inhibit the growth of untransformed plant cells and excess *Agrobacterium*, respectively. Once transformed callus developed, the callus was placed on media conducive to shoot and root development. The rooted plants (R_0) with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment. As demonstrated in this petition (Section VII), the use of disarmed *Agrobacterium tumefaciens* strain ABI, a designated plant pest, as the transformation vector has not imparted plant pest characteristics to MON 87403.

The R_0 plants generated through the transformation process described above had already been exposed to glyphosate in the selection medium and demonstrated glyphosate tolerance. The R_0 plants containing the *cp4 epsps* expression cassette, as well as the T-DNA cassette from the plasmid vector backbone, were self-pollinated to produce R_1 seed and R_1 plants (Huang et al. 2004). Subsequently, R_1 plants that were positive for the T-DNA and negative for the *cp4 epsps* expression cassette were identified by a polymerase chain reaction (PCR) based analysis (Huang et al. 2004). The R_1 plants homozygous for the T-DNA were selected for further development and their progenies were subjected to further molecular and phenotypic assessments. As is typical of a commercial event production and selection process, hundreds of different transformation events (regenerants) were generated in the laboratory using PV-ZMAP5714. After many months of careful selection and evaluation of these hundreds of events in the laboratory, greenhouse and field, MON 87403 was selected as the lead event based on superior agronomic, phenotypic, and molecular characteristics (Prado et al. 2014). Studies on MON 87403 were initiated to further characterize the genetic insertion and the expressed product, and to establish the food, feed, and environmental safety relative to commercial maize. The major steps involved in the development of MON 87403 are depicted in Figure III-2.

III.C. The *ATHB17* Coding Sequence and the ATHB17 Protein (T-DNA)

The *ATHB17* coding sequence is from *Arabidopsis thaliana* and encodes the ATHB17 protein (Figure III-3). For additional information about the truncated ATHB17 protein produced in MON 87403, see Section V.

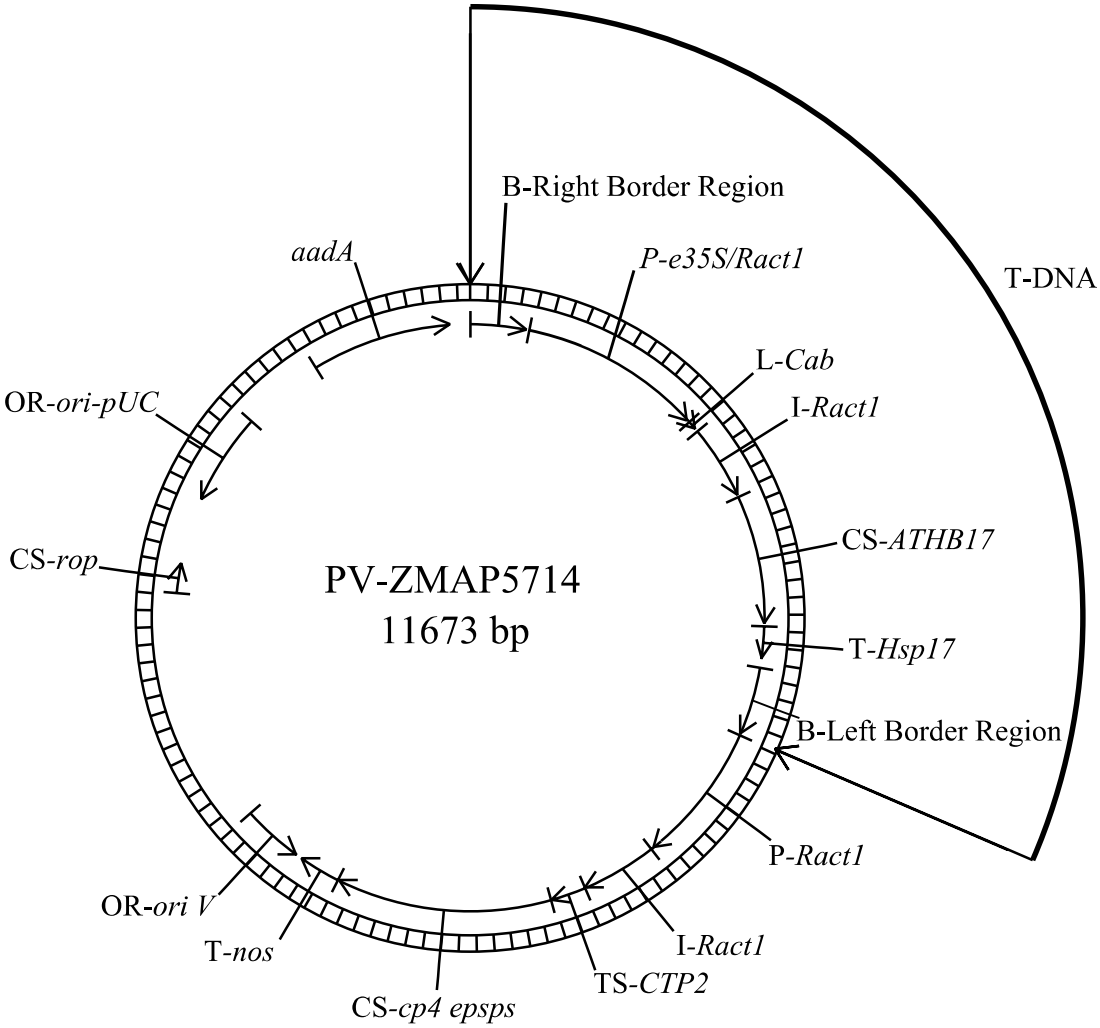

Figure III-1. Circular Map of PV-ZMAP5714
A circular map of PV-ZMAP5714 used to develop MON 87403 is shown. PV-ZMAP5714 contains a single T-DNA. Genetic elements are shown on the exterior of the map.

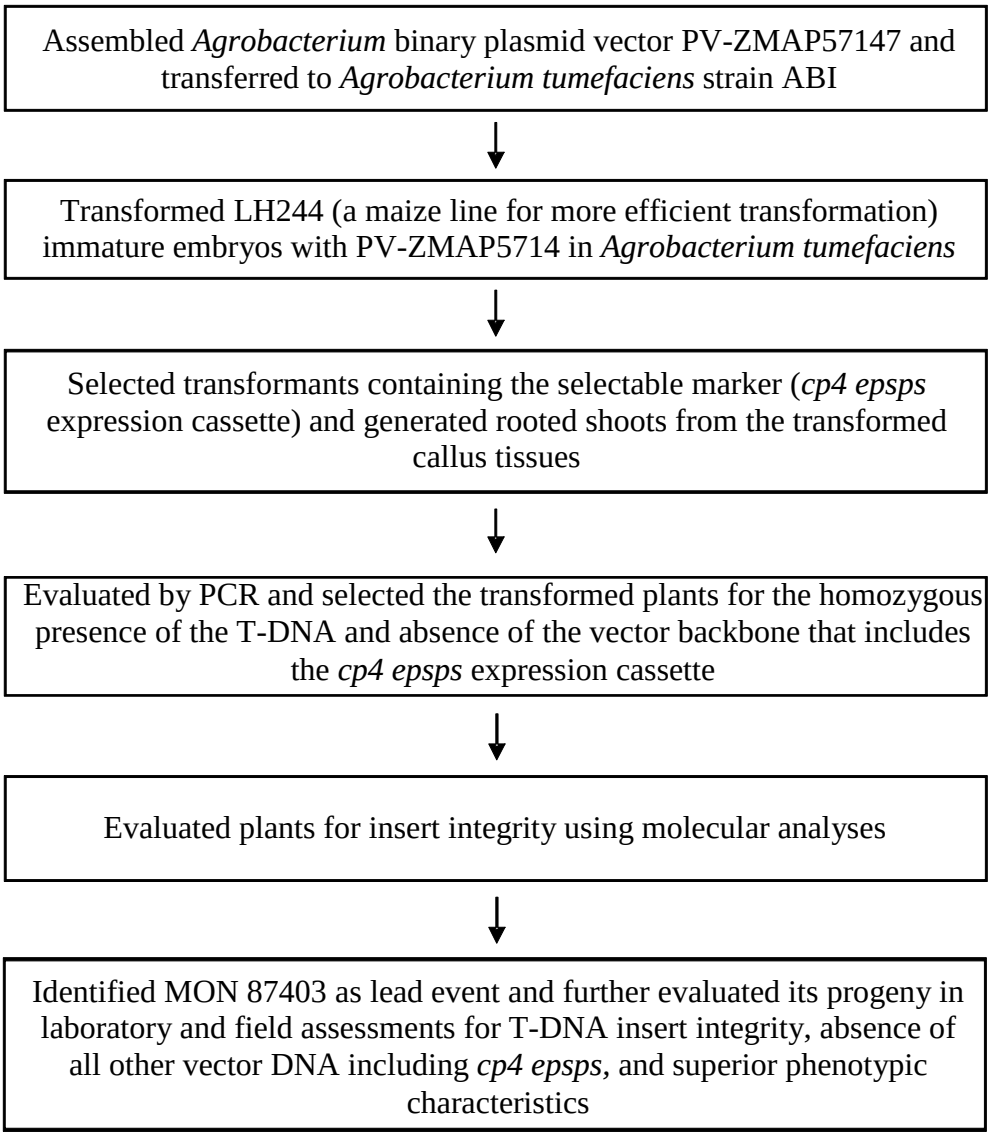

Figure III-2. Schematic of the Development of MON 87403

```
  1 MIKLLFTYIC TYTYKLYALY HMDYACVCMY KYKGIVTLQV CLFYIKLRVF
 51 LSNFTFSSSI LALKNPNNSL IKIMAILPEN SSNLDLTISV PGFSSSPLSD
101 EGSGGGRDQL RLDMNRLPSS EDGDDEEFSH DDGSAPPRKK LRLTREQSRL
151 LEDSFRQNHT LNPKQKEVLA KHLMLRPRQI EVWFQNRRAR SKLKQTEMEC
201 EYLKRWFGSL TEENHRLHRE VEELRAMKVG PTTVNSASSL TMCPRCERVT
251 PAASPSRAVV PVPAKKTFPP QERDR
```

Figure III-3. Deduced Amino Acid Sequence of the ATHB17 Protein
The amino acid sequence of the ATHB17 protein was deduced from the full-length coding nucleotide sequence present in PV-ZMAP5714. The amino-terminal 113 amino acids (highlighted in gray) are not predicted to be translated in MON 87403 as a result of mRNA splicing.

III.D. Regulatory Sequences

The *ATHB17* coding sequence in MON 87403 is under the regulation of the *e35S/Ract1* promoter, the chlorophyll a/b binding protein (CAB) leader, the *Ract1* intron, and the heat shock protein 17 (*Hsp17)* 3' untranslated region. The *e35S/Ract1* promoter, which directs transcription in plant cells, is a chimeric promoter consisting of the duplicated enhancer region from the cauliflower mosaic virus 35S RNA promoter (CaMV) (Kay et al. 1987) combined with the promoter of the *act1* gene from *Oryza sativa* that encodes Actin 1 (McElroy et al. 1990). The *Cab* leader sequence is the 5' untranslated region from the chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* and is involved in regulating gene expression (Lamppa et al. 1985). The *Ract1* intron is the intron from the *act1* gene from *Oryza sativa* (McElroy et al. 1990). The *Hsp17* 3' non-translated region is the 3' untranslated region from the heat shock protein, Hsp17, of *Triticum aestivum* (McElwain and Spiker 1989) that directs polyadenylation of the mRNA.

III.E. T-DNA Borders

PV-ZMAP5714 contains Left and Right Border regions (Figure III-1 and Table III-1) that were derived from *A. tumefaciens* plasmids. The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation (Barker et al. 1983; Depicker et al. 1982; Zambryski et al. 1982). The border regions separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer of T-DNA into the maize genome. As demonstrated in this petition (Section VII), the use of genetic elements from *A. tumefaciens*, a designated plant pest, has not imparted plant pest characteristics to MON 87403.

III.F. Genetic Elements Outside of the T-DNA Borders

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-ZMAP5714 in bacteria and are referred to as plasmid backbone. The origin of replication, *ori V*, is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid *RK2* (Stalker et al. 1981). The origin of replication, *ori-pUC*, is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pUC (Vieira and Messing 1987). Coding sequence *rop* encodes the repressor of primer (ROP) protein which is necessary for the maintenance of plasmid vector copy number in *E. coli* (Giza and Huang 1989). The backbone also contains the *cp4 epsps* expression cassette that codes for the CP4 EPSPS protein (conferring tolerance to glyphosate) that was used as the selectable marker during transformation (Huang et al. 2004). The *cp4 epsps* expression cassette is regulated by the *Ract1* promoter from *Oryza sativa*, the *Ract1* intron from *Oryza sativa*, the *CTP2* targeting sequence from *Arabidopsis thaliana*, and the *nos* 3′ untranslated region from *Agrobacterium tumefaciens*. The absence of the backbone and other unintended plasmid sequence in MON 87403 was confirmed by sequencing and bioinformatic analyses (see Section IV.A).

Table III-1. Summary of Genetic Elements in Plasmid PV-ZMAP5714

Genetic Element	Location in Plasmid	Function (Reference)
T-DNA		
B[1]-Right Border Region	1-357	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982)
Intervening Sequence	358-375	Sequence used in DNA cloning
P[2]-*e35S/Ract1*	376-1556	Chimeric promoter consisting of the duplicated enhancer region from the cauliflower mosaic virus 35S RNA promoter (CaMV) (Kay et al. 1987) combined with the promoter of the *act1* gene from *Oryza sativa* that encodes Actin 1 (McElroy et al. 1990) that directs transcription in plant cells
Intervening Sequence	1557-1561	Sequence used in DNA cloning
L[3]-*Cab*	1562-1622	5' UTR leader sequence from chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression (Lamppa et al. 1985)
Intervening Sequence	1623-1638	Sequence used in DNA cloning
I[4]-*Ract1*	1639-2118	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein. This sequence is involved in regulating gene expression (McElroy et al. 1990)
Intervening Sequence	2119-2130	Sequence used in DNA cloning
CS[5]-*ATHB17*	2131-2958	Coding sequence of the *ATHB17* gene from *Arabidopsis thaliana* encoding a member of the class II homeodomain-leucine zipper gene family (HD-Zip II) that is thought to act as a transcription factor (Ariel et al. 2007)
Intervening Sequence	2959-2971	Sequence used in DNA cloning
T[6]-*Hsp17*	2972-3181	3' UTR sequence from a heat shock protein, Hsp17, of *Triticum aestivum* (wheat) (McElwain and Spiker 1989) that directs polyadenylation of the mRNA
Intervening Sequence	3182-3234	Sequence used in DNA cloning
B-Left Border Region	3235-3676	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)

Table III-1 (continued). Summary of Genetic Elements in Plasmid PV-ZMAP5714

Genetic Element	Location in Plasmid	Function (Reference)
Backbone		
Intervening Sequence	3677-3682	Sequence used in DNA cloning
P-*Ract1*	3683-4603	Promoter and leader of the *act1* gene from *Oryza sativa* (rice) encoding the rice Actin 1 protein (McElroy et al. 1990) that directs transcription in plant cells
I-*Ract1*	4604-5081	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein (McElroy et al. 1990). This sequence is involved in regulating gene expression
Intervening Sequence	5082-5090	Sequence used in DNA cloning
TS[7]-*CTP2*	5091-5318	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann 1995; Klee et al. 1987)
CS-*cp4 epsps*	5319-6686	Coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance (Barry et al. 2001); (Padgette et al. 1996)
Intervening Sequence	6687-6701	Sequence used in DNA cloning
T-*nos*	6702-6954	3' UTR sequence of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS (Bevan et al. 1983; Fraley et al. 1983), that directs polyadenylation of the mRNA
Intervening Sequence	6955-7005	Sequence used in DNA cloning
OR[8]-*ori V*	7006-7402	Origin of replication from the broad host range plasmid *RK2*, used for maintenance of plasmid in *Agrobacterium* (Stalker et al. 1981)
Intervening Sequence	7403-8910	Sequence used in DNA cloning
CS-*rop*	8911-9102	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang 1989)
Intervening Sequence	9103-9529	Sequence used in DNA cloning
OR-*ori-pUC*	9530-10118	Origin of replication from plasmid pUC for maintenance of plasmid in *E. coli* (Vieira and Messing 1987)

Table III-1 (continued). Summary of Genetic Elements in Plasmid PV-ZMAP5714

Genetic Element	Location in Plasmid	Function (Reference)
Intervening Sequence	10119-10648	Sequence used in DNA cloning
aadA	10649-11537	Bacterial promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase from the transposon Tn7 (Fling et al. 1985). This sequence confers spectinomycin and streptomycin resistance
Intervening Sequence	11538-11673	Sequence used in DNA cloning

[1] B, Border
[2] P, Promoter
[3] L, Leader
[4] I, Intron
[5] CS, Coding Sequence
[6] T, Transcription Termination Sequence
[7] TS, Targeting Sequence
[8] OR, Origin of Replication

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

Characterization of the DNA insert in MON 87403 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87403 contains one copy of the intended transfer DNA (T-DNA) containing the *ATHB17* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions are based on several lines of evidence:

1. Molecular characterization of MON 87403 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87403 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87403 to determine the presence and identity of sequences derived from PV-ZMAP5714 (DuBose et al. 2013; Kovalic et al. 2012), demonstrating that MON 87403 contained a single T-DNA insert with no detectable backbone sequences.

2. Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87403 was used to determine the complete sequence of the single DNA insert from PV-ZMAP5714, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the inserted DNA is identical to the corresponding region in the PV-ZMAP5714 T-DNA. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87403 to the sequence of the insertion site in conventional maize. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 87403 upon DNA integration.

3. Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMAP5714 T-DNA insert in MON 87403 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87403.

4. Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the nature of the T-DNA as a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 87403 demonstrates that a single copy of the intended T-DNA was stably integrated at a single locus of the maize genome and that no plasmid backbone sequences are present in MON 87403.

A schematic representation of the NGS/JSA methodology and the basis of the characterization using NGS/JSA and PCR sequencing are illustrated in Figure IV-1 below. Appendix C provides an additional overview of these techniques, their use in DNA characterization in crop plants and the materials and methods.

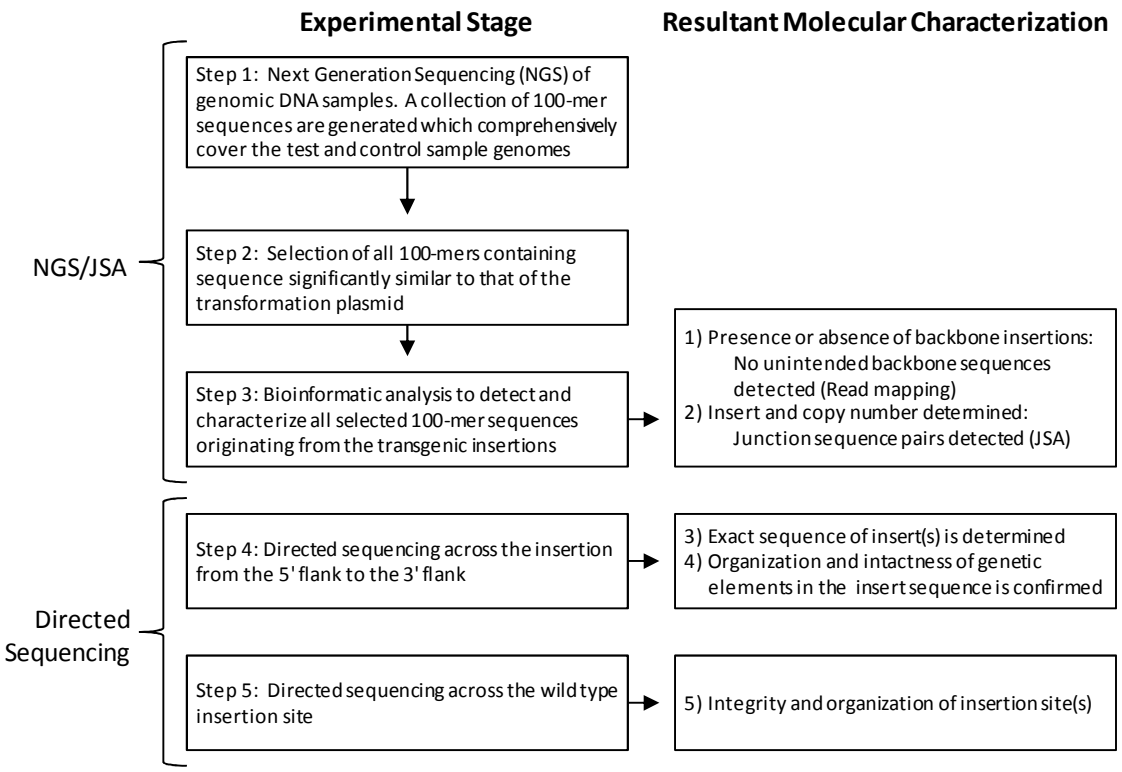

Figure IV-1. Molecular Characterization using Sequencing and Bioinformatics
Genomic DNA from the test and the conventional control was sequenced using technology that produces a set of short, randomly distributed sequence reads (each approximately 100 bp long) that comprehensively cover both genomes (Step 1). Utilizing these genomic sequence reads, bioinformatics search tools were used to select all sequence reads that were significantly similar to the transformation plasmid (Step 2) for use in read mapping to determine the presence/absence of backbone sequences and Junction Sequence Analysis (JSA) bioinformatics to determine the insert and copy number (Step 3). Overlapping PCR products are also produced which span any inserts and their wild type locus (Step 4 and Step 5 respectively); these overlapping PCR products are sequenced to allow for detailed characterization of the inserted DNA and insertion site(s).

The NGS/JSA method characterized the genomic DNA from MON 87403 and the conventional control using short (~100 bp) randomly distributed sequence fragments (sequencing reads) generated in sufficient number to ensure comprehensive coverage of the sample genomes. It has previously been demonstrated that 75× coverage of the soybean genome is adequate to provide comprehensive coverage and ensure detection of inserted DNA (Kovalic et al. 2012) and similarly 75× coverage provides comprehensive coverage of the maize genome (Clarke and Carbon 1976). To confirm sufficient sequence coverage of the genome, the 100-mer sequence reads are analyzed to determine the coverage of a known single-copy endogenous gene, this demonstrates the depth of coverage (the median number of times each base of the genome is independently sequenced). The level of sensitivity of this method was demonstrated by detection of a positive control spiked at 1 and $1/10^{th}$ copy-per-genome equivalent, thus confirming the

method's ability to detect any sequences derived from the transformation plasmid. Bioinformatics analysis was then used to select sequencing reads that contained sequences similar to the transformation plasmid, and these were analysed in depth to determine the number and the identity of sequence in the DNA inserts. NGS/JSA was run on all 5 generations of MON 87403 samples and the conventional controls. Results of NGS/JSA are shown in Sections IV.A and IV.D.

The number of DNA inserts was determined by analyzing sequence reads for novel junctions, while the identity of the inserted DNA and absence of backbone was assessed by sequence read mapping. The junctions of the DNA insert and the flanking DNA are unique for each insertion (Kovalic et al. 2012). An example is shown in Figure IV-2. Therefore, insertion sites can be recognized by analyzing for sequence reads containing such junctions.

Each insertion will produce two unique junction sequence classes characteristic of the genomic locus, one at the 5' end of the insert (Figure IV-3, named junction sequence class A, or JSC-A, in this case) and one at the 3' end of the insert (junction sequence class B, JSC-B) (Kovalic et al. 2012).

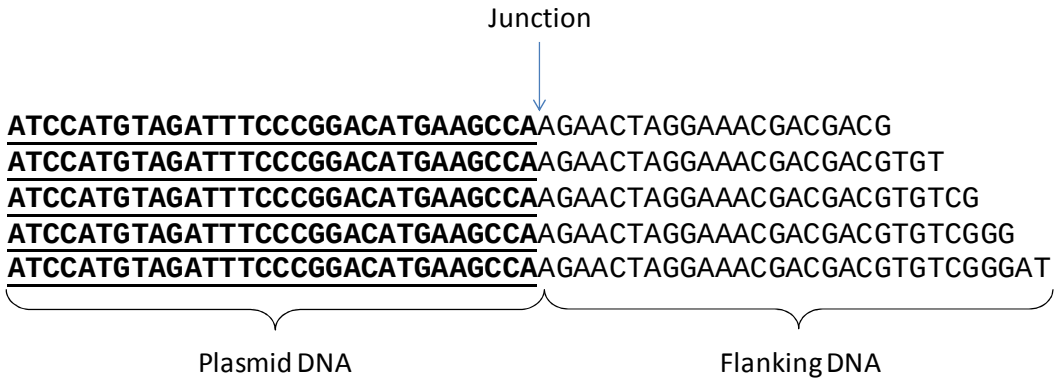

Figure IV-2. Junctions and Junction Sequences
Depicted above are five example junction sequences formatted and labeled to indicate the plasmid/flanking DNA portions of the sequences and with the junction point indicated (plasmid DNA is shown in bold, underlined text and flank DNA is shown in plain text). Junctions are detected by examining the NGS data for sequences having portions of plasmid sequences that span less than the full read. A group of junction sequences which share the same junction point and common flanking sequence is called a Junction Sequence Class (or JSC).

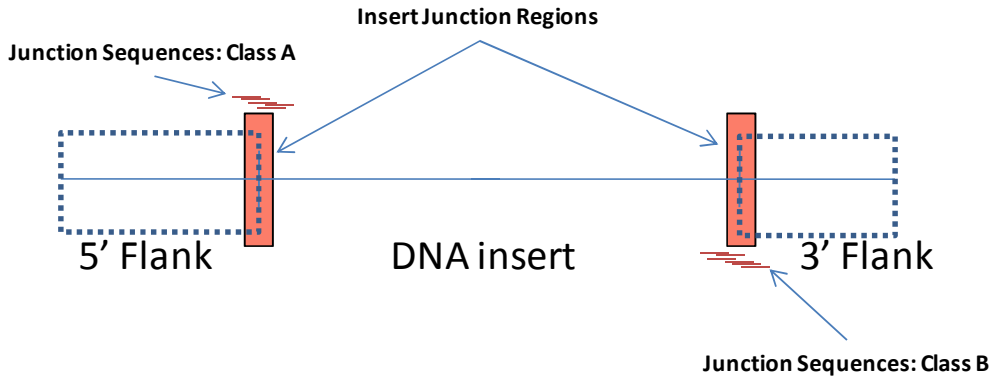

Figure IV-3. Two Unique Junction Sequence Classes are Produced by the Insertion of a Single Plasmid Region
A schematic representation of a single DNA insertion within the genome showing the inserted DNA, the 5' and 3' flanks (depicted as areas bounded by dotted lines), and the two distinct regions spanning the junctions between inserted DNA and flanking DNA (shaded boxes). The group of ~100-mer sequences in which each read contains sequences from both the DNA insert and the adjacent flanking DNA at a given junction is called a Junction Sequence Class. In this example, two distinct junction sequence classes (in this case: Class A at the 5' end and Class B at the 3' end) are represented.

By evaluating the number of unique junction classes detected, the number of insertion sites of the plasmid sequence can be determined. For a single insert, two junction sequence classes are expected, each originating from one end of the insert, both containing portions of plasmid DNA insert and flanking sequence.

Directed sequencing (locus-specific PCR and DNA sequencing analyses, Figure IV-1, step 5) complements the NGS/JSA analyses. Sequencing of the insert and flanking genomic DNA determined the complete sequence of the insert and flanks. This analysis evaluates if the sequence of the insert is identical to the corresponding sequence from the T-DNA in PV-ZMAP5714, if each genetic element in the insert is intact, if the T-DNA sequence is inserted as a single copy, and further confirms that no vector backbone or other unintended plasmid sequences were present within the T-DNA insert in MON 87403. Results are described in Sections IV.B and IV.C; methods are presented in Appendix C: .

The stability of the T-DNA present in MON 87403 across multiple generations was evaluated by NGS/JSA analyses as described above. This information was used to determine the number and identity of insertion sites. For a single insert, two junction sequence classes are expected; each one originates from either end of the insert, both containing portions of DNA insert and flanking sequence. All integrated sequences are expected to align to the T-DNA region of the plasmid. Results are described in Section IV.D.; methods are presented in Appendix C: .

Segregation analysis of the T-DNA was conducted to determine the inheritance and stability of the insert in MON 87403. Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the genetic behavior of the T-DNA. Results and methods are described in Section IV.E.

IV.A. Determining the Number and Identity of DNA Inserts in MON 87403

The number of insertion sites of PV-ZMAP5714 DNA in MON 87403 was assessed by performing NGS/JSA on MON 87403 genomic DNA. A plasmid map of PV-ZMAP5714 is shown in Figure III-1. Table IV-1 provides descriptions of the genetic elements present in MON 87403. A schematic representation of the insert and flanking sequences in MON 87403 is shown in Figure IV-4. For full details on materials and methods see Appendix C.

IV.A.1. Next Generation Sequencing of MON 87403 and Conventional Control Genomic DNA

Genomic DNA from five generations of MON 87403 and the appropriate conventional control (Figure IV-5) was isolated from seed and prepared for sequencing according to the manufacturer's protocol (Illumina, TruSeq library protocol. For material and method details see Appendix C:). These genomic DNA libraries were used to generate short (~100 bp) randomly distributed sequence fragments (sequencing reads) of the maize genome (see Figure IV-1, Step 1).

To demonstrate sufficient sequence coverage the 100-mer sequence reads were analyzed by mapping all reads to a known single-copy endogenous gene (*Zea mays* pyruvate decarboxylase (*pdc3*), GenBank accession version: AF370006.2). The analysis of sequence coverage plots showed that the depth of coverage (*i.e.*, the median number of times any base of the genome is expected to be independently sequenced) was 75× or greater for the five generations of MON 87403 (R_3, R_4, R_5, R_4F_1 and R_5F_1) and the conventional control. It has previously been demonstrated that 75× coverage of the soybean genome is adequate to provide comprehensive coverage and ensure detection of inserted DNA (Kovalic et al. 2012) and similarly 75× coverage provides comprehensive coverage of the maize genome (Clarke and Carbon 1976).

To demonstrate the method's ability to detect any sequences derived from the PV-ZMAP5714 transformation plasmid, a sample of conventional control DNA spiked with PV-ZMAP5714 DNA at 1 and $1/10^{th}$ genome equivalent was analyzed by NGS and bioinformatics. The level of sensitivity of this method was demonstrated to a level of 1 genome equivalent, 100% nucleotide identity was observed over 100% of PV-ZMAP5714 (Table C-2). This result demonstrates that all nucleotides of the transformation plasmid are observed by the sequencing and bioinformatic assessments performed. Also, observed coverage was adequate (Clarke and Carbon 1976) at a level $1/10^{th}$ genomic equivalent (99.97% coverage at 98.83% identity for the $1/10^{th}$ genome equivalent spiked control sample, Table C-2) and, hence, a detection level of at most $1/10^{th}$ genome equivalent was achieved for the plasmid DNA sequence assessment.

IV.A.2. Characterization of insert number in MON 87403 using Bioinformatic Analysis

The number of insertion sites of DNA from PV-ZMAP5714 in MON 87403 was assessed by performing NGS/JSA on MON 87403 genomic DNA using the R_3 generation (Figure IV-5).

Table IV-1. Summary of Genetic Elements in MON 87403

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
5' Flanking DNA	1-1345	DNA sequence flanking the 5' end of the insert
B[3]-Right Border Region[r1]	1346-1369	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982)
Intervening Sequence	1370-1387	Sequence used in DNA cloning
P[4]-*e35S/Ract1*	1388-2568	Chimeric promoter consisting of the duplicated enhancer region from the cauliflower mosaic virus 35S RNA promoter (CaMV) (Kay et al. 1987) combined with the promoter of the *act1* gene from *Oryza sativa* that encodes Actin 1 (McElroy et al. 1990) that directs transcription in plant cells
Intervening Sequence	2569-2573	Sequence used in DNA cloning
L[5]-*Cab*	2574-2634	5' UTR leader sequence from chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression (Lamppa et al. 1985)
Intervening Sequence	2635-2650	Sequence used in DNA cloning
I[6]-*Ract1*	2651-3130	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding Actin 1 protein. This sequence is involved in regulating gene expression (McElroy et al. 1990)

Table IV-1 (continued). Summary of Genetic Elements in MON 87403

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
Intervening Sequence	3131-3142	Sequence used in DNA cloning
CS[7]-*ATHB17*	3143-3970	Coding sequence of the *ATHB17* gene from *Arabidopsis thaliana* encoding a member of the class II homeodomain-leucine zipper gene family (HD-Zip II) that is thought to act as a transcription factor (Ariel et al. 2007)
Intervening Sequence	3971-3983	Sequence used in DNA cloning
T[8]-*Hsp17*	3984-4193	3' UTR sequence from a heat shock protein, Hsp17, of *Triticum aestivum* (wheat) (McElwain and Spiker 1989) that directs polyadenylation of the mRNA
Intervening Sequence	4194-4246	Sequence used in DNA cloning
B-Left Border Region[r1]	4247-4477	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)
3' Flanking DNA	4478-5744	DNA sequence flanking the 3' end of the insert

[1]Although flanking sequences and intervening sequences are not functional genetic elements; they comprise a portion of the sequence.
[2]Numbering refers to the sequence of the insert in MON 87403 and adjacent DNA.
[3]B, Border
[4]P, Promoter
[5]L, Leader Sequence
[6]I, Intron Sequence
[7]CS, Coding Sequence
[8]T, Transcriptional Terminator
[r1]Superscript in Left and Right Border Regions indicate that the sequence in MON 87403 was truncated compared to the sequences in PV-ZMAP5714.

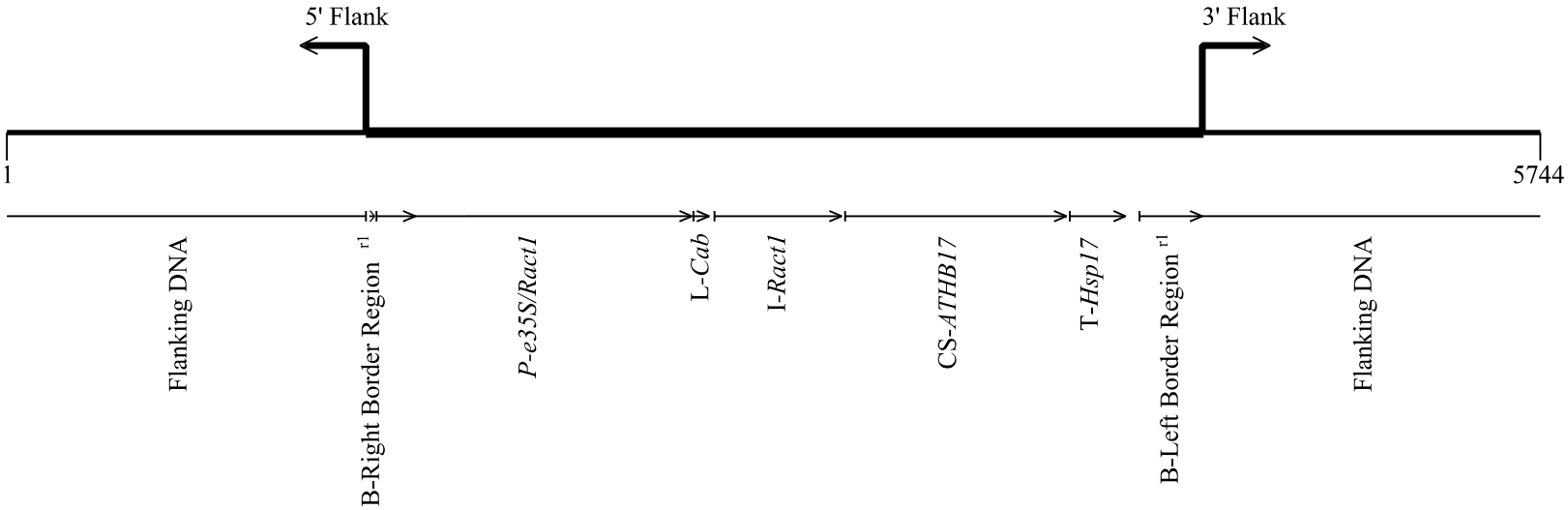

Figure IV-4. Schematic Representation of the Insert and Flanking Sequences in MON 87403
DNA derived from T-DNA of PV-ZMAP5714 integrated in MON 87403. Right-angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking sequence. Genetic elements within the insert are identified on the map. This schematic diagram is drawn to scale, the exact coordinates of every element is shown in Table IV-1.
[r1]Superscript in Left and Right Border Regions indicate that the sequence in MON 87403 was truncated compared to the sequences in PV-ZMAP5714.

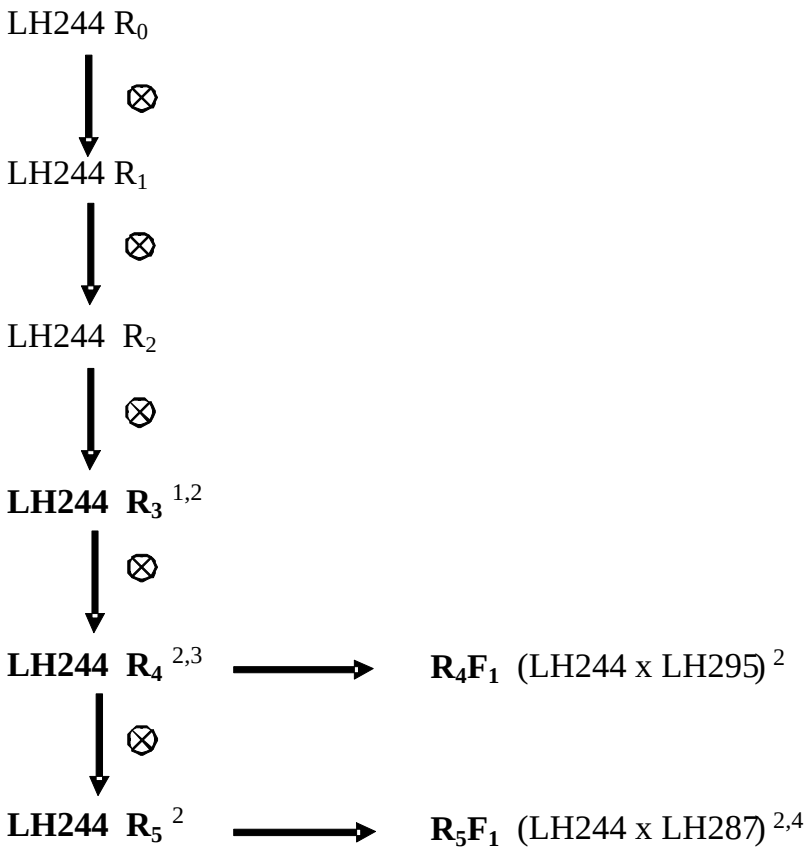

Figure IV-5. Breeding History of MON 87403
R_0 corresponds to the transformed plant, F# is the filial generation, ⊗ designates self-pollination.
[1]Generation used for molecular characterization
[2] Generations used to confirm insert stability
[3] Generation used for commercial development of MON 87403
[4] Generation used for agronomic/phenotypic and compositional analysis studies

IV.A.2.1. Selection of Sequence Reads Containing Sequence of the PV-ZMAP5714

PV-ZMAP5714 was transformed into the parental variety LH244 to produce MON 87403. Consequently, any DNA inserted into MON 87403 will consist of sequences that are similar to the PV-ZMAP5714 DNA sequence. Therefore, to fully characterize the DNA from PV-ZMAP5714 inserted in MON 87403, it is sufficient to completely analyze only the sequence reads that have similarity to the transformation plasmid (Figure IV-1, Step 2).

Using established criteria (described in the materials and methods, Appendix C:), sequence reads similar to the transformation plasmid were selected from MON 87403 and the conventional control sequence datasets and were then used as input data for bioinformatic junction sequence analysis.

IV.A.2.2. Determination of the Insert Number

The NGS/JSA method described above used the entire plasmid sequence as a query to determine the DNA insertion site number. Any inserted transformation plasmid sequence, regardless of origin, either T-DNA or backbone, can be identified by mapping reads to the transformation plasmid sequence while the number of inserted DNA molecules can be determined using JSA. Therefore unlike the traditional Southern blot analysis that separately hybridizes T-DNA or backbone probes, in NGS/JSA the determination of the T-DNA insert number and of the absence of backbone or unintended sequences are simply represented by the identification of sequence reads that match the transformation plasmid, the determination of the overall insert number in the genome followed by determination of the exact identity of any DNA insert using directed sequencing and sequence analysis.

By evaluating the number of unique junction classes, the number of DNA insertion sites can be determined (Figure IV-1, Step 3). If MON 87403 contains a single T-DNA insert, two junction sequence classes (JSCs), each containing portions of T-DNA sequence and flanking sequence, will be detected.

To determine the insert number in MON 87403, the selected sequence reads described above were analyzed using JSA (Kovalic et al. 2012). JSA uses bioinformatic analysis to find and classify partially matched reads characteristic of the ends of insertions. The number of resultant unique JSCs were determined by this analysis and are shown in Table IV-2.

Table IV-2. Unique Junction Sequence Class Results

Sample	Junction Sequence Classes Detected
MON 87403	2
LH244	0

The location and orientation of the junction sequences relative to the T-DNA insert determined for MON 87403 (as described in Section IV.B) are illustrated in Figure IV-6. As shown in the figure, there are two junction sequence classes identified in MON 87403. Junction Sequence Class A and Class B (JSC-A and JSC-B) both contain the T-DNA border sequence joined to genomic flanking sequence, indicating that they represent the sequences at the junctions of the intended T-DNA insert and genomic flanking sequence.

The presence of two, and only two, junction sequence classes (joining T-DNA border and flanking sequences) indicate this single pair of JSCs likely arises from the insertion of the intended PV-ZMAP5714 T-DNA at a single locus in the genome of MON 87403. JSC-A represents the junction of the T-DNA Left Border sequence to the 5' flank and JSC-B represents the junction of the T-DNA Right Border sequence to the 3' flank. Complete alignment of the JSCs to the full flank/insert sequence confirms that both of these JSCs originate from the same locus of the MON 87403 genome and are linked by contiguous, known and expected DNA that makes up the single insert.

IV.A.2.3. Determination of the Inserted DNA Identity

To determine the identity of inserted DNA, all selected sequences decribed in section IV.A.2.1 were mapped to the transformation plasmid sequence. While thousands of sequence reads from the R_3 generation mapped to the plasmid T-DNA sequence, only four reads mapped to the plasmid backbone. From this result it was determined that MON 87403 does not contain any sequence from the transformation plasmid backbone.

Based on the comprehensive NGS/JSA study, it was concluded that MON 87403 contains one T-DNA inserted into a single locus, as shown in Figure IV-6 and is devoid of backbone sequence. The identity of the DNA insert was confirmed by the sequencing and analysis of overlapping PCR products from this locus as described below in Section IV.B., which showed that the DNA insert contained only the T-DNA elements from the plasmid.

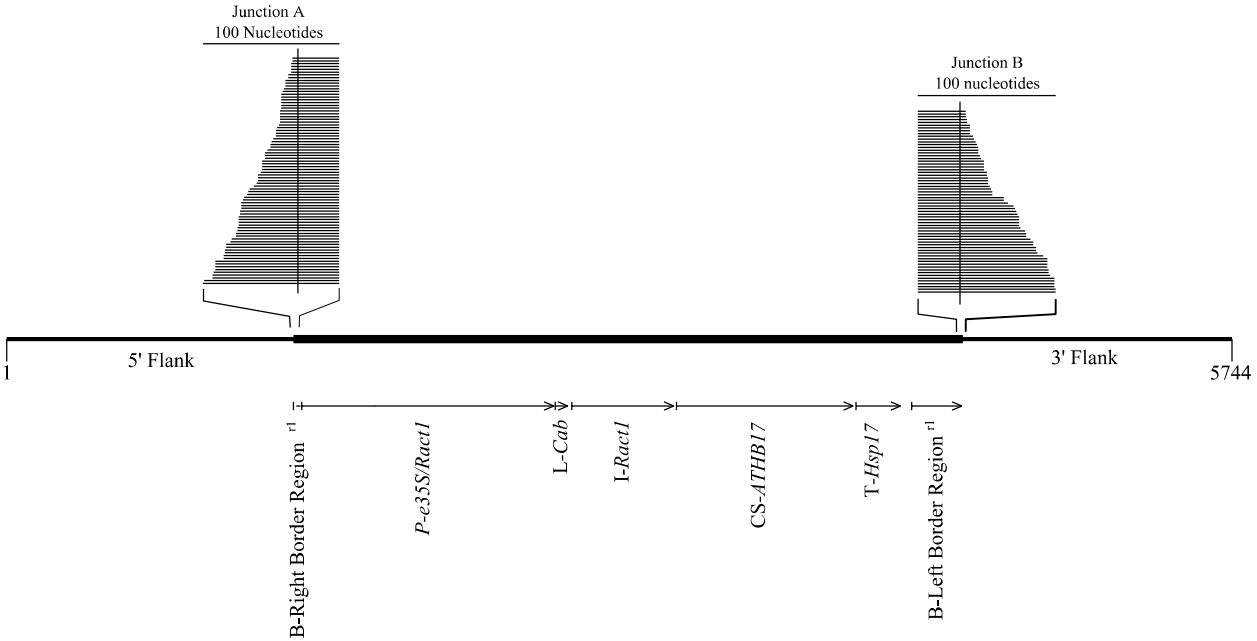

Figure IV-6. Junction Sequences Detected by NGS/JSA

Linear map of MON 87403 illustrating the relationship of the detected junction sequences to the insert locus. The individual junction sequences detected by JSA are illustrated as stacked bars.

[r1]Superscript in Left and Right Border Regions indicate that the sequence in MON 87403 was truncated compared to the sequences in PV-ZMAP5714.

IV.B. Organization and Sequence of the Insert and Adjacent DNA in MON 87403

The organization of the elements within the DNA insert and the adjacent genomic DNA was assessed using directed DNA sequence analysis (refer to Figure IV-1, Step 5). PCR primers were designed to amplify three overlapping regions of the MON 87403 genomic DNA that span the entire length of the insert (Figure IV-7). The amplified PCR products were subjected to DNA sequencing analyses. The results of this analysis confirm that the MON 87403 insert is 3,132 bp and that each genetic element within the T-DNA is intact compared to the transformation plasmid PV-ZMAP5714, with the exception of the border regions. The border regions both contain small terminal deletions with the remainder of the inserted border regions being identical to the sequence in PV-ZMAP5714. The sequence and organization of the insert was also shown to be identical to the corresponding T-DNA of PV-ZMAP5714, confirming that a single copy of the T-DNA was inserted as intended. This analysis also shows that only T-DNA elements (described in Table IV-1) were present. Moreover, the result, together with the conclusion of single DNA insert detected by NGS/JSA, demonstrated that no PV-ZMAP5714 backbone elements are present in MON 87403.

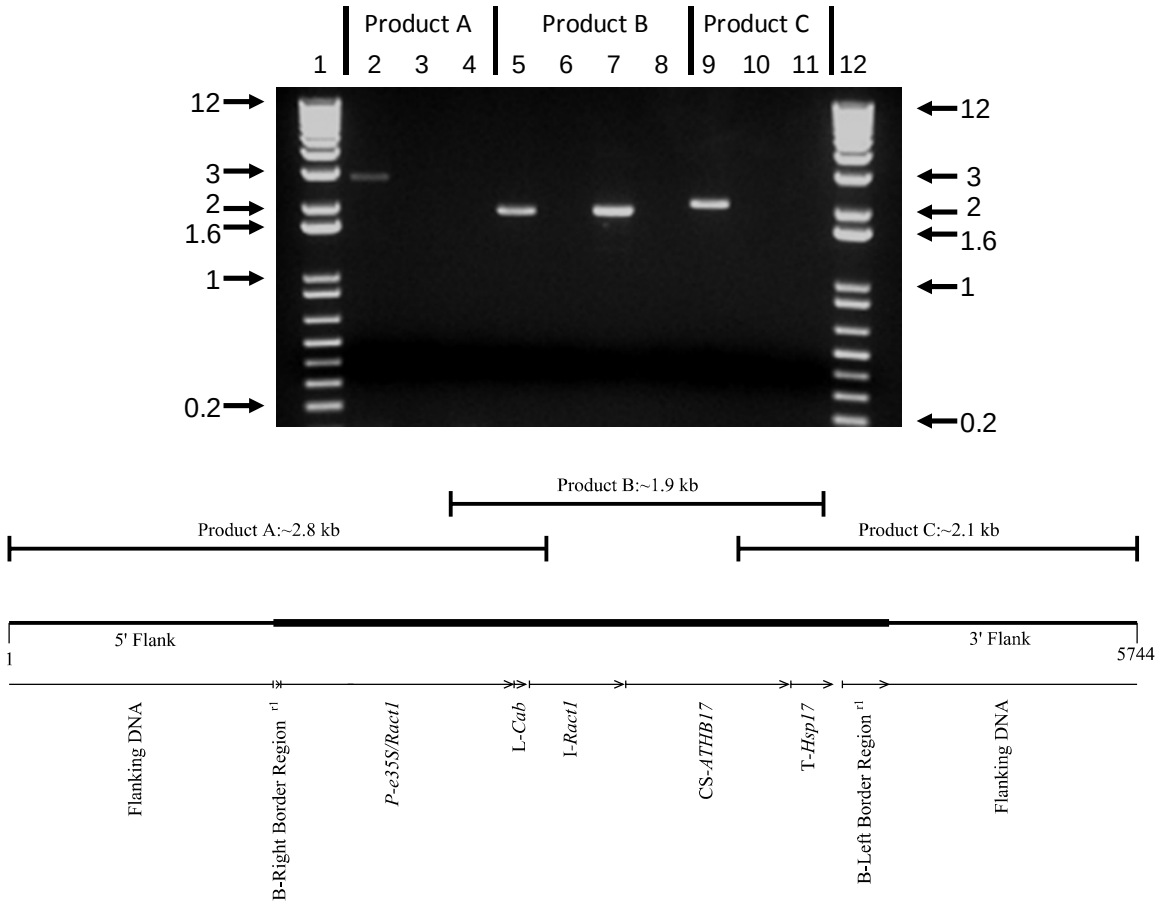

Figure IV-7. Overlapping PCR Analysis Across the Insert in MON 87403

PCR was performed on both conventional control genomic DNA and MON 87403 genomic DNA using three pairs of primers to generate overlapping PCR fragments from MON 87403 for sequencing analysis. To verify the PCR products, a portion of each PCR was loaded on a 1% (w/v) agarose gel and visualized by ethidium bromide staining. The expected product size for each amplicon is provided in the illustration. Lane designations are as follows:

Lane		**Lane**	
1	1 Kb Plus DNA Ladder	7	PV-ZMAP5714
2	MON 87403	8	Conventional Control
3	No Template Control	9	MON 87403
4	Conventional Control	10	No Template Control
5	MON 87403	11	Conventional Control
6	No Template Control	12	1 Kb Plus DNA Ladder
Arrows next to the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1 Kb DNA Plus Ladder (Invitrogen, Grand Island, NY) on the ethidium bromide stained gel.			

[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87403 was truncated compared to the sequences in PV-ZMAP5714.

IV.C. Sequencing of the MON 87403 Insertion Site

PCR and sequence analysis were performed on genomic DNA extracted from the conventional control to examine the insertion site in conventional maize (refer to Figure IV-1, Step 6). The PCR was performed with one primer specific to the genomic DNA sequence flanking the 5' end of the MON 87403 insert paired with a second primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-8). A sequence comparison between the PCR product generated from the conventional control and the sequence generated from the 5' and 3' flanking sequences of MON 87403 indicates that 149 bases of maize genomic DNA were deleted during integration of the T-DNA. The remainder of the flanks in MON 87403 are identical to the conventional control. Such changes are common during plant transformation and these changes presumably resulted from double-stranded break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process (Salomon and Puchta 1998).

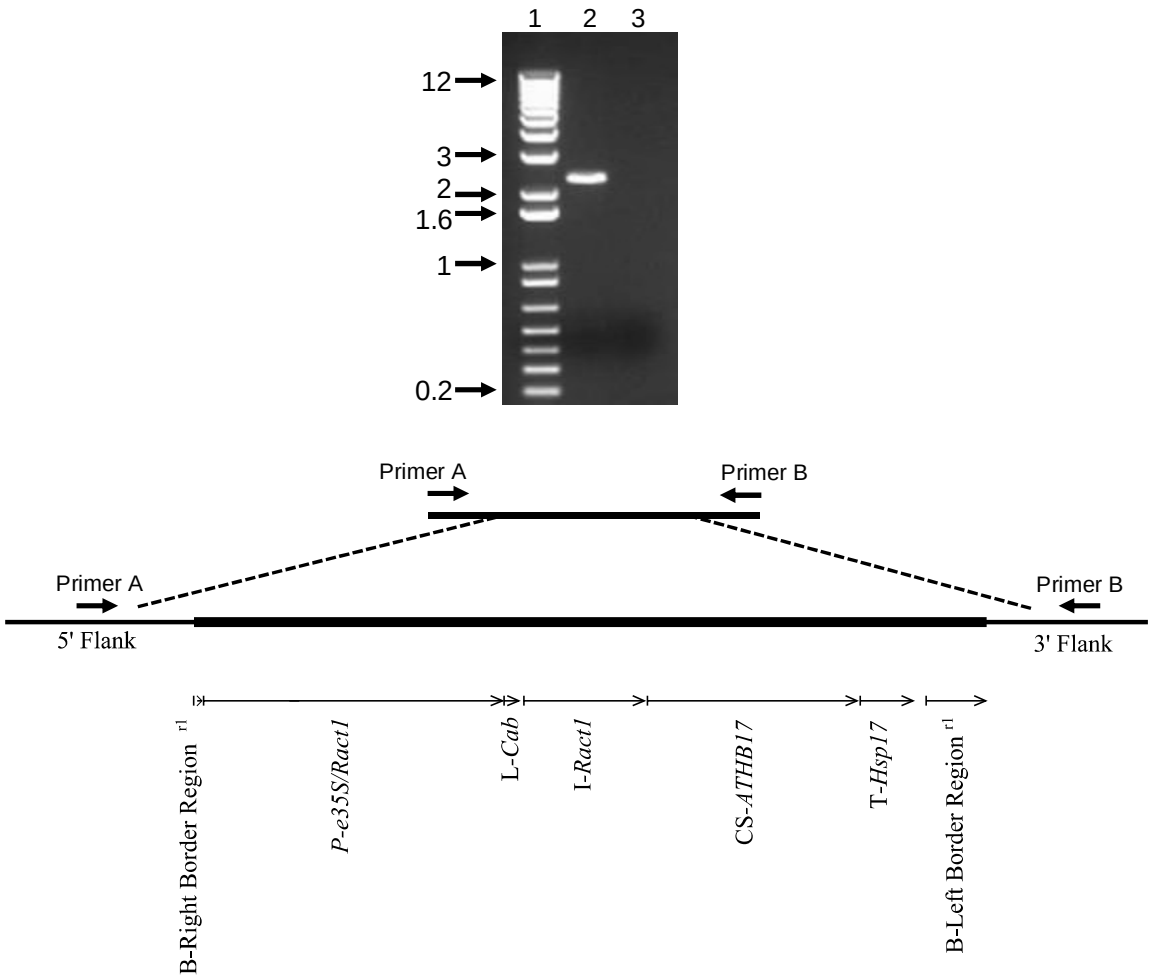

Figure IV-8. PCR Amplification of the MON 87403 Insertion Site

PCR analysis was performed to evaluate the insertion site. PCR was performed on conventional control DNA using Primer A, specific to the 5' flanking sequence, and Primer B, specific to the 3′ flanking sequence of the insert in MON 87403. The DNA generated from the conventional control PCR was used for sequencing analysis. This illustration depicts the MON 87403 insertion site in the conventional control (upper panel) and the MON 87403 insert (lower panel). To verify the PCR products, a portion of each PCR was loaded on the gel. Lane designations are as follows:

Lane	
1	1 Kb Plus DNA Ladder
2	Conventional Control
3	No template DNA control

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1 kb DNA Plus Ladder (Invitrogen, Grand Island, NY) on the ethidium bromide stained gel.

[r1]Superscript in Left and Right Border Regions indicate that the sequence in MON 87403 was truncated compared to the sequences in PV-ZMAP5714.

IV.D. Determination of Insert Stability over Multiple Generations of MON 87403

In order to demonstrate the stability of the T-DNA present in MON 87403 through multiple generations, NGS/JSA analysis was performed using DNA obtained from five breeding generations of MON 87403. The breeding history of MON 87403 is presented in Figure IV-5, and the specific generations tested are indicated in the figure legend. The MON 87403 R_3 generation was used for the molecular characterization analyses discussed in Sections IV.A-IV.C. and shown in Figure IV-5. To assess stability, four additional generations were evaluated by NGS/JSA analysis as previously described in Section IV.A, and compared to the fully characterized R_3 generation. The conventional controls used for the generational stability analysis included LH244, which included similar background genetics to the R_3, R_4 and the R_5 generations and represents the original transformation line; LH244 × LH295, a hybrid with similar background genetics to the R_4F_1 hybrid; and LH244 × LH287, a hybrid with similar background genetics to the R_5F_1 hybrid. Genomic DNA isolated from each of the selected generations of MON 87403 and conventional control was used for NGS/JSA analysis.

To determine the insert number in the MON 87403 generations, the sequences selected as described in Section IV.A.2.1 were analyzed using JSA (Kovalic et al. 2012). Table IV-3 shows the number of resultant JSCs containing PV-ZMAP5714 DNA sequence determined by this analysis.

Table IV-3. Junction Sequence Classes Detected

Sample	Junction Sequence Classes Detected
MON 87403 (R_3)	2
MON 87403 (R_4)	2
MON 87403 (R_4F_1)	2
MON 87403 (R_5)	2
MON 87403 (R_5F_1)	2
LH244	0
LH244 × LH295	0
LH244 × LH287	0

Alignment of the JSCs from each of the assessed MON 87403 generations (R_4, R_5, R_4F_1, and R_5F_1) to the full flank/insert sequence and JSCs determined for the MON 87403 R_3 generation, confirms that the pair of JSCs originates from the same region of the MON 87403 genome and is linked by contiguous, known and expected DNA sequence. This single identical pair of JSCs is observed as a result of the insertion of PV-ZMAP5714 T-DNA at a single locus in the genome of MON 87403. The consistency of these JSC data across all generations tested demonstrates that this single locus was stably maintained throughout the MON 87403 breeding process.

These results demonstrate that the MON 87403 single integration locus was maintained through several generations of breeding MON 87403; thereby confirming the stability of the insert.

Based on this comprehensive sequence data and bioinformatic analysis (NGS/JSA), it is concluded that MON 87403 contains a single and stable T-DNA insertion.

IV.E. Inheritance of the Genetic Insert in MON 87403

The MON 87403 T-DNA resides at a single locus within the maize genome and therefore should be inherited according to Mendelian principles of inheritance. During development of lines containing MON 87403, genotypic segregation data were recorded to assess the inheritance and stability of the MON 87403 T-DNA using Chi-square (χ^2) analysis over several generations. The χ^2 analysis is based on comparing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87403 breeding path for generating segregation data is described in Figure IV-9. The transformed R_0 plant was self-pollinated to generate R_1 seed. An individual plant homozygous for the MON 87403 T-DNA was identified in the R_1 segregating population via an End-Point TaqMan® PCR assay.

The homozygous positive R_1 plant was self-pollinated to give rise to R_2 seed. The R_2 plants were self-pollinated to produce R_3 seed. The R_3 plants were self-pollinated to produce R_4 seed. R_4 plants homozygous for the MON 87403 T-DNA were crossed via traditional breeding techniques to a Monsanto proprietary recurrent parent that does not contain the *ATHB17* coding sequence to produce hemizygous R_4F_1 seed. The R_4F_1 plants were crossed with the recurrent parent to produce BC_1F_1 seed. The BC_1F_1 generation was tested for the presence of the T-DNA by End-Point TaqMan PCR to select for hemizygous MON 87403 plants. BC_1F_1 plants hemizygous for MON 87403 T-DNA were crossed with the recurrent parent to produce the BC_2F_1 plants. BC_2F_1 plants hemizygous for MON 87403 T-DNA were crossed with the recurrent parent to produce the BC_3F_1 plants.

The inheritance of the MON 87403 T-DNA was assessed in the BC_1F_1, BC_2F_1, and BC_3F_1 generations. At the BC_1F_1, BC_2F_1, and BC_3F_1 generations, the MON 87403 T-DNA was predicted to segregate at a 1:1 ratio (hemizygous positive: homozygous negative) according to Mendelian inheritance principles.

A Pearson's chi-square (χ^2) analysis was used to compare the observed segregation ratios of the *ATHB17* coding sequence to the expected ratios.

The Chi-square was calculated as:

$$\chi^2 = \sum [(| o - e |)^2 / e]$$

® TaqMan is a registered trademark of Roche Molecular Systems, Inc.

where o = observed frequency of the genotype or phenotype and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$).

The results of the χ^2 analysis of the segregating progeny of MON 87403 are presented in Table IV-4. The χ^2 value in the BC_1F_1, BC_2F_1, and BC_3F_1 generations indicated no statistically significant difference between the observed and expected 1:1 segregation ratio (hemizygous positive: homozygous negative) of MON 87403 T-DNA. These results support the conclusion that the MON 87403 T-DNA resides at a single locus within the maize genome and is inherited according to Mendelian principles of inheritance. These results are also consistent with the molecular characterization data indicating that MON 87403 contains a single intact copy of the *ATHB17* expression cassette inserted at a single locus in the maize genome.

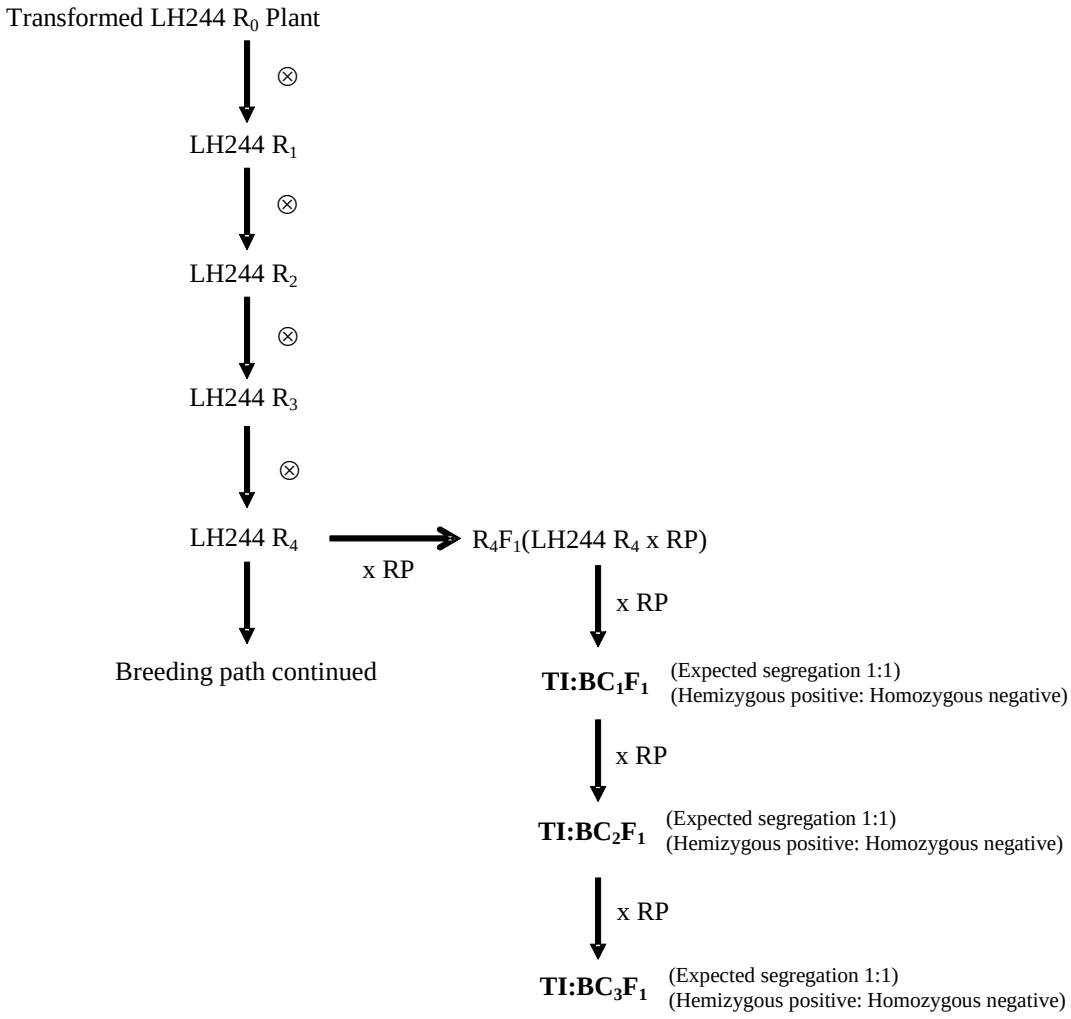

Figure IV-9. Breeding Path for Generating Segregation Data for MON 87403
Chi-square analysis was conducted on segregation data from BC_1F_1, BC_2F_1, and BC_3F_1 generations (bolded text).
TI: Trait Integration: Replacement of genetic background of MON 87403 by recurrent background except inserted gene.
RP: Recurring parent
BC: Back-Cross.
⊗: Self- Pollinated.

Table IV-4. Segregation of the Expression Cassette During the Development of MON 87403

Generation	Number of plants	Observed Positives	Observed Negatives	Expected Positives	Expected Negatives	χ^2	Probability
BC_1F_1	180	88	92	90.00	90.00	0.09	0.766
BC_2F_1	178	82	96	89.00	89.00	1.10	0.294
BC_3F_1	181	101	80	90.50	90.50	2.44	0.119

IV.F. Characterization of the Genetic Modification Summary and Conclusion

Molecular characterization of MON 87403 by NGS/JSA and directed sequencing demonstrated that a single copy of the intended transfer DNA (T-DNA) containing the *ATHB17* expression cassette from PV-ZMAP5714 was integrated into the maize genome at a single locus. These analyses also showed no PV-ZMAP5714 backbone elements were present in the event.

Directed sequence analyses performed on MON 87403 confirmed the organization and intactness of the full T-DNA and all expected elements within the insert, with the exception of incomplete Right and Left Border sequences that do not affect the functionality of the *ATHB17* expression cassette. Analysis of the T-DNA insertion site in maize showed that the 5' and 3' genomic DNA flanking the T-DNA insert in MON 87403 are identical to the conventional control, except for a 149 base pair deletion of genomic DNA at the insertion site in MON 87403. This deletion is not expected to affect food or feed safety.

Generational stability analysis by NGS/JSA demonstrated that the T-DNA in MON 87403 was maintained through five breeding generations, thereby confirming the stability of the insert. Results from segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 87403 at a single chromosomal locus.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE ATHB17Δ113 PROTEIN PRODUCED IN MON 87403

Characterization of the introduced protein(s) in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. As described in Sections IV and V, MON 87403 contains an *ATHB17* expression cassette that, when transcribed and translated, results in the expression of the ATHB17Δ113 protein.

This section summarizes: 1) the identity and function of the ATHB17Δ113 protein produced in MON 87403; 2) assessment of equivalence between the plant-produced and *E. coli*-produced proteins; 3) the level of the ATHB17Δ113 protein in plant tissues from MON 87403; 4) assessment of the potential allergenicity of the ATHB17Δ113 protein produced in MON 87403; and 5) the food and feed safety assessment of the ATHB17Δ113 protein produced in MON 87403. The data support a conclusion that the ATHB17Δ113 protein produced in MON 87403 is safe for human or animal consumption based on several lines of evidence summarized below.

V.A. Identity and Function of the ATHB17Δ113 Protein from MON 87403

V.A.1. Sequence Identity of the ATHB17Δ113 Protein from MON 87403

As described in Section IV, the *ATHB17* gene is derived from *Arabidopsis thaliana*. Compared to the wild type sequence of the ATHB17 protein, the amino acid sequence of the *ATHB17*-encoded protein expressed in MON 87403 lacks 113 amino acids due to alternative mRNA splicing, and has thus been designated ATHB17Δ113. The *ATHB17* expression cassette in MON 87403 includes the *ATHB17* coding sequence and the I-*Ract1* intron (Table IV-1). Typically, pre-mRNA processing includes mRNA splicing, which consists of the removal of the introns from pre-mRNA transcripts and ligation of exons to produce mature mRNA (Simpson and Filipowicz 1996). To determine the sequence of the mature *ATHB17* mRNA transcript in MON 87403, total RNA was extracted from leaf tissue and then PolyA$^+$ RNA was enriched. mRNA transcripts of *ATHB17* were amplified by RT-PCR and then analyzed by sequence analysis. In MON 87403, the *ATHB17* pre-mRNA is spliced to remove the majority of the I-*Ract1* intron as well as a portion of the *ATHB17* gene (Figure V-1). In order to predict the amino acid sequence of the protein produced from the *ATHB17* mRNA, a Basic Local Alignment Search Tool (BLASTX) search was performed to compare the *ATHB17* mRNA consensus sequence against the GenBank_Protein_Preferred protein sequence database. The top result of this homology search was the *Arabidopsis thaliana* ATHB17 protein sequence (GenBank ID 179876107). The first methionine (M) amino acid in the BLASTX alignment provides a predicted translation start site for the *ATHB17*-derived protein in MON 87403, and results in the protein ATHB17Δ113, which is lacking the N-terminal 113 amino acids found in wild type ATHB17 (Figure V-2). To confirm the presence of the ATHB17Δ113 protein in MON 87403, western blot analysis was conducted on MON 87403 with an anti-ATHB17 antibody. Western blots revealed the presence of an immunogenic band corresponding to the expected molecular weight of the predicted ATHB17Δ113 protein (Figure D-2).

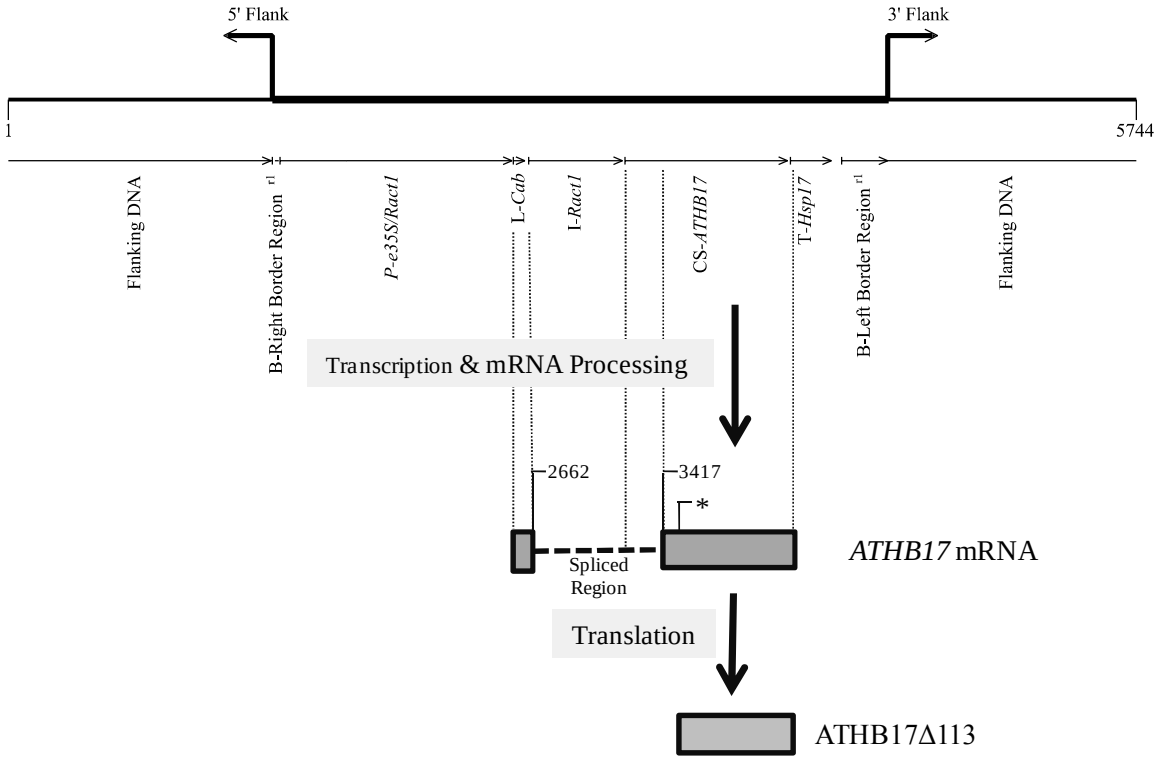

Figure V-1. Insert map of MON 87403 and schematic diagram of *ATHB17* transcription, mRNA processing, and predicted translation

The nucleotide sequence of the mature *ATHB17* mRNA was determined using RT-PCR followed by Sanger sequencing. The mRNA sequence that codes for the ATHB17 protein in MON 87403 was determined. This analysis allowed for the identification of the splice junction in MON 87403 *ATHB17* mRNA. The maize splicing of MON 87403 *ATHB17* mRNA results in the excision of the I-*Ract1* element (splice junction site 2662) and a portion of the CS-*ATHB17* coding region (splice junction site 3417). The observed CS-*ATHB17* coding region is predicted to produce a version of theATHB17 protein in MON 87403 without the first 113 amino acids at the N-terminus compared to the sequence of ATHB17 found in Arabidopsis (the translation initiation site is denoted by an *).

```
  1 MNRLPSSEDG DDEEFSHDDG SAPPRKKLRL TREQSRLLED SFRQNHTLNP KQKEVLAKHL
 61 MLRPRQIEVW FQNRRARSKL KQTEMECEYL KRWFGSLTEE NHRLHREVEE LRAIKVGPTT
121 VNSASSLTMC PRCERVTPAA SPSRAVVPVP AKKTFPPQER DR
```

Figure V-2. Predicted Amino Acid Sequence of the Protein Produced by MON 87403 *ATHB17* mRNA

The consensus sequence of MON 87403 mature *ATHB17* mRNA was used in a BLASTX 2.2.23 search of the GenBank_Protein_Preferred database. The predicted amino acid sequence of MON 87403 ATHB17Δ113, beginning with the first methionine amino acid in the top BLAST result is shown above.

V.A.2. Structure and Function of the ATHB17Δ113 Protein from MON 87403

The *Arabidopsis thaliana* ATHB17 protein is a member of the HD-Zip family of transcription factors. In plants, the family of HD-Zip proteins is further segregated into four distinct subfamilies designated as I, II, III, and IV, based upon the sequences of conservative structural domains and motifs that convey DNA specificity and physiological function (Ariel et al. 2007). As described in Section V.A.1, expression of the *Arabidopsis thaliana ATHB17* gene in MON 87403 results in production of the ATHB17Δ113 protein, which consists of a single polypeptide chain of 162 amino acids and has a predicted molecular weight of ~22 kDa. HD-Zip subfamily II members are characterized by highly conserved domains that include a homeodomain (HD) that recognizes a 9 bp DNA sequence CAAT(C/G)ATTG (Sessa et al. 1993), a leucine-zipper (LZ) domain responsible for protein dimerization, and a repression domain (Rice et al. 2014). ATHB17Δ113 retains the HD and LZ domains found in wild-type ATHB17 but lacks the repression domain.

V.B. Characterization and Equivalence of ATHB17Δ113 Protein from MON 87403

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties of the protein produced from the inserted DNA, and confirmation of the safety of the protein. For safety data generated using *E. coli*-produced protein to be applied to plant-produced protein, the equivalence of the plant- and *E. coli*-produced proteins must be assessed. As reported in Section V.C, the expression level of the ATHB17Δ113 protein in MON 87403 is very low in all tissues evaluated. Due to this low level expression, the MON 87403-produced ATHB17Δ113 protein was not isolated to a high level of purity, but instead limited amounts of the protein were enriched in leaf extract to a purity of approximately 3% (Appendix D:). Characterization of this preparation enabled the demonstration of equivalence between the MON 87403-produced ATHB17Δ113 protein and the *E. coli*-produced ATHB17Δ113 protein. A summary of these analytical results are shown below and the details of the materials, methods, and results are described in Appendix D: .

The ATHB17Δ113 protein purified from leaf tissue of MON 87403 was characterized and equivalence of the physicochemical properties between the MON 87403-produced

and *E. coli*-produced proteins was established using a panel of analytical techniques (Appendix D:), including: 1) SDS-PAGE analysis, to establish equivalence of apparent molecular weight between the MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins; 2) western blot analysis with antibodies specific for the ATHB17Δ113 protein to establish immunoreactive equivalence between the MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins; and 3) MALDI TOF MS analysis of MON 87403-produced ATHB17Δ113 to establish protein identity.

Due to the low quantity and purity of the enriched MON 87403-produced ATHB17Δ113, direct assessment of the ATHB17Δ113 N-terminal sequence, specific DNA binding activity, and glycosylation status were not feasible to be conducted for the MON 87403-produced ATHB17Δ113 protein. Instead, these characteristics of ATHB17Δ113 in MON 87403 were assessed indirectly. The N-terminal sequence of the *E. coli*-produced ATHB17Δ113 protein was directly analyzed and shown to match the N-terminus predicted by the RT-PCR analysis of MON 87403 *ATHB17* RNA as detailed in Section V.A.1. An SDS-PAGE assessment demonstrated that the apparent molecular weights of the MON 87403-produced and *E. coli*-produced ATHB17Δ113 were equivalent, which supports a conclusion that the N-termini are equivalent (Figure D-2). Analysis of the DNA binding activity of the *E. coli*-produced ATHB17Δ113 demonstrated that the protein specifically bound to the target DNA sequence CAAT(C/G)ATTG, as expected. While functional activity could not be directly measured for the isolated MON 87403-produced ATHB17Δ113 protein, the activity measured for *E. coli*-producted ATHB17Δ113 is consistent with the mechanism of action for ATHB17Δ113 in MON 87403.

There are no predicted N-terminal targeting or signal sequences in the ATHB17Δ113 amino acid sequence, which is expected due to the truncation of the N-terminus of the protein. Thus, although the ATHB17Δ113 protein sequence contains a consensus potential N-glycosylation sequence (NHT, starting at amino acid position 45), it lacks the N-terminal signal sequence required for transport to the endoplasmic reticulum, the site of N-glycosylation (Pattison and Amtmann 2009; Vitale and Denecke 1999). In total, this supports a conclusion that the MON 87403-produced ATHB17Δ113 protein is not glycosylated. This is similar to the CP4-EPSPS protein, which contains potential N-glycosylation sites but has been shown to not be glycosylated (Harrison et al. 1996).

Taken together, these data provide a characterization of the MON 87403-produced ATHB17Δ113 protein and establish its equivalence to the *E. coli*-produced ATHB17Δ113 protein.

V.C. Expression Levels of ATHB17Δ113 Protein in MON 87403

ATHB17Δ113 protein levels in various tissues of MON 87403 relevant to the risk assessment were determined by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 87403 were collected from four replicate plots planted in a randomized complete block field design during the 2012 growing season from the following five field sites in The United States: Jackson County, Arkansas

(site code ARNE); Story County, Iowa (site code IALL); Jefferson County, Iowa (site code IARL); Pawnee County, Kansas (site code KSLA) and Lehigh County, Pennsylvania (site code PAGR). The field sites were representative of maize-producing regions suitable for commercial production. OSL1[3], OSR1[4], forage, and grain tissue samples were collected from each replicated plot at all field sites.

Determining the protein expression levels serves to characterize MON 87403 and the levels are then used to assess food, feed and environmental exposure. Protein expression levels themselves do not provide information relevant to the assessment of the plant pest potential of MON 87403. Therefore, it is deemed appropriate to determine the ATHB17Δ113 protein levels for OSL1, OSR1, forage, and grain tissue samples, which were collected from each replicated plot at all field sites. Leaf and root tissues are distinct above and below ground plant tissues that may be important to environmental exposure. Forage and grain levels were determined to evaluate food and feed exposure in humans and animals and are required for margin of exposure calculations for both.

The ELISA results obtained for each sample were averaged across the five sites and are summarized in Table V-1. The details of the materials and methods are described in Appendix E. The individual ATHB17Δ113 protein levels in MON 87403 across all samples analyzed from all sites ranged from <LOD (<0.00028 µg/g dwt) to 0.017 µg/g dwt. The mean ATHB17Δ113 protein level among all tissue types was highest in OSL1 at 0.014 µg/g dwt and lowest in grain at <LOD µg/g dwt.

[3] Over season leaf 1
[4] Over season root 1

Table V-1. Summary of ATHB17Δ113 Protein Levels in Tissues from MON 87403 Grown in 2012 United States Field Trials

Tissue Type[1]	Development Stage[2]	Mean(SD) Range (µg/g dwt)[3]	LOQ/LOD[4] (µg/g dwt)
OSL1	V3-V4	0.014 (0.0020) 0.0096 – 0.017	0.00109/0.00049
OSR1	V3-V4	0.0023 (0.0016) 0.00083 – 0.0058	0.00078/0.00065
Forage	R5	0.0018 (0.00064) 0.0011 – 0.0035	0.00078/0.00063
Grain	R6	<LOD (N/A) N/A – N/A	0.00156/0.00028

[1]OSL= over season leaf
OSR= over season root
[2]The crop development stage each tissue was collected.
[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dwt). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites. (n=20, except OSR1 where n=16 due to four samples resulting in inconclusive levels)
[4]LOQ=limit of quantitation; LOD=limit of detection.

V.D. Assessment of Potential Allergenicity of the ATHB17Δ113 Protein

The allergenic potential of an introduced protein is assessed by comparing the biochemical characteristics of the introduced protein to biochemical characteristics of known allergens (Codex Alimentarius 2009). Using a weight of evidence approach, a protein is not likely to be associated with allergenicity if: 1) the protein is from a non-allergenic source; 2) the protein represents a small portion of the total plant protein; 3) the protein does not share structural similarities to known allergens based on the amino acid sequence; and 4) the protein does not show resistance to pepsin digestion. The ATHB17Δ113 protein has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) The ATHB17Δ113 protein originates from *Arabidopsis thaliana* an organism that has not been reported to be a source of known allergens.

2) The ATHB17Δ113 protein level in grain is < the limit of detection of the assay and, therefore, conservatively is less than 0.0000003% of the total protein in the grain that could be consumed from MON 87403 maize.

3) Bioinformatics analyses demonstrated that the ATHB17Δ113 protein does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes.

4) Finally, *in vitro* digestive fate experiments conducted with the ATHB17Δ113 protein demonstrate that the protein is rapidly digested by pepsin in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that the ATHB17Δ113 protein does not pose a significant allergenic risk to humans.

V.E. Safety Assessment Summary of ATHB17Δ113 Protein in MON 87403

V.E.1. The Donor Organism has a History of Safe Use

The *Arabidopsis* genus is a member of the mustard (Brassicaceae) family, which includes many well known food crops including cabbage, cauliflower, radish, turnip and canola/rapeseed. The most well known member of the *Arabidopsis* genus is a small annual flowering plant *Arabidopsis thaliana* (L.) which is also known under the common name of mouse-ear cress (Meyerowitz and Somerville 1994). *Arabidopsis thaliana* offers important advantages for basic research in genetics and molecular biology due to the small size of its sequenced genome, short life cycle, well developed classical genetics, and many naturally occurring mutants. *Arabidopsis thaliana* thus serves as an excellent model system for studying complex processes in plants.

Arabidopsis thaliana is not purposely consumed as a food source by humans. *Camelina sativa*, an emerging oilseed crop, is reported to be the cultivated species most closely related to Arabidopsis (Flannery et al. 2006). *Camelina sativa* leaves are consumed as fresh greens by humans in the country of Georgia (Facciola 1998).

Arabidopsis thaliana is generally not considered an allergenic or toxic source organism. Although *Arabidopsis thaliana* contains homologs of proteins previously described as allergens in other plant species (e.g., germins, lipid transfer protein, profilins, and small molecular weight calcium binding proteins), no Arabidopsis proteins have been reported in a peer-reviewed database of known allergens (FARRP 2013). One case of occupational asthma has been reported in a laboratory worker due to exposure to Arabidopsis pollen (Yates et al. 2008). In a recent internal bioinformatic assessment, none of the HD-Zip family of proteins in Arabidopsis, including the ATHB17 protein, showed any homology to any known allergens, toxins, or any other protein with known adverse effects.

V.E.2. ATHB17Δ113 Protein Belongs to a Common Class of Plant Proteins

The *Arabidopsis thaliana HB17* (*ATHB17*) gene encodes a member of the HD-Zip protein family of transcription factors (Ariel et al. 2007). The HD-Zip protein family is characterized by the presence of a leucine zipper (LZ) domain adjacent to the C-terminus of a homeodomain (HD) (Mukherjee et al. 2009; Ruberti et al. 1991). These two domains, HD and LZ, are individually present in transcription factors found across eukaryotic organisms, however such combination of HD and LZ domains in a single transcription factor is unique to plants; HD-Zip genes are found in all plant groups, but not in animals or fungi (Ariel et al. 2007). A bioinformatic search for homeobox genes (i.e. genes encoding proteins containing a HD) performed across several plant genomes (Mukherjee et al. 2009) yielded a comprehensive list of HD-Zip family members present in several plant species. It identified 48 HD-Zip genes in the Arabidopsis genome, 47 genes in the rice genome, and 70 genes in the maize genome. The HD-Zip family comprises 63% of all homeobox genes in maize and 40% - 60% of the homeobox genes in other plant species, making it the most abundant class of homeobox genes in many plant species.

Bioinformatic searches using the ATHB17Δ113 amino acid sequence as the query identify homologous sequences from several different plant species, including the food crops soybean, rice, corn tomato, potato, orange, papaya, grape, and cruciferous vegetables. Overall the protein sequence identity of ATHB17Δ113 to homologs in these species range from ~58-83%, with the highest identity to the homologs in the Brassica species *Brassica rapa* (a species including common food crops such as turnip and napa cabbage) and *Brassica oleracea* (a species including common food crops such as cabbage and Brussels sprouts). The amino acid sequence alignment between ATHB17Δ113 and its food crop homologs spans the length of the ATHB17Δ113 protein. Thus ATHB17Δ113 shares sequence identity and structural similarity with proteins present in plants currently consumed, establishing that humans and animals are exposed to this class of proteins and that no adverse effects have been attributed to this class of proteins.

V.E.3. ATHB17Δ113 Protein in MON 87403 is Not Homologous to Known Allergens or Toxins

Bioinformatics analyses were performed to assess the potential for allergenicity, toxicity, or biological activity of ATHB17Δ113. The analysis demonstrated that ATHB17Δ113

protein does not share amino acid sequence similarity with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health.

V.E.4. ATHB17Δ113 Protein in MON 87403 is Susceptible to Pepsin and Pancreatin Digestion

ATHB17Δ113 was readily digestible by pepsin in simulated gastric fluid and pancreatin in simulated intestinal fluid. Rapid degradation of ATHB17Δ113 by pepsin and pancreatin makes it highly unlikely that ATHB17Δ113 in MON 87403 would be absorbed in the digestive tract and have any adverse effects on human or animal health.

V.E.5. ATHB17Δ113 Protein in MON 87403 is Not Acutely Toxic

An acute oral toxicology study was conducted with the ATHB17Δ113 protein. Results indicated that ATHB17Δ113 did not cause any adverse effects in mice, with a No Observable Adverse Effect Level (NOAEL) of 1335 mg/kg body weight, the highest dose tested. As described below, this NOAEL (*i.e.*, known safe dose) is approximately one billion times (1×10^9) higher than a conservative estimate of high end exposure through dietary consumption of MON 87403.

V.E.6. Human and Animal Exposure to the ATHB17Δ113 Proteins

A common approach used to assess potential health risks for potentially toxic materials is to calculate a Margin of Exposure (MOE) between the lowest NOAEL from an appropriate animal toxicity study and an estimate of human exposure. Since no evidence of mammalian toxicity has been reported for ATHB17Δ113, and the expression levels in grain are extremely low (2.5×10^{-4} µg/g fwt[5]), dietary risk assessments would normally not be considered necessary. Nevertheless, a dietary risk assessment was still conducted for this protein in order to provide further assurances of safety by calculating a MOE between the NOAEL (1335 mg/kg body wt/day) for the ATHB17Δ113 protein in a mouse acute oral toxicity study (Section V.E.5 and 95th percentile consumption estimate of acute dietary exposure determined using the Dietary Exposure Evaluation Model - Food Commodity Intake Database (DEEM-FCID) (U.S. EPA 2013). DEEM-FCID utilizes food consumption data from the National Health and Nutrition Examination Survey (NHANES) conducted in 2003-2008 (Included: vegetable maize, popcorn, and field maize (flour, meal, bran, and starch). Based on levels of expressed protein on a fresh weight basis, 95th percentile exposure to ATHB17Δ113 for the general U.S. population was estimated to be 0.51 ng/kg body weight (bw). For non-nursing infants in the U.S., the most highly exposed sub-population, 95th percentile exposure to ATHB17Δ113 was estimated to be 1.21 ng/kg bw. For the ATHB17Δ113 protein, MOEs for acute dietary intake were estimated to be 2.6×10^9 and 1.1×10^9 for the general

[5] Fresh weight basis was calculated by multiplying the dry weight value of 2.8×10^{-4} µg/gram (Section V.C) by a dry weight correction factor of 0.882 to account for 11.8% moisture content in the grain.

population and non-nursing infants, respectively. The MOEs reflect that a human would have to eat millions of kilograms of maize in one day to achieve exposures to the expressed proteins in MON 87403 that were not toxic to mice, which would be a physical impossibility. These very large MOEs further indicate that there is no meaningful risk to human health from dietary exposure to the ATHB17Δ113 protein produced by MON 87403.

Actual MOEs will likely be much higher because the exposure estimates utilized are conservative (95^{th} percentile, assume 100% market penetration of MON 87403) and as described in Section V.E.4., ATHB17Δ113 is rapidly digested, further minimizing exposures. In addition, there are a number of steps in the processing of maize to make food ingredients, including high temperature treatments, hydrolyses, soaking in slightly acidic water, and drying that can denature a protein. Changes in temperature, pH, and physical disruptions associated with food processing and cooking/preparation generally lead to loss of protein structure and functionality (Hammond and Jez 2011). Like other proteins, the ATHB17Δ113 protein in MON 87403 is expected to be similarly susceptible to denaturation when exposed to high temperatures, pH extremes, and digestive environments encountered during processing and cooking of foods containing MON 87403. Thus, there are likely to be significantly lower exposures to the functionally active form of this protein through consumption of MON 87403 than the already negligible levels estimated above.

The potential ATHB17Δ113 protein exposure to animals from consumption of MON 87403 in feeds was evaluated by calculating an estimate of daily dietary intake (DDI) and relating that value to total daily dietary protein intake. The highest percentage of ATHB17Δ113 protein was in the lactating dairy cow, which was 0.000001% (g/g) of the total daily dietary protein intake (6.8×10^{-8} g of ATHB17Δ113/kg bw divided by 5.9 g/kg bw total daily dietary protein intake for lactating dairy cow). The percentage of the ATHB17Δ113 protein consumed as part of the daily protein intake for chickens and pigs is much less than for the lactating dairy cow. Therefore, in the maximal intake scenario, poultry, swine and lactating dairy cattle would be consuming 0.000001% (g/g) or less of their total protein as ATHB17Δ113 protein from MON 87403 maize.

In summary, there is no significant risk to human and animal health associated with dietary exposure to the ATHB17Δ113 protein in food and feed products derived from MON 87403.

V.F. ATHB17Δ113 Protein Characterization and Safety Conclusion

The data and information provided in this section address the questions important to the food and feed safety of the ATHB17Δ113 protein in MON 87403, including the potential allergenicity and toxicity. To summarize, ATHB17Δ113 belongs to the HD-Zip class of plant transcription factors, and has high amino acid sequence homology to HD-Zip proteins encoded in several common food crops. The donor organism for the ATHB17Δ113 coding sequence, *Arabidopsis thaliana*, is ubiquitous in the environment and not commonly known for human or animal pathogenicity, or allergenicity. The physicochemical characteristics of the ATHB17Δ113 protein from MON 87403 have

been characterized and shown to be equivalent to those of *E. coli*-produced ATHB17Δ113, which was used for the described safety studies. The expression level of ATHB17Δ113 protein in selected tissues of MON 87403 were determined and shown to be below the limit of detection of a sensitive assay in grain. An assessment of the allergenic potential of the protein supports the conclusion that the ATHB17Δ113 protein does not pose a meaningful safety risk to humans and animals or an allergenic risk to humans. The ATHB17Δ113 protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. In addition, the ATHB17Δ113 protein is rapidly digested in simulated digestive fluids and demonstrated no oral toxicity in mice at a test level that corresponds to a very large margin of exposure when considering the protein is undetectable in grain. Based on the above information, the consumption of the ATHB17Δ113 protein from MON 87403 or its progeny is considered safe for humans and animals.

The protein safety data presented herein support the conclusion that food and feed products containing MON 87403 or derived from MON 87403 are as safe as maize currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 87403

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process (Codex Alimentarius 2009) in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional control that has a history of safe use. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus document for maize composition (OECD 2002b).

A recent review of compositional assessments conducted according to OECD guidelines which encompassed a total of seven biotechnology-derived crop varieties, nine countries and eleven growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition. Most compositional variation is attributable to growing region, agronomic practices, and genetic background (Harrigan et al. 2010). Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients, anti-nutrients, and secondary metabolites that reflect the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics, and yield (Harrigan et al. 2010; Harrigan et al. 2009; Ridley et al. 2011; Zhou et al. 2011)

Compositional equivalence between biotechnology-derived and conventional crops supports an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD 2002a). OECD consensus documents on compositional considerations for new crop varieties emphasize quantitative measurements of essential nutrients and known anti-nutrients. These quantitative measurements effectively discern any compositional changes that imply potential nutritional or safety (*e.g.,* anti-nutritional) concerns. Levels of the components in grain and/or other raw agricultural commodities of the biotechnology-derived crop product are compared to: 1) corresponding levels in a conventional comparator, a genetically similar conventional line, grown concurrently, under similar field conditions, and 2) natural ranges generated from an evaluation of commercial reference hybrids grown concurrently and from data published in the scientific literature. The comparison to data published in the literature places any potential differences between the assessed crop and its comparator in the context of the well-documented variation in the concentrations of crop nutrients, anti-nutrients, and secondary metabolites.

This section provides analyses of concentrations of key nutrients, anti-nutrients, and secondary metabolites of MON 87403 compared to that of a conventional control grown and harvested under similar conditions, as appropriate. In addition, conventional commercial maize reference varieties (hereafter refered to as reference hybrids) were included in the composition analyses to establish a range of natural variability for each component, defined by the 99% tolerance interval. The production of materials for compositional analyses used a sufficient variety of field trial sites, robust field designs (randomized complete block design with four blocks), and sensitive analytical methods that allow accurate assessments of compositional characteristics over a range of environmental conditions under which MON 87403 is expected to be grown.

VI.A. Compositional Equivalence of MON 87403 Grain and Forage to Conventional Maize

Grain and forage samples were collected from MON 87403, a conventional control maize, and a total of 17 different reference hybrids (Table F-1) grown in the United States during a 2012 field production. The reference hybrids were included in the composition analyses to provide data on the natural variability for each component. The field production was conducted at eight sites. The field sites were planted in a randomized complete block design with four blocks per site. MON 87403, the conventional control, and reference hybrids were grown in areas of the United States that were typical for maize production and under normal agronomic field conditions for their respective geographic regions.

The evaluation of MON 87403 followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD 2002b). Grain samples were analyzed for levels of nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals, and vitamins. The anti-nutrients analyzed in grain included phytic acid and raffinose. Secondary metabolites analyzed in grain included furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber, and minerals. In total, 78 different components were assayed (nine in forage and 69 in grain).

Of those 78 components, 14 fatty acids (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, palmitoleic, heptadecanoic, heptadecenoic, gamma linolenic, eicosadienoic, eicosatrienoic, and arachidonic acids), sodium, and furfural had more than 50% of observations below the assay limit of quantitation (LOQ) and were excluded from statistical analysis. Moisture in grain and forage was measured for conversion of components to dry weight, but was not statistically analyzed. Therefore, 60 components were statistically analyzed.

The statistical comparison of MON 87403 and the conventional control was based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). The compositional data from the reference hybrids were combined across all field sites to calculate a 99% tolerance interval for each component to estimate the natural variability of each component in maize.

A statistically significant difference between MON 87403 and the conventional control does not necessarily imply biological relevance from a food and feed safety perspective. Therefore, statistically significant differences observed are typically evaluated in the context of natural variability to determine whether a detected difference indicates a biologically relevant compositional change. However, in this study, no significant differences between MON 87403 and the conventional control were observed in any of the measured components. Measurements of grain nutrients, including protein, amino acids (18 components), total fat, fatty acids (22 components), carbohydrates by calculation, fiber (3 components), ash, minerals (9 components), vitamins (7 components), antinutrients (phytic acid and raffinose), and secondary metabolites (furfural, ferulic acid, and p-coumaric acid) demonstrated that values in MON 87403

were not statistically significantly different from those in the conventional control (Table VI-1 to Table VI-6). Forage component levels, including ash, protein, total fat, carbohydrates by calculation, fiber (ADF and NDF), and minerals (calcium and phosphorus) were also not statistically significantly different between MON 87403 and the conventional control (Table VI-7). International Life Sciences Institute Crop Composition Database (ILSI-CCDB) and published literature values for all analytes are provided in Table VI-8.

The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients, and secondary metabolites in maize grain and forage of MON 87403 and the conventional control. The lack of any statistically significant differences between MON 87403 and the conventional control demonstrated that MON 87403 was not a major contributor to variation in nutrient, antinutrient, or secondary metabolite component levels in maize grain or forage and confirmed the compositional equivalence of MON 87403 to the conventional control in levels of these components.

Table VI-1. Summary of Maize Grain Protein and Amino Acids for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	p-Value	% Relative[6]
Protein	10.13 (0.34) 8.41 - 11.90	10.15 (0.34) 8.49 - 12.50	(8.58 - 12.63) 7.72, 12.67	4.01	-0.016 (0.14)	0.911	-0.16
Alanine	0.77 (0.030) 0.63 - 0.91	0.77 (0.030) 0.62 - 0.98	(0.63 - 0.99) 0.55, 1.01	0.36	0.00036 (0.012)	0.975	0.05
Arginine	0.48 (0.0093) 0.41 - 0.53	0.48 (0.0093) 0.41 - 0.54	(0.43 - 0.61) 0.41, 0.59	0.13	-0.00007 (0.0051)	0.988	-0.01
Aspartic Acid	0.63 (0.018) 0.54 - 0.72	0.63 (0.018) 0.52 - 0.74	(0.56 - 0.79) 0.52, 0.78	0.21	0.00058 (0.0080)	0.943	0.09
Cystine/Cysteine	0.21 (0.0041) 0.18 - 0.23	0.21 (0.0041) 0.18 - 0.24	(0.17 - 0.26) 0.16, 0.25	0.06	0.0017 (0.0032)	0.589	0.83
Glutamic Acid	1.85 (0.077) 1.48 - 2.26	1.86 (0.077) 1.43 - 2.34	(1.50 - 2.47) 1.26, 2.52	0.91	-0.0056 (0.033)	0.870	-0.30

Table VI-1 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	p-Value	% Relative[6]
Glycine	0.37 (0.0065) 0.32 - 0.41	0.37 (0.0065) 0.33 - 0.42	(0.32 - 0.44) 0.32, 0.44	0.08	0.00016 (0.0036)	0.966	0.04
Histidine	0.28 (0.0068) 0.24 - 0.33	0.28 (0.0068) 0.24 - 0.33	(0.22 - 0.35) 0.20, 0.35	0.09	0.0016 (0.0034)	0.648	0.55
Isoleucine	0.36 (0.014) 0.29 - 0.43	0.36 (0.014) 0.28 - 0.46	(0.28 - 0.44) 0.26, 0.46	0.17	-0.00044 (0.0061)	0.944	-0.12
Leucine	1.27 (0.059) 0.98 - 1.58	1.27 (0.059) 0.93 - 1.64	(0.98 - 1.65) 0.81, 1.73	0.71	0.0015 (0.026)	0.956	0.12
Lysine	0.27 (0.0048) 0.24 - 0.30	0.27 (0.0048) 0.23 - 0.31	(0.23 - 0.33) 0.24, 0.31	0.07	0.0013 (0.0027)	0.625	0.50
Methionine	0.21 (0.0063) 0.17 - 0.23	0.20 (0.0063) 0.17 - 0.25	(0.16 - 0.26) 0.15, 0.26	0.08	0.00071 (0.0028)	0.803	0.35

Table VI-1 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	p-Value	% Relative[6]
Phenylalanine	0.52 (0.022) 0.41 - 0.63	0.52 (0.022) 0.40 - 0.64	(0.42 - 0.65) 0.36, 0.68	0.24	0.0024 (0.0093)	0.801	0.47
Proline	0.95 (0.030) 0.80 - 1.10	0.95 (0.030) 0.76 - 1.18	(0.73 - 1.12) 0.64, 1.17	0.43	0.0032 (0.015)	0.830	0.34
Serine	0.44 (0.015) 0.35 - 0.51	0.45 (0.015) 0.37 - 0.55	(0.37 - 0.58) 0.34, 0.57	0.18	-0.0068 (0.0072)	0.356	-1.51
Threonine	0.36 (0.0098) 0.30 - 0.40	0.35 (0.0098) 0.30 - 0.41	(0.31 - 0.44) 0.28, 0.43	0.11	0.0025 (0.0043)	0.570	0.69
Tryptophan	0.077 (0.0015) 0.063 - 0.086	0.077 (0.0015) 0.069 - 0.084	(0.067 - 0.096) 0.064, 0.093	0.01	-0.00019 (0.00085)	0.827	-0.25
Tyrosine	0.41 (0.016) 0.32 - 0.48	0.41 (0.016) 0.32 - 0.52	(0.34 - 0.55) 0.30, 0.53	0.20	0.00043 (0.0081)	0.959	0.10

Table VI-1 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87403, Conventional Control, and Conventional References

					Difference (Test minus Control)		
Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Mean (S.E.)	p-Value	% Relative[6]
Valine	0.46 (0.013) 0.38 - 0.52	0.46 (0.013) 0.39 - 0.56	(0.38 - 0.57) 0.35, 0.57	0.17	-0.00092 (0.0061)	0.880	-0.20

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids. Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

[6]The relative magnitude of the difference in mean values between MON 87403 and the control, expressed as a percent of the control.

Table VI-2. Summary of Maize Grain Total Fat and Fatty Acids for MON 87403, Conventional Control, and Conventional References

Component	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Total Fat (% dwt)[1]	3.56 (0.072) 3.10 - 3.90	3.54 (0.072) 3.11 - 4.09	(2.49 - 4.70) 1.93, 5.49	0.98	0.026 (0.060)	0.682
16:0 Palmitic[6]	12.77 (0.14) 12.27 - 13.50	12.68 (0.14) 10.59 - 13.59	(9.90 - 13.12) 7.90, 14.94	2.99	0.097 (0.072)	0.219
18:0 Stearic	2.04 (0.022) 1.87 - 2.14	2.06 (0.022) 1.82 - 2.28	(1.46 - 2.42) 1.05, 2.72	0.46	-0.025 (0.019)	0.206
18:1 Oleic	21.84 (0.30) 20.77 - 23.46	21.77 (0.30) 20.50 - 28.08	(21.13 - 34.04) 14.77, 38.25	7.59	0.070 (0.19)	0.716
18:2 Linoleic	61.23 (0.30) 59.60 - 62.78	61.39 (0.30) 57.12 - 63.36	(51.19 - 62.88) 45.88, 70.60	6.25	-0.15 (0.16)	0.372
18:3 Linolenic	1.31 (0.014) 1.22 - 1.40	1.30 (0.014) 1.16 - 1.40	(0.78 - 1.48) 0.69, 1.67	0.24	0.012 (0.010)	0.267

Table VI-2 (continued). Summary of Maize Grain Total Fat and Fatty Acids for MON 87403, Conventional Control, and Conventional References

Component	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	p-Value
20:0 Arachidic	0.45 (0.012) 0.41 - 0.52	0.45 (0.012) 0.40 - 0.54	(0.30 - 0.52) 0.26, 0.55	0.14	-0.0017 (0.0034)	0.634
20:1 Eicosenoic	0.20 (0.0030) 0.18 - 0.22	0.20 (0.0030) 0.18 - 0.23	(0.19 - 0.30) 0.14, 0.33	0.05	-0.0020 (0.0017)	0.243
22:0 Behenic	0.16 (0.0081) 0.062 - 0.21	0.16 (0.0081) 0.069 - 0.20	(0.055 - 0.23) 0, 0.26	0.13	0.0029 (0.0045)	0.544

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids. Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

[6]Expressed as % total fatty acid. Prefix numbers refer to number of carbon atoms and number of carbon-carbon double bonds in the fatty acid molecule; 16:0 means sixteen carbon atoms and zero double bonds. Numbers are not included in text discussion for reasons of clarity. The following fatty acids with more than 50% of observations below the assay LOQ were excluded from statistical analysis: caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, gamma linolenic acid, eicosadienoic acid, eicosatrienoic acid, and arachidonic acid.

Table VI-3. Summary of Maize Grain Carbohydrates by Calculation and Fiber for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Carbohydrates by	84.98 (0.34)	84.98 (0.34)	(82.55 - 86.97)	3.84	0.0021 (0.12)	0.986
Calculation	83.09 - 86.73	82.77 - 86.61	81.80, 87.71			
Acid Detergent Fiber	3.63 (0.068)	3.62 (0.068)	(2.52 - 4.42)	1.49	0.0087 (0.062)	0.893
	3.16 - 4.07	2.91 - 4.40	2.36, 4.43			
Neutral Detergent Fiber	9.47 (0.19)	9.41 (0.19)	(6.86 - 12.18)	3.48	0.063 (0.16)	0.696
	8.11 - 10.67	7.53 - 11.01	5.32, 12.85			
Total Dietary Fiber	13.04 (0.15)	12.95 (0.15)	(9.83 - 17.30)	4.19	0.098 (0.22)	0.657
	11.53 - 14.70	11.50 - 15.69	10.05, 15.51			

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids.
Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

Table VI-4. Summary of Maize Grain Ash and Minerals for MON 87403, Conventional Control, and Conventional References

Component	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Ash (% dwt)[1]	1.31 (0.026) 1.09 - 1.52	1.33 (0.026) 1.10 - 1.51	(1.02 - 1.53) 1.08, 1.60	0.42	-0.017 (0.019)	0.371
Calcium (% dwt)	0.0037 (0.00021) 0.0026 - 0.0049	0.0037 (0.00021) 0.0025 - 0.0049	(0.0011 - 0.0058) 0.0011, 0.0059	0.00	0.00006 (0.00006)	0.321
Copper (mg/kg dwt)	1.57 (0.11) 1.13 - 3.35	1.55 (0.11) 1.04 - 2.91	(0.92 - 6.11) 0.29, 3.17	1.87	0.023 (0.12)	0.852
Iron (mg/kg dwt)	19.33 (0.82) 15.60 - 24.15	19.60 (0.82) 15.81 - 23.90	(14.66 - 25.54) 10.87, 27.03	8.09	-0.27 (0.21)	0.240
Magnesium (% dwt)	0.12 (0.0033) 0.10 - 0.14	0.12 (0.0033) 0.096 - 0.14	(0.093 - 0.14) 0.092, 0.15	0.04	-0.00010 (0.0013)	0.939
Manganese (mg/kg dwt)	6.17 (0.31) 4.36 - 7.85	6.14 (0.31) 4.55 - 8.55	(4.02 - 9.46) 2.59, 10.23	3.99	0.029 (0.11)	0.787

Table VI-4 (continued). Summary of Maize Grain Ash and Minerals for MON 87403, Conventional Control, and Conventional References

Component	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Phosphorus (% dwt)	0.30 (0.0068) 0.25 - 0.33	0.30 (0.0068) 0.24 - 0.34	(0.24 - 0.36) 0.24, 0.38	0.10	-0.0017 (0.0041)	0.683
Potassium (% dwt)	0.33 (0.0076) 0.30 - 0.40	0.33 (0.0076) 0.30 - 0.40	(0.27 - 0.42) 0.23, 0.42	0.10	-0.0023 (0.0044)	0.611
Zinc (mg/kg dwt)	20.52 (0.83) 16.30 - 25.20	20.98 (0.83) 16.49 - 27.14	(15.56 - 30.10) 9.09, 32.95	10.65	-0.46 (0.30)	0.165

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids.
Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

Table VI-5. Summary of Maize Grain Vitamins for MON 87403, Conventional Control, and Conventional References

Component (mg/kg dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Vitamin A (β-Carotene)	1.16 (0.039) 0.89 - 1.37	1.14 (0.039) 0.88 - 1.37	(0.48 - 2.85) 0, 3.10	0.49	0.025 (0.023)	0.319
Vitamin B1 (Thiamin)	3.48 (0.13) 2.72 - 4.19	3.52 (0.13) 2.82 - 4.23	(2.54 - 4.99) 1.73, 5.12	1.41	-0.046 (0.042)	0.277
Vitamin B2 (Riboflavin)	1.83 (0.059) 1.48 - 2.43	1.71 (0.059) 1.22 - 2.22	(1.35 - 2.35) 1.25, 2.22	1.00	0.12 (0.053)	0.057
Vitamin B3 (Niacin)	16.47 (0.78) 12.74 - 21.74	16.59 (0.78) 12.61 - 26.88	(12.77 - 30.15) 7.36, 30.18	14.27	-0.12 (0.46)	0.788
Vitamin B6 (Pyridoxine)	7.11 (0.17) 5.50 - 10.63	6.89 (0.17) 5.85 - 9.36	(4.66 - 8.80) 4.51, 8.98	3.51	0.22 (0.19)	0.255
Vitamin B9 (Folic Acid)	0.39 (0.016) 0.27 - 0.50	0.39 (0.016) 0.28 - 0.47	(0.22 - 0.77) 0.038, 0.69	0.20	0.00065 (0.0086)	0.940

Table VI-5 (continued). Summary of Maize Grain Vitamins for MON 87403, Conventional Control, and Conventional References

Component (mg/kg dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Vitamin E (α-Tocopherol)	17.87 (0.65) 14.70 - 20.76	18.33 (0.65) 15.03 - 23.23	(8.68 - 25.90) 2.50, 27.12	8.20	-0.47 (0.24)	0.095

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids.
Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

Table VI-6. Summary of Maize Grain Anti-nutrients and Secondary Metabolites for MON 87403, Conventional Control, and Conventional References

Component	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Anti-nutrient (% dwt[1])						
Phytic Acid	0.89 (0.017) 0.70 - 1.04	0.87 (0.017) 0.67 - 1.07	(0.68 - 1.15) 0.68, 1.18	0.40	0.023 (0.016)	0.164
Raffinose	0.22 (0.012) 0.14 - 0.30	0.23 (0.012) 0.17 - 0.31	(0.062 - 0.35) 0.00088, 0.40	0.14	-0.0030 (0.0041)	0.487
Secondary Metabolite (µg/g dwt)						
Ferulic Acid	2262.60 (61.27) 1833.71 - 2587.97	2213.54 (61.27) 1344.15 - 2694.48	(1381.65 - 2990.97) 827.07, 3473.40	1350.33	49.06 (48.06)	0.341
p-Coumaric Acid	216.73 (5.25) 160.59 - 242.25	212.58 (5.25) 173.81 - 260.72	(103.35 - 383.41) 6.62, 433.65	86.91	4.15 (5.09)	0.441

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids.
Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

Table VI-7. Summary of Maize Forage Proximates, Fiber, and Minerals for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Ash	4.00 (0.35) 2.17 - 5.87	4.14 (0.35) 2.14 - 6.24	(2.05 - 7.98) 0.67, 7.56	4.10	-0.14 (0.11)	0.199
Carbohydrates by Calculation	86.98 (0.43) 84.01 - 90.21	86.76 (0.43) 83.73 - 89.74	(82.09 - 90.15) 81.04, 92.37	6.01	0.22 (0.28)	0.449
Protein	7.08 (0.27) 5.60 - 8.92	7.19 (0.27) 4.42 - 9.34	(4.14 - 10.27) 3.56, 10.69	4.92	-0.12 (0.19)	0.548
Total Fat	1.96 (0.12) 0.92 - 2.89	1.98 (0.12) 0.53 - 2.94	(0.62 - 3.18) 0.81, 3.33	2.41	-0.023 (0.12)	0.851
Acid Detergent Fiber	23.01 (0.89) 16.18 - 33.12	22.44 (0.89) 17.33 - 30.17	(16.01 - 37.25) 17.89, 28.94	12.85	0.57 (0.83)	0.513
Neutral Detergent Fiber	36.83 (1.06) 28.67 - 44.62	37.23 (1.06) 29.77 - 56.33	(27.09 - 54.66) 30.85, 44.85	26.57	-0.40 (1.33)	0.768

Table VI-7 (continued). Summary of Maize Forage Proximates, Fiber, and Minerals for MON 87403, Conventional Control, and Conventional References

Component (% dwt)[1]	MON 87403 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Conventional Reference (Range)[3] Tolerance Interval[4]	Control Range Value[5]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Calcium	0.22 (0.015) 0.12 - 0.34	0.23 (0.015) 0.16 - 0.33	(0.12 - 0.40) 0.10, 0.36	0.17	-0.013 (0.0083)	0.150
Phosphorus	0.16 (0.0058) 0.12 - 0.22	0.16 (0.0058) 0.13 - 0.22	(0.10 - 0.30) 0.045, 0.30	0.09	0.0011 (0.0049)	0.828

[1]dwt = dry weight.

[2]Mean (S.E.) = least-square mean (standard error).

[3]Range is the minimum and maximum raw values for the conventional reference maize hybrids.

[4]With 95% confidence, interval contains 99% of the values expressed in the population of conventional reference maize hybrids. Negative limits set to zero.

[5]Maximum value minus minimum value for the control maize hybrid.

Table VI-8. Literature and ILSI Database Ranges for Components in Maize Forage and Grain

Grain Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Grain Nutrients		
Proximates (% dwt)		
Ash	1.17 – 2.01[a]; 1.27 – 1.63[b]	0.616 – 6.282
Carbohydrates by calculation	81.31 – 87.06[a]; 82.10 – 85.98[b]	77.4 – 89.5
Fat, total	2.95 – 4.40[a]; 3.18 – 4.23[b]	1.742 – 5.900
Protein	8.27 – 13.33[a]; 9.17 – 12.19[b]	6.15 – 17.26
Fiber (% dwt)		
Acid detergent fiber	1.82 – 4.48[a]; 1.83 – 3.39[b]	1.82 – 11.34
Neutral detergent fiber	6.51 –12.28[a]; 6.08 – 10.36[b]	5.59 – 22.64
Total dietary fiber	10.65 – 16.26[a]; 10.57 – 14.56[b]	9.01 – 35.31
Amino Acids (% dwt)		
Alanine	0.60 – 1.04[a]; 0.68 – 0.96[b]	0.44 - 1.39
Arginine	0.34 – 0.52[a]; 0.34 – 0.50[b]	0.12 - 0.64
Aspartic acid	0.52 – 0.78[a]; 0.59 – 0.76[b]	0.33 – 1.21
Cystine	0.19 – 0.26[a]; 0.20 – 0.26[b]	0.13 – 0.51
Glutamic acid	1.54 – 2.67[a]; 1.71 – 2.44[b]	0.97 – 3.54
Glycine	0.33 – 0.43[a]; 0.33 – 0.42[b]	0.18 – 0.54
Histidine	0.25 – 0.37[a]; 0.27 – 0.34[b]	0.14 – 0.43
Isoleucine	0.30 – 0.48[a]; 0.32 – 0.44[b]	0.18 – 0.69
Leucine	1.02 – 1.87[a]; 1.13 – 1.65[b]	0.64 – 2.49
Lysine	0.26 – 0.33[a]; 0.28 – 0.31[b]	0.17 – 0.67
Methionine	0.17 – 0.26[a]; 0.16 – 0.30[b]	0.12 – 0.47
Phenylalanine	0.43 – 0.72[a]; 0.45 – 0.63[b]	0.24 – 0.93
Proline	0.74 – 1.21[a]; 0.78 – 1.11[b]	0.46 – 1.63
Serine	0.39 – 0.67[a]; 0.43 – 0.60[b]	0.24 – 0.77
Threonine	0.29 – 0.45[a]; 0.31 – 0.39[b]	0.22 – 0.67
Tryptophan	0.047 – 0.085[a]; 0.042 – 0.070[b]	0.027 – 0.215
Tyrosine	0.13 – 0.43[a]; 0.12 – 0.41[b]	0.10 – 0.64
Valine	0.42 – 0.62[a]; 0.45 – 0.58[b]	0.27 – 0.86
Fatty Acids (% Total FA)		
16:0 Palmitic	8.80 – 13.33[a]; 9.84 – 12.33[b]	7.94 – 20.71
18:0 Stearic	1.36 – 2.14 [a]; 1.30 – 2.10[b]	1.02 – 3.40
18:1 Oleic	19.50 – 33.71[a]; 19.59 – 29.13[b]	17.4 – 40.2
18:2 Linoleic	49.31 – 64.70[a]; 56.51 – 65.65[b]	36.2 – 66.5
18:3 Linolenic	0.89 – 1.56[a]; 1.03 – 1.38[b]	0.57 – 2.25
20:0 Arachidic	0.30 – 0.49[a]; 0.30 – 0.41[b]	0.279 – 0.965
20:1 Eicosenoic	0.17 – 0.29[a]; 0.17 – 0.27[b]	0.170 – 1.917
22:0 Behenic	0.069 – 0.28[a]; 0.059 – 0.18[b]	0.110 – 0.349
Minerals		
Calcium (% dwt)	0.0030 – 0.0083[c]	0.00127 – 0.02084
Copper (mg/kg dwt)	0.85 – 3.54[c]	0.73 – 18.50
Iron (mg/kg dwt)	10.58 – 30.97[c]	10.42 – 49.07
Magnesium (% dwt)	0.085 – 0.15[c]	0.0594 – 0.194
Manganese (mg/kg dwt)	3.67 – 9.39[c]	1.69 – 14.30
Phosphorous (% dwt)	0.25 – 0.38[c]	0.147 – 0.533
Potassium (% dwt)	0.29 – 0.47[c]	0.181 – 0.603
Zinc (mg/kg dwt)	16.67 – 31.38[c]	6.5 – 37.2

Table VI-8 (continued). Literature and ILSI Database Ranges for Components in Maize Forage and Grain

Grain Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Vitamins (mg/kg dwt)		
Folic acid	0.19 – 0.35[a]; 0.23 – 0.42[b]	0.147 – 1.464
Vitamin A [β–Carotene]	122 – 4740[d]	0.19 – 46.81
Vitamin B_1 [Thiamine]	2.33 – 4.17[a]; 2.71 – 4.33[b]	1.26 – 40.00
Vitamin B_2 [Riboflavin]	0.94 – 2.42[a]; 1.64 – 2.81[b]	0.50 – 2.36
Vitamin B_3 [Niacin]	15.07 – 32.38[a]; 13.64 – 42.06[b]	10.37 – 46.94
Vitamin B_6 [Pyridoxine]	4.93 – 7.53[a]; 4.97 – 8.27[b]	3.68 – 11.32
Vitamin E [α–Tocopherol]	5.96 – 18.44[a]; 2.84 – 15.53[b]	1.537 – 68.672
Grain Anti–Nutrients (% dwt)		
Phytic acid	0.69 – 1.09[a]; 0.60 – 0.94[b]	0.111 – 1.570
Raffinose	0.079 – 0.22[a]; 0.061 – 0.15[b]	0.020 – 0.320
Grain Secondary Metabolites (µg/g dwt)		
Ferulic acid	1205.75 – 2873.05[a]; 1011.40 – 2539.86[b]	291.9 – 3885.8
p–Coumaric acid	94.77 – 327.39[a]; 66.48 – 259.68[b]	53.4 – 576.2

Forage Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Forage Nutrients		
Proximates (% dwt)		
Ash	2.67 – 8.01[a]; 4.59 – 6.90[b]	1.527 – 9.638
Carbohydrates by calculation	81.88 – 89.26[a]; 84.11 – 87.54[b]	76.4 – 92.1
Fat, total	1.28 – 3.62[a]; 0.20 – 1.76[b]	0.296 – 4.570
Protein	5.80 – 10.24[a]; 5.56 – 9.14[b]	3.14 – 11.57
Fiber (% dwt)		
Acid detergent fiber	19.11 – 30.49[a]; 20.73 – 33.39[b]	16.13 – 47.39
Neutral detergent fiber	27.73 – 49.62[a]; 31.81 – 50.61[b]	20.29 – 63.71
Minerals (% dwt)		
Calcium	0.12 – 0.33[a]; 0.21 – 0.41[b]	0.07139 – 0.57679
Phosphorous	0.090 – 0.26[a]; 0.13 – 0.21[b]	0.09362 – 0.37041

[1]dwt=dry weight; FA = fatty acids.
[2]Literature range references: [a]US and [b]Chile (Harrigan et al. 2009), [c]France (Ridley et al. 2011), [d](Safawo et al. 2010).
[3]ILSI range is from ILSI Crop Composition Database, 2011 [Accessed 9 January 2013] (ILSI 2011).

VI.B. Compositional Assessment of MON 87403 Conclusion

Compositional analysis was conducted on grain and forage of MON 87403 grown at eight sites in a 2012 field production in the United Sates that are representative of typical agricultural regions for maize production. The compositional analysis, based on the OECD consensus document for maize, included measurement of nutrients, anti-nutrients and secondary metabolites in conventional reference hybrids to provide data on the natural variability of each compositional component analyzed.

Of the 60 components statistically assessed for MON 87403, none of the components showed a significant difference between MON 87403 and the conventional control. These results support the overall conclusion that MON 87403 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87403 to the conventional control in levels of these components.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides a comparative assessment of the phenotypic, agronomic, and environmental interaction characteristics of MON 87403 compared to the conventional control. The data support a conclusion that MON 87403 is not meaningfully different from the conventional control with the exception of the increased ear biomass trait, and therefore, is not expected to pose a plant pest risk compared to conventional maize. These conclusions are based on the results of multiple evaluations from laboratory and field assessments.

Phenotypic, agronomic, and environmental interaction characteristics of MON 87403 were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, observations of plant responses to abiotic stress, plant-disease and plant-arthropod interactions, and pollen characteristics. Results from these assessments demonstrate that MON 87403 does not possess a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stresses, diseases, or arthropods; or c) characteristics that would confer a plant pest risk compared to the conventional control.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87403, data were collected to evaluate altered plant pest potential. A detailed description of the regulated article phenotype is requested as part of the petition for determination of nonregulated status in 7 CFR § 340.6. That description includes differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived." As part of the characterization of MON 87403, data were collected to provide a detailed description of the phenotypic, agronomic, and environmental interaction characteristics of MON 87403. A subset of these data were included in an evaluation of specific characteristics related to altered plant pest potential (e.g., seed dormancy, lodging, ear drop, and environmental interactions data).

The plant characterization of MON 87403 encompassed six general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) lodging and seed retention on the plant; 5) plant response to abiotic stress and interactions with diseases and arthropods; and 6) intended phenotype (increased ear biomass). An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD 1993) and were comprised of a combination of field and laboratory studies conducted by scientists who are familiar with the production and evaluation of maize. In each of these assessments, MON 87403 was compared to an appropriate conventional control that had a genetic background similar to MON 87403 but did not possess increased ear biomass trait. In addition, multiple commercial maize hybrids developed through conventional breeding and selection (see Appendices G, H,

and I and Tables G-1, H-1, and I-1) were included to provide a range of comparative values for each characteristic that are representative of the variability in existing commercial maize hybrids. Data collected for the various characteristics from the commercial reference hybrids provided context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in U.S. Field Trials, and Laboratory Studies

Data category	Characteristics measured (associated section where discussed)	Evaluation timing[1] (Setting of evaluation)	Evaluation description (measurement endpoints)
Germination, dormancy, and emergence	Normal germinated (VII.C.1)	Day 4 and 7: 20/30°C (Laboratory)	Percentage of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated (VII.C.1)	Day 7: 20/30°C (Laboratory)	Percentage of seed producing seedlings that could not be classified as normal germinated
	Germinated (VII.C.1)	Day 4, Day 7, and Day 12: 5, 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Percentage of seed that had germinated (both normally or abnormally)
	Dead (VII.C.1)	Day 4 and 7: 5, 10, 20, 30, 10/20, 10/30, and 20/30°C. Day 12: 5, 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Percentage of seed that had visibly deteriorated and become soft to the touch (also included non-viable hard and non-viable firm-swollen seed)
	Viable hard (VII.C.1)	Day 7: 20/30°C Day 12: 5, 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test[2])
	Viable firm-swollen (VII.C.1)	Day 7: 20/30°C Day 12: 5, 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Percentage of seed that imbibed water and were firm to the touch but did not germinate (viability determined by a tetrazolium test[2])
	Early stand count (VII.C.2.1)	V2 – V5[1] growth stage (Field)	Number of emerged plants in two rows
Vegetative growth	Final stand count (VII.C.2.1)	Pre-harvest (Field)	Number of plants in two rows
	Plant vigor (VII.C.2.1)	V2 – V5[1] growth stage (Field)	Rated on a 1-9 scale, where 1 = excellent vigor and 9 = poor vigor
	Stay green (VII.C.2.1)	Maturity[1] (Field)	Rated on a 1-9 scale, 1 = 90 to 100%; 2 = 80-89%; 3 = 70-79%; 4 = 60-69%; 5 = 50-59%; 6 = 40-49%; 7 = 30-39%; 8 = 20-29%; and 9 = 0 – 19% green tissue
	Ear height (VII.C.2.1)	R1 – Maturity[1] (Field)	Distance from the soil surface at the base of the plant to the ear attachment node on five plants per plot
	Plant height (VII.C.2.1)	R1 – Maturity[1] (Field)	Distance from the soil surface at the base of the plant to the flag leaf collar on five plants per plot

Table VII-1 (continued). Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials and Laboratory Studies

Data category	Characteristics measured	Evaluation timing[1] (Setting of evaluation)[1]	Evaluation description
Reproductive growth	Days to 50% pollen shed (VII.C.2.1)	Pollen shed[1] (Field)	Days from planting until 50% of the plants have begun to shed pollen
	Days to 50% silking (VII.C.2.1)	Silking[1] (Field)	Days from planting until 50% of the plants have multiple silks exposed
	Pollen viability (VII.C.3)	Tasseling (Laboratory)	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen morphology (VII.C.3)	Tasseling (Laboratory)	Diameter of viable pollen grains and visual observations
	Grain moisture (VII.C.2.1)	Harvest (Field)	Percentage moisture of harvested shelled grain
	Test weight (VII.C.2.1)	Harvest (Field)	Test weight (kilogram/hecto liter) of harvested shelled grain
	Yield (VII.C.2.1)	Harvest (Field)	Calculated in Mg/ha, adjusted to 15.5% grain moisture content
Lodging and Seed retention	Stalk lodged plants (VII.C.2.1)	Pre-harvest (Field)	Number of plants per plot broken below the ear
	Root lodged plants (VII.C.2.1)	Pre-harvest (Field)	Number of plants per plot leaning at the soil surface at >30° from the vertical
	Dropped ears (VII.C.2.1)	Pre-harvest (Field)	Number of mature ears dropped from plants
Environmental interactions	Abiotic stress response (VII.C.2.2.1)	Four times during growing season (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)
	Disease damage (VII.C.2.2.1)	Four times during growing season (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)
	Arthropod damage (VII.C.2.2.1)	Four times during growing season (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)
	Stalk rot disease (VII.C.2.2.1)	Harvest (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)
	Ear/kernel rot disease (VII.C.2.2.1)	Harvest (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)

Table VII-1 (continued). Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials and Laboratory Studies

	Characteristics measured	Evaluation timing[1] (Setting of evaluation)[1]	Evaluation description
Environmental interactions	Corn earworm damage (VII.C.2.2.2)	R5 growth stage[1] (Field)	Quantitative assessment on 10 plants per plot by examining ears for damaged area using a plastic film grid (each grid cell = 0.5 cm^2)
	European corn borer damage (VII.C.2.2.2)	R6 growth stage[1] (Field)	Quantitative assessment on 10 plants per plot by counting number of feeding galleries and total length of feeding galleries in each stalk
	Arthropod abundance (VII.C.2.2.2)	Five collection times during growing season (Field)	Quantitative assessment of arthropod abundance via sticky traps collections and visual counts
Intended Phenotype	R1 ear biomass (VII.C.2.1)	R1 Growth Stage[1] (Field)	Dry weight in grams of whole ears from 1 m in length of row

[1] Plant growth stages were determined using descriptions and guidelines outlined in Corn Growth and Development (Ritchie et al. 1997).
[2] Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA/SCST 2010).

VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments, and are considered from a basis of familiarity. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a well-characterized conventional crop whose biological properties and plant pest potential are well-known. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors, and provides a basis for comparative environmental risk assessment between a biotechnology-derived plant and its conventional counterpart.

Expert knowledge and experience with conventionally bred maize was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for maize. As such, MON 87403 was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interaction characteristics. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weedy characteristics (e.g., seed dormancy, pre-harvest seed loss characteristics, and lodging) was used to assess whether there was an increase in weediness potential of MON 87403 compared to conventional maize. Evaluation of environmental interaction characteristics (e.g., plant abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest assessment. Prior to analysis, the overall dataset was evaluated for possible evidence of biologically-relevant changes and unexpected plant responses. No unexpected observations or issues were identified. Based on all of the data collected, an assessment was made to determine if MON 87403 could be expected to pose an increased plant pest risk compared to conventional maize.

VII.B.1. Interpretation of Criteria for Detected Differences

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest/weed potential. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest/weed potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in a step-wise method (Figure VII-1) or in a similar fashion. All detected differences for a characteristic are considered in the context of whether or not the difference would increase the crop's pest/weed potential. Ultimately, a weight of evidence approach considering all characteristics and data is used for the overall risk assessment of differences and their significance. In detail, Figure VII-1 illustrates the stepwise assessment process employed:

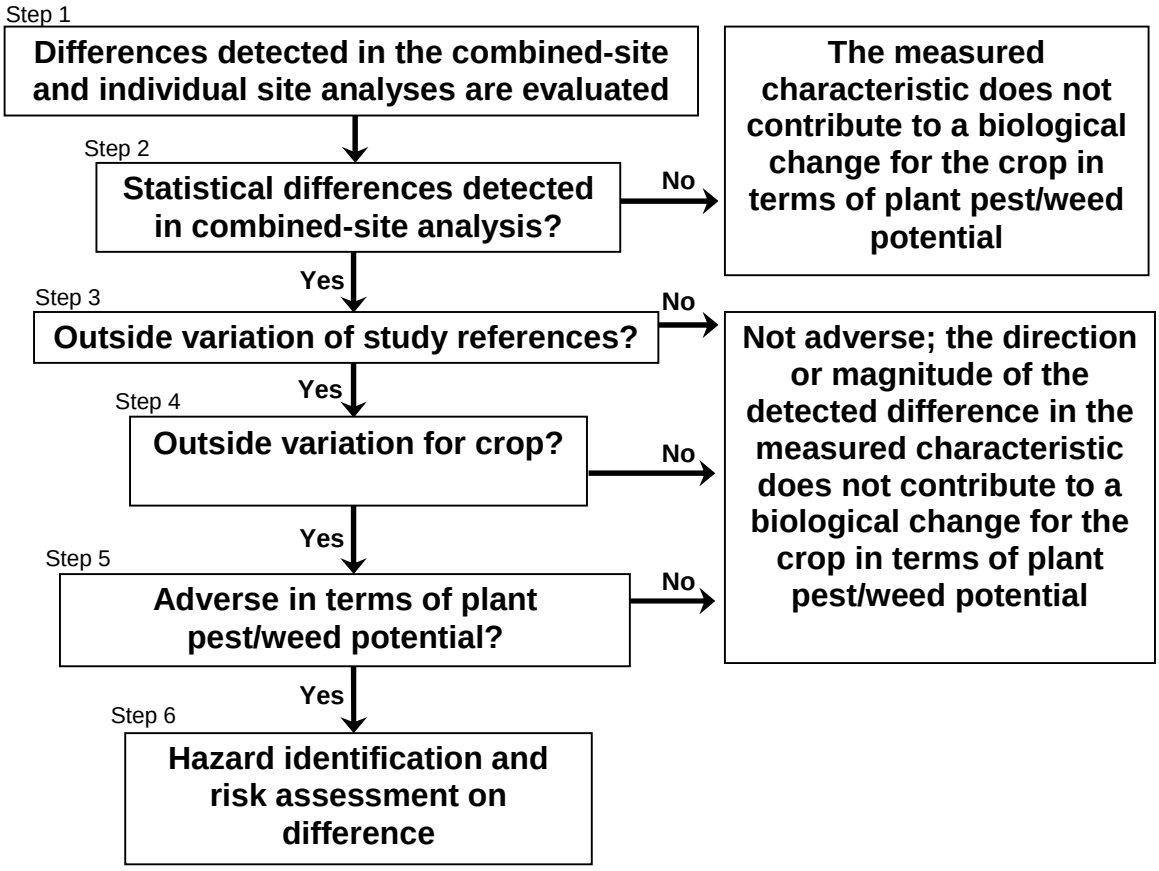

Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

Note: A "no" answer at any step indicates that the characteristic does not contribute to a biological change for the crop in terms of plant pest potential and subsequent steps are not considered. If the answer is "yes" or "uncertain," the subsequent step is considered.

Steps 1 and 2 - Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed, where appropriate, within each individual site and in a combined-site analysis, in which the data are pooled among sites. All statistically significant differences are evaluated and considered in the context of a change in plant pest/weed potential. Differences detected in individual-site analyses that are not detected when data across multiple environments are pooled in the combined-site analysis are considered not biologically meaningful in terms of plant pest/weed potential and, therefore, are not further considered in subsequent steps. Any difference detected in the combined-site analysis is further assessed.

Step 3 - Evaluate Differences in the Context of Reference Hybrids Included in the Study

If a difference for a characteristic is detected in the combined-site analysis across multiple environments, then the mean value of the biotechnology-derived crop for the characteristic is assessed relative to the range of variation of the reference hybrids included in the study (e.g., reference range).

Step 4 - Evaluate Differences in the Context of the Crop

If the mean value of the characteristic for a biotechnology-derived crop is outside the variation of the reference hybrids in the study, the mean value of the biotechnology-derived crop is assessed relative to known values common for the crop (e.g., published values).

Step 5 - Relevance of Differences to Plant Pest/Weed Potential

If the mean value of the characteristic for a biotechnology-derived crop is outside the range of values common for the crop, the difference is then assessed for whether or not it is meaningful in terms of plant pest/weed potential.

Step 6 - Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, a risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest/weed potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait introgression into any populations growing outside of cultivated environments or into a sexually-compatible species.

VII.B.2. Interpretation of Vigor and Environmental Interactions Data

For the qualitative assessments of vigor, abiotic stress response, disease damage, and arthropod damage, the biotechnology-derived crop and the conventional control are considered different in susceptibility or tolerance if the range of injury symptoms of each did not overlap across all four replications. Any observed differences are assessed for biological significance in the context of the range of the reference hybrids, and for consistency in other observation times and sites. Differences that are not consistently observed in multiple environments are not considered to be biologically meaningful in terms of plant pest potential.

Quantitative assessments of corn earworm and European corn borer damage are analyzed within individual sites and pooled across sites in a combined-site analysis. Statistically significant differences detected between the biotechnology-derived crop and the conventional control are evaluated using the method outlined in Figure VII-1.

Quantitative assessments of arthropod abundance are only analyzed within each individual site. Statistically significant differences between the biotechnology-derived crop and the conventional control were assessed for biological significance in the context of the range of the reference hybrids, and for consistency with other collection times and collection sites and in the context of pest potential. Differences that are not consistently detected in multiple environments are not considered biologically meaningful in terms of plant pest potential.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interaction Characteristics of MON 87403

This section provides the results of comparative assessments conducted in replicated laboratory and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interactions description of MON 87403. The characteristics for MON 87403 evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic, agronomic, and environmental interaction observations under field conditions (Section VII.C.2), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendices G, H, and I.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest factor (7 CFR § 340.6). Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson 1996; Lingenfelter and Hartwig 2007). Information on germination and dormancy characteristics is therefore useful when assessing a plant for increased weediness potential. To assess germination characteristics, standardized germination assays are available and routinely used. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of 20/30 °C as optimal for testing the germination and dormancy characteristics of maize seed (AOSA 2012b; a). Additional temperature regimes were also evaluated.

A comparative assessment of seed germination and dormancy characteristics was conducted on MON 87403 and the conventional control. The seed lots used for the germination testing of MON 87403 (selfed F2 grain), the conventional control, and the reference hybrids (4 per site, 9 unique across all locations) were produced in replicated field trials during 2012 in Story County, Iowa (IA), Warren County, Illinois (IL), and Lehigh County, Pennsylvania (PA). These geographic areas represent a broad range of environmental conditions for maize production for this product. The experiments were arranged as separate split-plot experiments with four replications for each temperature regime.

Descriptions of the evaluated germination and dormancy characteristics and the timing of the evaluations for all temperature regimes are listed in Table VII-1. Additional details on the materials and experimental methods used in this evaluation are presented in Appendix G: .

In the combined-site analysis, in which data were pooled across the three seed production sites, no statistically significant differences (α=0.05) were detected between MON 87403 and the conventional control for any characteristic at the AOSA temperature regime (20/30 °C), or at the temperature regimes of 5, 10, 20, 30, 10/20, 10/30 °C (Table VII-2). In addition, no hard seed were observed at any temperature.

The germination and dormancy characteristics evaluated were used to assess MON 87403 in the context of plant pest/weed potential. The results of this assessment, particularly the lack of increased hard seed, and no changes in other germination and dormancy characteristics, support the conclusion that the introduction of increased ear biomass trait is not expected to result in increased plant pest/weed potential compared to conventional maize.

Table VII-2. Germination Characteristics of MON 87403 and the Conventional Control

Temperature (°C)	Assessment Category	Mean % (S.E.)[1] MON 87403	Control	Reference Range[2]
5	Germinated	5.7 (0.90)	6.2 (1.24)	2.3 – 9.5
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	4.1 (0.60)	3.5 (0.69)	1.5 – 6.1
	Viable Firm-Swollen	90.3 (1.21)	90.3 (1.15)	86.8 – 94.8
10	Germinated	95.0 (0.73)	94.0 (0.79)	95.0 – 99.0
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	1.8 (0.58)	2.0 (0.51)	0.5 - 3.6
	Viable Firm-Swollen	3.2 (0.66)	4.0 (0.98)	0.0 – 3.8
20[3]	Germinated	99.3 (0.26)	99.1 (0.23)	96.3 - 99.8
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	0.7 (0.26)	0.9 (0.23)	0.3 – 3.7
	Viable Firm- Swollen†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
30	Germinated	99.4 (0.23)	99.6 (0.19)	97.5 - 99.8
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	0.6 (0.23)	0.4 (0.19)	0.3 - 2.5
	Viable Firm- Swollen†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
10/20	Germinated	99.4 (0.19)	99.5 (0.19)	96.0 - 100.0
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	0.4 (0.15)	0.5 (0.19)	0.0 – 3.8
	Viable Firm- Swollen	0.2 (0.11)	0.0 (0.00)	0.0 - 0.5
10/30	Germinated	99.6 (0.19)	99.5 (0.19)	97.0 – 100.0
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	0.4 (0.19)	0.5 (0.19)	0.0 - 3.0
	Viable Firm- Swollen†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
20/30 (AOSA)	Normal Germinated	98.9 (0.31)	98.8 (0.32)	95.6 – 99.8
	Abnormal Germinated	0.8 (0.25)	0.7 (0.22)	0.0 - 2.0
	Viable Hard†	0.0 (0.00)	0.0 (0.00)	0.0 - 0.0
	Dead	0.3 (0.13)	0.5 (0.23)	0.3 – 2.5
	Viable Firm- Swollen	0.1 (0.08)	0.0 (0.00)	0.0 - 0.1

Note: The experimental design was a split-plot with four replications.
No statistically significant differences were detected between MON 87403 and the conventional control (α =0.05) using ANOVA.
[1]MON 87403 and the conventional control values represent means with standard error (S.E.) in parentheses. N = 12. In some instances, the total percentage of both MON 87403 and the conventional control did not equal 100% due to numerical rounding of the means.
[2]Reference range is the minimum and maximum mean values observed among the 9 commercially available reference hybrids.
[3]Three replicates of the reference Midland Phillips 799 were used from sites ILMN and PAGR.
†No statistical comparison could be made due to lack of variability in the data.

VII.C.2. Field Phenotypic, Agronomic, and Environmental Interactions Characteristics

Phenotypic and agronomic characteristics, and environmental interactions were evaluated under field conditions as part of the plant characterization assessment of MON 87403. These data were developed to provide USDA-APHIS with a detailed description of MON 87403 relative to the conventional control and reference hybrids. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "a detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 87403 and the conventional control that may impact its plant pest/weed potential. Specific characteristics that are related to weediness, (e.g., lodging and ear drop), were used to assess whether there is a potential increase in weediness of MON 87403 compared to conventional maize. Environmental interactions including plant response to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance were also assessed as an indirect indicator of changes to MON 87403 and are also considered in the plant pest assessment.

The results of the assessments of agronomic and phenotypic characteristics demonstrated that the introduction of the increased ear biomass trait did not meaningfully alter the plant pest/weed potential of MON 87403 compared to conventional maize. Furthermore, the lack of meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance also support the conclusion that the introduction of the increased ear biomass trait is not expected to result in increased plant pest/weed potential for MON 87403 compared to conventional maize.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

Data were collected at 13 sites (Table VII-3) in the U.S. during 2012 to evaluate phenotypic and agronomic characteristics of MON 87403 compared to the conventional control. These 13 sites provided a diverse range of environmental and agronomic conditions representative of commercial maize production areas in North America. The experimental design at each site was a randomized complete block with four replications. At all sites, MON 87403, the conventional control, and four reference hybrids were evaluated. A total of 21 unique reference hybrids were evaluated among the 13 sites (Table H-1). The planted plot dimensions varied between sites due to variability in available planting equipment (Table H-2). All plots of MON 87403, the conventional control, and the reference hybrids at each site were uniformly managed in order to assess whether the introduction of the increased ear biomass maize trait altered the phenotypic and agronomic characteristics of MON 87403 compared to the conventional control.

Descriptions of the evaluated phenotypic characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, details concerning the timing of phenotypic assessments, and detailed results of the individual-site data comparisons are presented and discussed in Appendix H (Table H-4). The results of the combined site analyses are summarized below.

In the combined-site analysis, no statistically significant differences were detected between MON 87403 and the conventional control for 12 of 13 characteristics including

early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, plant height, dropped ear count, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and yield (Table VII-4). One significant difference was detected for ear height in the combined-site analysis. MON 87403 had increased ear height (111.1 vs. 107.5 cm) compared to the conventional control. However, the mean value of MON 87403 for ear height was within the reference range for this characteristic and a small ear height difference would not be expected to influence plant weediness. Thus, the measured phenotypic characteristics of MON 87403 were not altered in terms of pest/weed potential compared to conventional maize (Figure 1, step 2, "no" answer) and (Figure 1, step 3, "no" answer).

Plant vigor data were summarized as ranges within individual sites. MON 87403 and the conventional control were considered different if the range of vigor values did not overlap across all four replications. There were no differences observed between MON 87403 and the conventional control in the plant vigor ratings across sites (Table H-4).

The phenotypic and agronomic characteristics evaluated in this study were used to provide a detailed description of MON 87403 compared to the conventional control. A subset of these characteristics was used to assess the weediness of MON 87403. The results of the agronomic and phenotypic assessment demonstrate that there were no changes indicating altered weediness/pest potential of MON 87403 compared to the conventional control. Thus, the introduction of the increased ear biomass trait is not expected to result in increased plant pest/weed potential from MON 87403 compared to conventional maize.

Evaluation of Intended Phenotypic Characteristic:

In other specifically designed experiments, ear biomass data were collected at 13 sites in the U.S. during 2012 to evaluate the intended phenotype of MON 87403 compared to the conventional control (Table VII-1). These 13 sites provided a diverse range of environmental and agronomic conditions representative of commercial maize production areas in North America. The experimental design at each site was a randomized complete block with four replications. At all sites, MON 87403 and the conventional control were evaluated. The harvested area within each plot was 1 m in length. All plots of MON 87403 and the conventional control were uniformly managed in order to assess whether the introduction of the increased ear biomass maize trait demonstrated the intended phenotypic characteristic. Descriptions of the evaluated phenotypic characteristic and the timing of the evaluation are listed in Table VII-1).

As noted previously (Section I.B), in the combined-site analysis, a statistically significant difference was detected between MON 87403 and the conventional control for increased ear biomass (Table I-1). MON 87403 had increased ear biomass (mean 144.5 g/m of row vs. 129.3 g/m of row) compared to the conventional control thereby demonstrating the intended phenotype.

Table VII-3. Field Phenotypic Evaluation Sites for MON 87403 during 2012

Site Code	County, State
ARNE	Jackson, Arkansas
IABG	Greene, Iowa
IARL	Jefferson, Iowa
ILCX	Vermilion, Illinois
ILMN	Warren, Illinois
INSH	Boone, Indiana
KSLA	Pawnee, Kansas
NCBD	Perquimans, North Carolina
NEDC	Butler, Nebraska
NESH	Polk, Nebraska
NEYO	York, Nebraska
PAGR	Lehigh, Pennsylvania
PAHM	Berks, Pennsylvania

Table VII-4. Combined-Site Comparison of MON 87403 to Conventional Control for Phenotypic and Agronomic Characteristics During 2012

	Mean (S.E.)[1]		Reference Range[2]	
Phenotypic Characteristic (units)	MON 87403	Control	Min	Max
Early stand count (#/plot)	83.0(0.92)	83.6 (0.90)	75.4	96.8
Days to 50% pollen shed	63.5 (0.67)	63.0 (0.65)	56.4	71.3
Days to 50% silking	63.9 (0.65)	64.0 (0.70)	56.0	68.8
Stay green rating (1-9 scale)	5.3 (0.36)	5.2 (0.35)	1.0	9.0
Ear height (cm)	111.1 (2.24)*	107.5 (2.25)	86.9	133.2
Plant height (cm)	240.5 (3.69)	239.5 (3.48)	184.2	260.3
Dropped ears (#/plot)	1.2 (0.45)	0.9 (0.36)	0.0	6.1
Stalk lodged plants (#/plot)	2.4 (0.58)	2.2 (0.53)	0.0	10.8
Root lodged plants (#/plot)	0.7 (0.26)	0.5 (0.17)	0.0	1.0
Final stand count (#/plot)	72.5 (0.72)	72.0 (0.70)	63.8	76.0
Grain moisture (%)	19.1 (0.61)	19.0 (0.58)	14.0	28.7
Test weight (kg/hl)	72.6 (0.69)	72.7 (0.67)	67.9	79.2
Yield (Mg/ha)	10.1 (0.45)	10.3 (0.52)	6.3	14.3

* Indicates statistical difference between MON 87403 and the conventional control (α =0.05).
Note: The experimental design was a randomized complete block with four replicates per site.
[1] Test and control values represent means with standard error in parentheses. N = 51.
[2] Reference range is calculated from the minimum and maximum mean values from among 21 unique reference materials.

VII.C.2.2. Environmental Interaction Characteristics

USDA-APHIS considers the environmental interaction of the biotechnology-derived crop compared to its conventional control to determine the potential for increased plant pest characteristics. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 87403. In the 2012 U.S. field trials conducted to evaluate the phenotypic and agronomic characteristics of MON 87403, data were also collected on plant response to abiotic stress (e.g., drought, wind, nutrient deficiency, etc.), disease damage, arthropod-related damage, and arthropod abundance (Tables VII-5 through VII-7 and H-5 through H-10). These data were used to assess plant pest potential (Section IX) compared to the conventional control. The results of the field evaluations showed that the increase ear biomass trait did not unexpectedly alter the assessed environmental interactions of MON 87403 compared to the conventional control. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance support the conclusion that the introduction of the increased ear biomass trait is not expected to result in increased plant pest potential from MON 87403 compared to commercial maize.

VII.C.2.2.1. Qualitative Environmental Interactions Assessment

Plant responses to abiotic stressors, disease damage, and arthropod damage were assessed at natural levels, i.e., no artificial infestation or imposed abiotic stress; therefore these levels typically varied between observations at a site and among sites. Plant responses to abiotic stress, disease damage, and arthropod damage data were collected from each plot using a categorical scale (none, slight, moderate, and severe) of increasing severity of observed damage for each stressor. This scale was utilized to allow for the evaluation of the wide variety of potential abiotic stressors, disease damage, and arthropod damage symptoms potentially occurring across the season and across sites. These data were categorical and therefore were summarized and not subjected to ANOVA. For a particular stressor, all comparisons of the range of responses for MON 87403 to the range of responses for the conventional control across all observation times and sites are reported.

Descriptions of the evaluated environmental interactions characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, additional details concerning the qualitative environmental interactions assessments, and detailed results of the qualitative data comparisons are presented and discussed in Appendix H (Tables H-5 through H-7).

In the qualitative assessment, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 143 comparisons of plant response to abiotic stressors, including cold, drought, flood, frost, hail, heat, nutrient deficiency, soil compaction, sunscald, and wind (Tables VII-5 and H-5). Additionally, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 176 comparisons for plant damage caused by diseases, including anthracnose, bacterial leaf spot, ear rot, eyespot, Fusarium sp., Goss's bacterial wilt, gray leaf spot, leaf blight, maize rough dwarf virus,

Pythium sp., *Rhizoctonia* sp., rust, seedling blight, smut, stalk rot, and Stewart's bacterial wilt (Tables VII-5 and H-6). Finally, no differences in the range of responses were observed between MON 87403 and the conventional control for any of the 150 comparisons for plant damage caused by arthropods, including aphid, armyworm, billbug, cutworm, corn earworm, corn flea beetle, rootworm beetle, European corn borer, grasshopper, Japanese beetle, sap beetle, spider mite, stink bug, and wireworm adult (Tables VII-5 and H-7).

The lack of differences observed between MON 87403 and the conventional control for plant responses to abiotic stressors, disease damage, and arthropod-related damage in multiple environments across the U.S. supports the conclusion that the introduction of the increased ear biomass trait is not expected to cause a biologically meaningful change in terms of plant pest potential compared to the conventional control (See Section VII.B.2).

Table VII-5. Summary of Qualitative Environmental Interactions Assessments during 2012

Stressor	Number of observations across all sites	Number of observations with no differences between MON 87403 and the conventional control across all sites[1]
Abiotic stressors	143	143
Disease damage	176	176
Arthropod-related damage	150	150
Total	469	469

Note: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 87403 and the conventional control during any observation for damage caused by any of the assessed stressors.
[1]MON 87403 and the conventional control were considered different in susceptibility or tolerance if the range of injury symptoms across four replications did not overlap between MON 87403 and the conventional control.

VII.C.2.2.2. Quantitative Environmental Interactions Assessment

Quantitative arthropod assessments on corn earworm (CEW: *Helicoverpa zea*) damage, European corn borer (ECB: *Ostrinia nubilalis*) damage, and arthropod abundance were conducted at four sites (Table VII-3: IABG, NEYO, NCBD, and PAHM). CEW and ECB damage was assessed once during the growing season at each site. Arthropod abundance was assessed from collections performed five times during the growing season at each site using sticky traps and visual counts.

Descriptions of the evaluated environmental interactions characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, additional details concerning the specific arthropod damage assessments, sticky traps, and visual counts with detailed results of the individual-site data comparisons are presented and discussed in Appendix H (Tables H-8 through H-10). The results of the combined-site analysis are summarized below.

In the combined-site analysis, no statistically significant differences (α=0.05) were detected between MON 87403 and the conventional control for plant damage caused by CEW or ECB (Table VII-6). Considering this lack of significant differences, the results indicate no changes in susceptibility or resistance of MON 87403 to these common maize pests (See Figure VII-1, Step 2, answer "no").

In an assessment of arthropod abundance from sticky traps, no statistically significant differences were detected between MON 87403 and the conventional control for 130 out of 144 comparisons among the collections at the four sites (Tables VII-7 and H-9). The mean abundance values for MON 87403 were within the respective ranges of commercial reference hybrids for seven of the 14 detected differences. For the remaining seven differences, the mean abundance values from MON 87403 were outside the reference range; however, these differences were not consistently detected across collections or sites. Thus, these differences were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of increased pest potential of MON 87403 compared to the conventional control (See Section VII.B.2)

In an assessment of arthropod abundance from visual counts, no statistically significant differences were detected between MON 87403 and the conventional control for 61 out of 66 comparisons among the collections at the four sites (Tables VII-7 and H-10). The mean abundance values for MON 87403 were within the respective ranges of commercial reference hybrids for three of the five detected differences. For the remaining two differences, the mean abundance values from MON 87403 were outside the reference range; however, these differences were not consistently detected across collections or sites. Thus, these differences were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of increased pest potential of MON 87403 compared to the conventional control (See Section VII.B.2).

Table VII-6. Combined-Site Comparison of CEW and ECB Damage to MON 87403 Compared to the Conventional Control during 2012

Pest Arthropod	Assessment Timing	Damage assessment	Mean (S.E.)[1]		Reference range[2]
			MON 87403	Control	
Corn earworm (*H. zea*)	R5	Damage area of 10 plants per plot (cm^2)	1.8 (0.41)	2.7 (0.78)	0.0 – 5.0
European corn borer (*O. nubilalis*)	R6	Number of stalk galleries of 10 plants per plot	0.3 (0.12)	0.3 (0.11)	0.0 – 1.2
European corn borer (*O. nubilalis*)	R6	Stalk gallery length (cm) of 10 plants per plot	1.3 (0.57)	1.4 (0.50)	0.0 – 4.7

Note: The experimental design was a randomized complete block with four replicates per site.
No statistically significant differences were detected between MON 87403 and the conventional control (α=0.05) using ANOVA.
[1] MON 87403 and the conventional control values represent means with standard error in parentheses. N = 16.
[2] Reference range is calculated from the minimum and maximum mean values from among 11 unique reference materials across all sites (IABG, NCBD, NEYO, and PAHM).

Table VII-7. Summary of Arthropod Abundance Assessments and Detected Differences during 2012

Summary of Statistical Comparisons[1]				Summary of Detected Differences[2]				
Arthropod Abundance Assessment	Number of sites	Number of comparisons across sites	Number of comparisons where no differences were detected	Arthropod	Site	Collection Number	Within reference range?	Consistently detected across collections or sites?
Sticky Traps	4	144	130	Lacewings	IABG	1	Yes	No
				Lacewings	NEYO	3	Yes	No
				Lacewings	NEYO	5	No	No
				Macro-parasitic hymenoptera	PAHM	4	No	No
				Micro-parasitic hymenoptera	NCBD	2	Yes	No
				Aphids	NEYO	5	Yes	No
				Corn flea beetles	IABG	3 & 5	No	No
				Corn rootworm beetles	NEYO	3	Yes	No
				Thrips	NCBD	2	Yes	No
				Thrips	NEYO	2 & 4	No	No
				Thrips	NEYO	5	Yes	No
				Thrips	PAHM	3	No	No
Visual Counts	4	66	61	Minute pirate bugs	PAHM	4	No	No
				Corn rootworm beetles	PAHM	1	No	No
				Sap beetles	NCBD	5	Yes	No
				Sap beetles	NEYO	3	Yes	No
				Shining flower beetles	NCBD	2	Yes	No

[1]Quantitative arthropod abundance assessments were statistically analyzed at α=0.05 using ANOVA. Lack of sufficient arthropod abundance precluded statistical comparisons between MON 87403 and the conventional control for additional 92 comparisons (sticky traps) and 171 comparisons (visual counts); however, descriptive statistics were provided for these comparisons in Appendix H (Tables H-9 and H-10).

[2]Nineteen statistically significant differences were detected. These differences are further discussed in Section VII.C.2.2 using the approach outlined in Section VII.B.2.

VII.C.3. Pollen Characteristics

USDA-APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait into sexually compatible plants and wild relatives to determine the potential for increased weedy or invasive characteristics of the receiving species. Pollen morphology and viability information are pertinent to this assessment and, therefore, were assessed for MON 87403. In addition, morphological characterization of pollen produced by MON 87403 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87403 compared to the conventional control.

The viability and morphology of pollen collected from MON 87403 compared to that of the conventional control were assessed. Pollen was collected from MON 87403, the conventional control, and four commercial references (Table I-1) grown under similar agronomic conditions at the field site in Story County, Iowa, a geographic area that represents environmentally relevant conditions for maize production for this product. The trial was arranged in a randomized complete block design with four replications. Once all plants across the replications reached the flowering stage, pollen was collected from three non-systematically selected plants per plot and stained for assessment. Descriptions of the evaluated pollen viability and morphology characteristics and the timing of the evaluations are listed in Table VII-1. The details of the materials and experimental methods used in this evaluation are presented in Appendix I: .

No statistically significant differences (α=0.05) were detected between MON 87403 and the conventional control for percent viable pollen or pollen grain diameter (Table VII-8). Furthermore, no visual differences in general pollen morphology were observed between MON 87403 and the conventional control (Figure I-1).

The pollen characterization data contribute to the detailed phenotypic description of MON 87403 compared to the conventional control. Based on the assessed characteristics, the results support a conclusion that neither pollen viability nor morphology of MON 87403 were altered compared to conventional maize.

Table VII-8. Pollen Characteristics of MON 87403 Compared to the Conventional Control during 2012

Pollen Characteristic (unit)	Mean (S.E.)[1]		Reference Range[2]
	MON 87403	Control	
Viability[3] (%)	97.8 (0.70)	98.7 (0.36)	97.3 – 99.3
Diameter[4] (µm)	81.8 (1.20)	84.6 (2.06)	80.5 – 83.7

Note: No significant differences were detected between the MON 87403 and the conventional control (α=0.05).

[1] MON 87403 and the conventional control values represent means with standard error (S.E.) in parentheses. N=4.

[2] Reference range is the minimum and maximum mean value observed among the four reference hybrids.

[3] Evaluated from three sub-samples per replication at 40X magnification.

[4] Evaluated from 10 representative viable pollen grains per replication at 200X magnification.

VII.D. Conclusions for Phenotypic, Agronomic, and Environmental Interactions Evaluation

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential as assessed by USDA-APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest potential of the biotechnology-derived crop compared to the conventional crop. Ultimately, a weight of evidence approach that considers all characteristics and data is used for the overall risk assessment of differences and their significance.

An extensive and robust set of agronomic, phenotypic, and environmental interactions data, including specific weedy characteristics, were used to assess whether the introduction of the increased ear biomass trait altered the plant pest potential of MON 87043 compared to the conventional control, considered within the context of the variation among the reference hybrids. These assessments included six general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) lodging and seed retention on the plant; 5) plant response to abiotic stress and interactions with diseases and arthropods; and 6) intended phenotype (increased ear biomass). Within these data categories, data relevant to understanding specific characteristics associated with weediness were also assessed to determine whether there was a potential increase in weediness of MON 87403 compared to conventional maize.

Results from these assessments comparing MON 87403 and the conventional control demonstrate that MON 87403 does not possess: 1) increased weediness characteristics; 2) increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods; or 3) characteristics that would confer a plant pest risk compared to conventional maize. Therefore, based on the results of multiple assessments discussed above and presented in the appendices, the weight of evidence indicates that MON 87403 is not meaningfully different from conventional maize with the exception of the increased ear biomass trait and is not expected to pose a plant pest risk compared to conventional maize.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts of deregulation on agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. and North America for producing maize, and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 87403. Discussions include maize production, plant growth and development, general management practices during the season, management of weeds, insects and diseases, maize rotational crops, and volunteer management. Information presented in the previous section demonstrated that MON 87403 is no more susceptible to diseases or pests than commercially cultivated maize. Additionally, data presented in this section show that MON 87403 is not expected to pose a plant pest risk compared to conventional maize. MON 87403 provides an increased ear biomass at the R1 reproductive stage derived from the ATHB17Δ113 protein and hence an increased yield opportunity. Thus, there are no expected changes to the inputs needed for MON 87403, and no expected impacts to agronomic practices employed for production of maize compared to the current situation.

VIII.B. Overview of U.S. Maize Production

VIII.B.1. Maize Production

The U.S., China, Brazil, Ukraine, and Argentina are the top five countries producing maize globally (USDA-FAS 2014). As noted in Section I.B, maize (*Zea mays* L.) is the largest crop grown in the U.S. in terms of acreage planted (95.4 million acres in 2013) (Table VIII-1) (USDA-NASS 2014c), exceeding soybean and wheat with acreages of 76.5 (USDA-NASS 2014b) and 56.2 million acres (USDA-NASS 2014a), respectively. The value of maize reached $62.7 billion in the United States in 2013, exceeding soybeans and wheat with values of $41.8 and $14.4 billion, respectively (USDA-NASS 2013a). The principal uses of maize are feed and residual, ethanol fuel, export, and high-fructose corn syrup (Capehart et al. 2012).

The planting of 95.4 million acres of maize in 2013 was down 1.8 million acres from 2012 (Table VIII-1). Much of that production occurs in upper Midwest states (Figure VIII-1). Of the 2013 acreage planted, approximately 87.7 million acres were harvested for grain and 6.3 million acres were harvested for silage (USDA-NASS 2013b). Total production was approximately 13.9 billion bushels with an average yield of 158.8 bushels per acre (Figure VIII-1). The value of maize grain production in the U.S. has ranged from $46.7 to $77.4 billion in the past 6 years (Table VIII-1).

Table VIII-1. Maize Production in the U.S., 2008-2013

Year	Acres Planted (x 1,000)	Acres Harvested (x 1,000)	Production (x 1,000 $)	Production (x 1,000 bushels)	Yield (bushels/acre)
2013	95,365	87,668	62,716,048	13,925,147	158.8
2012	97,155	87,375	74,330,610	10,780,296	123.4
2011	91,936	83,989	76,939,603	12,359,612	147.2
2010	88,192	81,446	64,643,295	12,446,865	152.8
2009	86,382	79,490	46,734,066	13,091,862	164.7
2008	85,982	78,570	49,312,615	12,091,648	153.9

Source: (USDA-NASS 2013b)

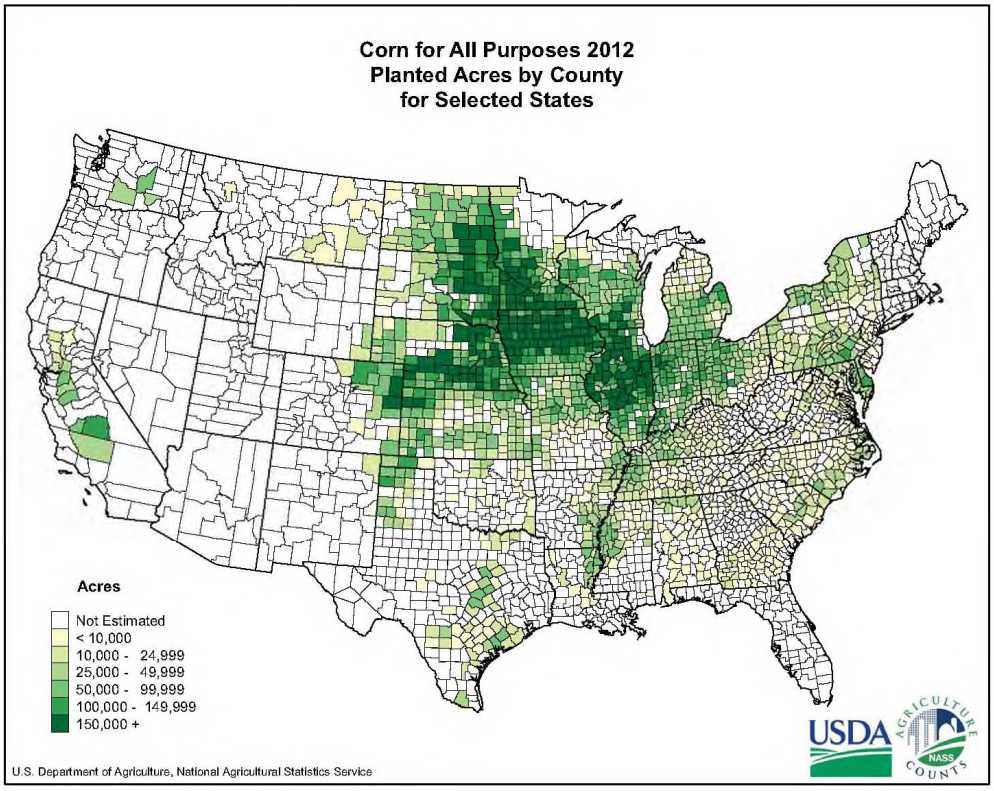

Figure VIII-1. Planted Maize Acres by County in the U.S. in 2012
Source: (USDA-NASS 2012)

VIII.C. Management of Insect Pests

Monsanto summarized major issues associated with the management of insect pests in its petition for nonregulated status for Corn Rootworm-Protected Maize MON 87411 (Petition #13-290-01p) (Monsanto 2013). None of the information on this subject has changed in any substantive way and is incorporated here by reference (Monsanto 2013) (Section IX.D). In brief, insect pests continue to cause damage to maize and are commonly addressed by insecticide treatment of seeds, soil, or over-the-top application of insecticides, or use of a number of crop rotation or integrated pest management practices.

MON 87403 was developed to increase the yield opportunity of commercial maize hybrids and has no unique pest control attributes. Thus, no changes to insect pest control practices are expected from use of MON 87403.

VIII.D. Management of Diseases and Other Pests

Monsanto summarized major issues associated with the management of diseases and other pests in its petition for nonregulated status for Corn Rootworm-Protected Maize MON 87411 (Petition #13-290-01p) (Monsanto 2013). None of the information on this subject has changed in any substantive way and is incorporated here by reference (Monsanto 2013) (Section IX.E). Briefly, management of diseases and pests of maize are important to protecting the yield of harvested grain. Disease and pest incidence varies from year to year and growers may choose to use pesticides or a variety of management practices to control problem diseases or pests.

Environmental observations in field studies have demonstrated no apparent impact of MON 87403 on diseases of maize (Section VII.C.2). Therefore, no changes in current disease management practices are anticipated from the introduction of MON 87403.

VIII.E. Weed Management

Monsanto summarized major issues associated with the management of weeds in its Petition for nonregulated status for corn rootworm-protected MON 87411 (Petition #13-290-01p) (Monsanto 2013). None of the information on this subject has changed in any substantive way and is incorporated here by reference (Monsanto 2013) (Section IX.F). Briefly, weed control in maize is essential to optimizing yield because weeds compete with maize for light, nutrients, and moisture.

MON 87403 was developed to increase the yield opportunity of commercial maize hybrids and has no unique herbicide tolerance traits. Thus, no changes to herbicide use or weed resistance management practices are expected from use of MON 87403.

VIII.F. Crop Rotation Practices in Maize

Crop rotation is a well-established farming practice and a useful management tool for maize production. Crop rotations are used to diversify farm income, spread labor requirements throughout the year, and spread the crop loss risk associated with weather

and pest damage across two or more crops. In terms of soil and pest management, rotations are used to 1) manage weed, insect, and disease pests, 2) reduce soil erosion by wind and water, 3) maintain or increase soil organic matter, 4) provide biologically fixed nitrogen when legumes are used in the rotation, and 5) manage excess nutrients (Singer and Bauer 2009). Studies in U.S. corn belt states indicate maize yield is about 10-15% higher in maize grown following soybean than maize grown following maize (Singer and Bauer 2009). Despite the many benefits of crop rotations, crop price fluctuations, input costs, rental agreements, government price supports, weather, choice of farming system and on-farm resources, and other factors all contribute to decisions regarding crop rotations. Market conditions such as U.S. government-mandated ethanol use and record high commodity maize prices have increased the demand for maize grain and resulted in increases in maize acreage in recent years (Singer and Bauer 2009; USDA-NASS 2013b). Introduction of MON 87403 is not, however, expected to impact crop rotation practices any more so than current biotechnology-derived products available to growers.

VIII.G. Maize Volunteer Management

Volunteer maize commonly occurs in rotational crops in the season following cultivation of conventional or biotechnology-derived maize. Viable grain is not produced on the approximately 8% of U.S. maize acres that are cultivated for the production of silage, and volunteer maize plants typically do not occur in the rotational crops that follow maize harvested as silage. In the warmer climates of the Southeast and Southwest, volunteer maize is rare because maize grain remaining after harvest is likely to germinate in the fall and the resulting plants can usually be controlled by tillage or by freezing temperatures in the winter. In northern maize-growing regions, volunteer maize does not always occur in rotational crops because of seed decomposition over the winter, efficient harvest procedures, and tillage prior to planting rotational crops. None of the agronomic or phenotypic data collected for MON 87403 (Section VIII.C), including data regarding germination and seed dormancy, indicates that MON 87403 is any more likely to volunteer than existing commercial maize hybrids.

Management of volunteer maize in rotational crops involves minimizing or reducing the potential for volunteers through practices that include: 1) adjusting harvest equipment to minimize the amount of grain lost in the field; 2) planting hybrids that reduce the extent of ear drop; 3) choosing hybrids with superior stalk strength and reduced lodging; and 4) practicing no-till production to significantly reduce the potential for volunteer growth in rotational crops. If volunteer maize does occur in subsequent crops, pre-plant tillage, in-crop cultivation and the use of selective herbicides are very effective management tools. Introduction of MON 87403 is not expected to impact maize volunteer management practices any more so than existing commercial maize hybrids.

VIII.H. Stewardship of MON 87403

Monsanto develops effective products and technologies that deliver value to growers and conserve resources that agriculture depends on, and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes

throughout the lifecycle of a product and by participation in the Excellence Through Stewardship® (ETS) Program (BIO 2010). ETS policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides (Monsanto Company 2013) and Monsanto Technology Stewardship Agreements that are signed by growers who utilize Monsanto branded traits, to ensure stewardship compliance.

As an integral action of fulfilling this stewardship commitment, Monsanto will seek biotechnology regulatory approvals for MON 87403 in all key maize importing countries with functioning regulatory systems to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization Policy on Product Launch (BIO 2010). Monsanto continues to monitor other countries that are key importers of maize from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

Monsanto also commits to industry best practices on seed quality assurance and control to ensure the purity and integrity of MON 87403 seed. As with all of Monsanto's products, before commercializing MON 87403 products in any country, the appropriate detection methods will be made available to maize producers, processors, and buyers.

VIII.I. Impact of the Introduction of MON 87403 on Agricultural Practices

MON 87403 has been developed to provide an increased yield opportunity in maize hybrids that will be grown in the U.S. The introduction of MON 87403 is not expected to have major impacts on current agronomic, cultivation and management practices for maize. No changes are anticipated in crop rotations, tillage practices, planting practices, fertility management, weed and disease management, and volunteer management from the introduction of MON 87403.

MON 87403 has been shown to be comparable to conventional maize in its compositional, phenotypic, and agronomic characteristics (Sections VI and VII). When introgressed into existing biotechnology-derived maize hybrids that contain insect protection and herbicide tolerance traits, MON 87403 is expected to continue to provide benefits to growers, that include reduced use of insecticides , increased yield protection and opportunity, water conservation , and increased worker safety.

IX. PLANT PEST ASSESSMENT

This section provides a brief review and assessment of the plant pest potential of MON 87403 and its impact on agronomic practices and the environment. USDA-APHIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. Regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

According to PPA, the definition of "plant pest" includes the living stage of any of the following, or a similar article that can directly or indirectly injure, damage, or cause disease in any plant or plant product: (A) a protozoan; (B) a nonhuman animal; (C) a parasitic plant; (D) a bacterium; (E) a fungus; (F) a virus or viroid; or (G) an infectious agent or other pathogens (7 U.S.C. § 7702[14]).

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not expected to pose a plant pest risk. Information in this petition related to plant pest risk characteristics includes: 1) mode-of-action and changes to plant metabolism; 2) composition; 3) expression and characteristics of the gene product; 4) potential for weediness of the regulated article; 5) impacts to NTOs; 6) disease and pest susceptibilities; 7) impacts on agronomic practices; and 8) impacts on the weediness of any other plant with which it can interbreed, as well as the potential for gene flow. Using the assessment above, the data and analysis presented in this petition lead to a conclusion that MON 87403 is not expected to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR § 340.

IX.A. Characteristics of the Genetic Insert and Expressed Product

IX.A.1. Genetic Insert

As described in Section III, MON 87403 was developed by *Agrobacterium*-mediated transformation of maize embryos using plasmid vector PV-ZMAP5714. Characterization of the DNA insert in MON 87403 was conducted using a combination of sequencing, PCR, and bioinformatics methods. The results of this characterization demonstrate that MON 87403 contains one copy of the intended T-DNA containing the *ATHB17* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These methods also confirmed that no vector backbone or other unintended plasmid sequences are present in MON 87403. Additionally, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87403 to the sequence of the insertion site in conventional maize. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 87403 upon DNA integration.

IX.B. Expression and Characterization of Gene Product

IX.B.1. Protein Safety and Expression Levels

The safety and expression of the ATHB17Δ113 protein is detailed in Section V. Expression levels were determined from four tissue types from trials conducted in 2012 in the United States and are presented in Section V.C. The expression in the various tissues ranged from <LOD (<0.00028 µg/g dw) to 0.017 µg/g dw. ATHB17Δ113 represents a very small percentage of the total protein in maize seed (no more than 0.001%). The ATHB17Δ113 protein originates from *Arabidopsis thaliana,* and no Arabidopsis proteins have been reported in the peer-reviewed database of known allergens (FARRP 2013). Futhermore, bioinformatic assessments show that the ATHB17Δ113 protein does not show homology to any known allergens, toxins, or any other protein with known adverse effects. These assessments also showed that the amino acid sequence of the ATHB17Δ113 protein shares 58%-83% sequence identity to proteins present in a variety of food crops including corn, rice, sorghum, cruciferous vegetables, tomato, potato, papaya, orange and grape, indicating that ATHB17Δ113 is very similar to proteins that have a robust history of safe use. Finally, the protein is rapidly digested in simulated gastric fluid and simulated intestinal fluid assays (Section V.D) and a mouse gavage study demonstrated no acute oral toxicity with a NOAEL for ATHB17Δ113 of 1335 mg/kg, further supporting a conclusion that this protein is safe for consumption.

IX.C. Compositional Characteristics

Compositional comparisons based on OECD guidance were presented in Section VI to assess whether levels of nutrients, anti-nutrients, and secondary metabolites in grain and forage derived from MON 87403 are comparable to levels in the conventional control and several reference hybrids for which there is an established history of safe consumption. Nutrients assessed in this analysis included proximates, carbohydrates by calculation, acid detergent fiber, neutral detergent fiber, total dietary fiber, minerals, amino acids, and vitamins. Anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid. Forage samples were assessed for levels of proximates, fiber, minerals (calcium and phosphorus), and carbohydrates by calculation. In all, 78 different components were assayed (nine in forage and 69 in grain). Of those 78 components, 14 fatty acids, sodium, and furfural had more than 50% of observations below the assay limit of quantitation (LOQ) and were excluded from statistical analysis. Moisture in grain and forage was measured for conversion of components to dry weight, but was not statistically analyzed. Therefore, 60 components were statistically analyzed.

A combined-site analysis of the data was conducted to determine statistically significant differences (at 5%) between MON 87403 and the conventional control. The biological significance of differences from the data was reviewed using considerations relevant to food and feed safety and nutritional quality. These considerations included: 1) a determination of the mean differences of nutrient and anti-nutrient components of MON 87403 and the conventional control; 2) consideration of the mean differences

between MON 87403 and the conventional control in the context of the range of values for the conventional control, 3) comparison of the mean differences between MON 87403 and the conventional control to variation in conventional maize as estimated by in-study reference hybrid values and assessing whether the mean values for MON 87403 were within 99% tolerance intervals, literature values, and/or ILSI-CCDB values. These evaluations of variation within the conventional control and conventional maize, including reference hybrids, are important as crop composition is known to be influenced by environment and germplasm.

The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients, and secondary metabolites in maize grain and forage of MON 87403 and the conventional control. Of the 60 components statistically assessed there were no statistically significant differences in any component. These results support the overall conclusion that MON 87403 was compositionally equivalent to the conventional control.

IX.D. Phenotypic, Agronomic, and Environmental Interaction Characteristics

An extensive set of comparative plant characterization data were used to assess whether the introduction of an increased ear biomass trait altered the plant pest potential of MON 87403 compared to the conventional control (Section VII). Phenotypic, agronomic, and environmental interaction characteristics of MON 87403 were evaluated and compared to those of the conventional control. As described previously, these assessments included: seed dormancy and germination characteristics; agronomic and plant phenotypic characteristics; observations for abiotic stress response, disease damage, arthropod-related damage, arthropod abundance, and pollen characteristics. Results from all phenotypic, agronomic, and environmental interaction assessments demonstrated that MON 87403 does not possess weedy characteristics, or increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors compared to the conventional control. Taken together, the results of the analysis support a determination that MON 87403 is no more likely to pose a plant pest risk than conventional maize.

IX.D.1. Seed Dormancy and Germination

A comparative assessment of seed germination and dormancy characteristics was conducted on MON 87403 and the conventional control. The results of this assessment, particularly the lack of increased hard seed, and no changes in other germination and dormancy characteristics, support the conclusion that the introduction of MON 87403 is not expected to result in increased plant pest/weed potential compared to conventional maize.

IX.D.2. Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as stalk and root lodging. Phenotypic characteristics such as early stand count, days to 50% pollen shed and silking, stay green,

ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, R1 ear biomass, and yield were assessed. In the combined-site analysis in which the data were pooled among the sites, no statistically significant differences, except for ear height, were detected between MON 87403 and the conventional control for any of the assessed characteristics. Thus, the phenotypic and agronomic characteristics of MON 87403 were not altered in terms of pest/weed potential compared to conventional maize.

IX.D.3. Response to Abiotic Stressors

No differences were observed during comparative field observations between MON 87403 and the conventional control with respect to responses to abiotic stressors such as drought, mineral and nutrient toxicity, or temperature stress. The lack of significant differences in the MON 87403 response to abiotic stress supports the conclusion that the introduction of the ATHB17Δ113 protein is unlikely to result in increased pest/weed potential compared to conventional maize.

IX.D.4. Pollen Morphology and Viability

Evaluations of pollen morphology and viability from field-grown plants provide useful information in a plant pest assessment as it relates to the potential for gene flow to, and possible introgression of a biotechnology-derived trait into sexually-compatible plants and wild relatives. No statistically significant differences were detected between MON 87403 and the conventional control for percent viable pollen or pollen grain diameter. Additionally, no visual differences in general pollen morphology were observed between MON 87403 and the conventional control. Based on the assessed characteristics, the results support a conclusion that neither pollen viability nor morphology of MON 87403 were altered and therefore do not contribute to an increased pest/weed potential compared to conventional maize.

IX.E. Impact to Non-target Organisms Including Those Beneficial to Agriculture or Threatened and Endangered Species

Data from 2012 U.S. phenotypic and agronomic studies and observational data on environmental interactions such as plant-disease interactions, arthropod damage and arthropod abundance, were collected at select sites for MON 87403 and conventional controls. Results from these extensive studies support conclusions of no adverse impacts to non-target arthropod populations and no changes to plant-disease interactions.

As also noted, the ATHB17Δ113 protein belongs to a common class of plant protein transcription factors (HD-Zip) and shares protein sequence homology with a number of common food crops that are consumed widely by humans and animals without noted allergenic or toxic effects. Bioinformatics analyses also assessed the potential for allergenicity, toxicity, or biological activity of ATHB17Δ113. Those analyses demonstrated that ATHB17Δ113 protein does not share amino acid sequence similarity with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health. Additionally, a mouse gavage study using

ATHB17Δ113 protein also supports a conclusion of safety from consumption of MON 87403 by vertebrate organisms. Taken together, these data, along with the compositional equivalence data noted above (Section VI.A), support the conclusion that MON 87403 has no reasonable mechanism for harm to NTOs, or impact on threatened and endangered species compared to cultivation of conventional maize.

IX.F. Weediness Potential of MON 87403

Maize is not listed as a weed in the major weed references (Crockett 1977; University of Montana 2011), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR § 360). In addition, maize has been grown throughout the world without any report that it is a serious weed. During domestication of maize, traits often associated with weediness, such as, seed dormancy, a seed dispersal mechanism, or the ability to form reproducing populations outside of cultivation, have not been selected. Even if individual kernels of maize were distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable volunteer maize populations are not found growing in fence rows, ditches, or road sides. Maize is poorly suited to survive without human assistance and is not capable of surviving as a weed (Galinat 1988; Keeler 1989).

In comparative studies between MON 87403 and a conventional control, phenotypic, agronomic and environmental interaction data were evaluated (Section VII) for changes that would impact the plant pest potential, in particular, plant weediness potential. Results of these evaluations show that there is no fundamental difference between MON 87403 and the conventional control for traits potentially associated with weediness. The only difference noted related to ear height and that characteristic is not known to contribute to weediness. Furthermore, comparative field observations between MON 87403 and its conventional control and their response to abiotic stressors indicated no differences and, therefore, no increased weediness potential. Collectively, these findings support the conclusion that MON 87403 has no increased weed potential compared to conventional maize and it is no more likely to become a weed than conventional maize.

IX.G. Potential for Pollen Mediated Gene Flow and Introgression

Pollen mediated gene flow is a process whereby one or more genes successfully integrate into the genome of a recipient plant. Introgression is affected by both biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and relative humidity, and field architecture. Because gene introgression is a natural biological process, it does not constitute an environmental risk in and of itself. Gene introgression must be considered in the context of the transgenes inserted into the biotechnology derived plant, and the likelihood that the presence of the transgenes and their subsequent transfer to recipient plants will result in increased plant pest potential. The potential for gene introgression from MON 87403 is discussed below.

IX.G.1. Hybridization with Cultivated Maize

Maize morphology fosters cross pollination, therefore, high levels of pollen mediated gene flow can occur in this species. In addition, researchers recognize that (1) the amount of gene flow that occurs can be high because of open pollination; (2) the percent gene flow can vary by population, hybrid or inbred; (3) the level of gene flow decreases with greater distance between the source and recipient plants; (4) environmental factors affect the level of gene flow; (5) maize pollen is viable for a short period of time under field conditions; (6) maize produces ample pollen over an extended period of time; and, (7) maize is almost exclusively wind pollinated.

Based on several studies conducted on the extent of pollen mediated gene flow between maize fields, results were found to vary depending on the experimental design, environmental conditions, and detection method, as expected. In general, the percent of gene flow diminished with increasing distance from the source field, generally falling below 1% at distances >200 m (~660 feet) (Table IX-1). This information is useful for managing gene flow during maize breeding, seed production, identity preservation or other applications; in addition, it forms the basis for the USDA-APHIS performance standards for maize. All testing and production of regulated MON 87403 seed or grain have been conducted under USDA notification according to these standards. Gene flow from fields planted with MON 87403 to other maize would not be of concern because of the lack of potential to cause harm to humans and to the environment.

Table IX-1. Summary of Published Literature on Maize Cross Pollination

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
~1 25 75 125 200 300 400 500	28.6 14.2 5.8 2.3 1.2 0.5 0.2 0.2	Frequencies of outcrossing by distance. Three year study. Pollen source was a yellow dent and the pollen recipient was a white sweet maize.	USA	(Jones and Brooks 1950)
100 150 200 300 400	0.01 0.00 0.01 0.00 0.00	Frequencies of outcrossing by distance and pollen viability. Two year study. A purple gene marker was utilized to measure pollen mobility.	Mexico	(Luna et al. 2001)
1 5 10 14 19 24 28 33 36	9.7-19.0 1.3-2.6 0.7-2.0 0.3-0.6 0.4 0-0.3 0.1-0.5 0-0.3 0-0.1	Frequency of outcrossing by distance. Conducted over three years and three sites. Single male and female per location.	Canada	(Ma et al. 2004)

Table IX-1 (continued). Summary of Published Literature on Maize Cross Pollination

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
24-32 60-62 123-125 244-254 486-500 743-745	0.01-0.7 0.01-0.2 0.001-0.08 0-0.02 0-0.005 0-0.002	Frequencies of outcrossing by distance. One pollen donor and 7 pollen recipients had different relative maturities. The pollen donor had the genetic markers P1-rr and R1-nj. Pollination of the pollen recipient caused the female with typically a yellow kernel to produce a kernel with a purple coloration. Conducted over two years/two sites. Values reported here are from one site.	USA	(Halsey et al. 2005)
1 10 35 100 150 200 250	17.0-29.9 1.5-2.5 0.4 0.03-0.05 0.01-0.03 0.007-0.03 0.002-0.03	Frequency of outcrossing by distance. Pollination was quantified by measuring outcrossing from a transgenic hybrid plot to a conventional grain production field. A combination of three marker genes were utilized to detect outcrosses: y1 (seed color gene), *Bt* and glyphosate tolerance. Two years/two sites.	USA	(Goggi et al. 2006)
~1 2 5 10 20 40 80	3-13 0.2-10 0.1-2.3 0.2-3.7 0.1-0.8 0-0.7 0.1-0.2	The main objective of the study was to compare a PCR based method to measure outcrossing rates determined by phenotypic analysis. Four *Bt* hybrids and a single non-*Bt* hybrid were used as a pollen donor and reciepient, respectively. Conducted in one year/one site.	Spain	(Pla et al. 2006)

Table IX-1 (continued). Summary of Published Literature on Maize Cross Pollination

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
2 5 10 20 25 50 150 200	34.9 9.9 12.2 8.2 4 5.9 5.4 0.24	Frequency of outcrossing (expressed as % GM DNA) by distance. The study was conducted in a large farm scale evaluation across the UK. Values reported here are maximum raw values	UK	(Weekes et al. 2007)
52 85 105 125 149 150 200 287 371 402 458 4125 4440	0.009 0.015 0.003 0.01 0.016 0.007 0.009 0.005 0.008 0.005 0.0002 0.006 0.0005	Frequency of outcrossing by distance. Outcrossing was measured using the occurrence of yellow kernels in 13 white kernel maize fields.	Switzerland	(Bannert and Stamp 2007)

IX.G.2. Hybridization with Wild Annual Species of *Zea mays* subsp. *mexicana*

For gene flow to occur by normal sexual transmission, the following conditions must exist: (1) the two parents must be sexually compatible; (2) there must be overlapping flowering times; and (3) a suitable factor (such as wind or insects) must be present and capable of transferring pollen between the two parents.

Maize and annual teosinte (*Zea mays* subsp. *mexicana*), are genetically compatible, wind-pollinated and teosinte pollen can pollinate maize silks when in close proximity to each other, *e.g.* in areas of Mexico and Guatemala (Wilkes 1972). Maize crosses with teosinte; however, teosinte is not present in the U.S. other than as an occasional botanical garden specimen or small feral populations in Florida, Alabama, and Maryland. In experimental studies where maize and teosinte species were planted together, very low hybridization rates were observed for maize and *Zea mays* subsp. *mexicana* (Baltazar et al. 2005; Ellstrand et al. 2007). Differences in factors such as flowering time, geographical distribution, and development factors make natural crosses in the U.S. highly unlikely.

IX.G.3. Hybridization with the Wild Perennial Species of Subgenus Tripsacum

In contrast with maize and teosinte, which hybridize under certain conditions, it is only with extreme difficulty and special techniques that maize and the related perennial species, *Tripsacum dactyloides* (gamma grass) hybridize. Furthermore, hybrids of the cross are male sterile, even after several backcrosses to maize (Russell and Hallauer 1980).

A single species, *Tripsacum floridanum* (Florida gamma grass), found in the extreme southern Florida counties of Miami-Dade, Collier and Monroe has been categorized as a threatened species by the state of Florida and listed on the USDA Natural Resources Conservation Service (USDA-NRCS) database (USDA-NRCS 2012). Another species, *Tripsacum dactyloides* (Eastern gamma grass), found primarily throughout the eastern U.S., has been categorized as endangered in Massachusetts and Pennsylvania, and as threatened in New York (USDA-NRCS 2013). However, given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea* as mentioned above, the occurrence of *T. floridanum* primarily in both highly urbanized and non-agricultural, swampy areas of the state where commercial maize is not typically grown, as well as the preference of *T. dactyloides* for wet habitats where hybrid maize production would not occur, it is very unlikely there would be any impact on this species due to the introduction of MON 87403.

IX.G.4. Transfer of Genetic Information to Species with which Maize Cannot Interbreed (Horizontal Gene Flow)

Monsanto is aware of no reports confirming the transfer of genetic material from maize to other species with which maize cannot interbreed. The probability for horizontal gene flow to occur is judged to be exceedingly small. Even if it were to occur, the

consequences would be negligible since the ATHB17Δ113 protein is similar to other HD-Zip proteins found in maize that are not known to have meaningful toxicity to humans and NTOs. Similar to the situation with transfer of the protein genes, the likelihood of horizontal transfer of the ATHB17Δ113 coding sequence from MON 87403 is also exceedingly small. The consequence of such transfer, given the known lack of toxicity of these classes of proteins would also be expected to be inconsequential. In either case, the presence of this coding sequence would not be expected to increase the pest/weed potential of the recipient species.

IX.H. Potential Impact on Maize Agronomic Practices

An assessment of current maize agronomic practices was conducted to determine whether the cultivation of MON 87403 has the potential to impact current maize management practices (Section VIII). Maize fields are typically highly managed agricultural areas that are dedicated to crop production. Other than the specific insertion of the ATHB17 coding sequence that provides the potential for increased ear biomass at an early reproductive phase and hence increased yield opportunity, MON 87403 is similar to other high-yielding maize hybrids available commercially in the U.S.

The data presented demonstrate that MON 87403 is similar to commercially cultivated maize in its agronomic, phenotypic, ecological, and compositional characteristics, and has levels of resistance to insect pests and diseases comparable to other commercially cultivated maize. Based on this assessment, the introduction of MON 87403 is not likely to impact current U.S. maize agronomic or cultivation practices or lead to an increased plant pest potential compared to other maize hybrids widely available to growers.

IX.I. Conventional Breeding with Other Biotechnology-derived or Conventional Maize

Numerous biotechnology-derived maize products have been deregulated or are under consideration for deregulation by USDA-APHIS. Once deregulated, MON 87403 may be bred with these deregulated maize products, as well as with conventional maize, creating new improved hybrids. APHIS has determined that none of the individual biotechnology-derived maize products it has previously deregulated displays increased plant pest characteristics. APHIS has also concluded that progeny derived from crosses of these deregulated maize products with conventional or previously deregulated maize are unlikely to exhibit new plant pest properties. This presumption, that combined-trait biotechnology products are unlikely to exhibit new characteristics that would pose new plant pest risks not observed in the single event biotech product, is based upon several facts. Namely: 1) stability of the genetic inserts is confirmed in each approved biotech-derived maize product across multiple generations; 2) stability of each of the introduced traits is continually and repeatedly assessed as new combined-trait hybrids are created by plant breeders and tested over multiple seasons prior to commercialization; 3) combined-trait products are developed using conventional breeding that has been safely used for thousands of years to generate new varieties (Steiner et al. 2013; WHO 1995); 4) worldwide organizations, such as World Health Organization, Food and Agriculture Organization/ World Health Organization, International Seed Federation, CropLife

International and U.S. FDA, conclude that the safety of the combined-trait product can be based on the safety of the parental GE events (CLI 2005; FAO-WHO 1996; ISF 2005; WHO 1995); and 5) practical applications in the field have shown that two unrelated biotechnology traits combined together by conventional breeding do not display new characteristics or properties distinct from those present in the single event biotech products (Pilacinski et al. 2011).

Therefore, based on the considerations above and the conclusion that MON 87403 is no more likely to pose a plant pest risk than commercially cultivated maize, it can be concluded that any progeny derived from crosses between MON 87403 and conventional maize or deregulated biotechnology-derived maize are no more likely to pose a plant risk than commercially cultivated maize.

IX.J. Summary of Plant Pest Assessments

A plant pest, as defined in the PPA, is the living stage of any of the following that can directly or indirectly injure, damage, or cause disease in any plant or plant product: (A) a protozoan; (B) a nonhuman animal; (C) a parasitic plant; (D) a bacterium; (E) a fungus; (F) a virus or viroid; (G) an infectious agent or other pathogen, or (H) any article similar to or allied with any of the articles specified in the preceding subparagraphs (7 U.S.C. § 7702[14]). Characterization data presented in Sections III through VII and Section IX of this petition confirm that MON 87403, with the exception of the increased ear biomass at R1 phenotype, is not fundamentally different from conventional maize, in terms of plant pest potential. Monsanto is not aware of any study results or observations associated with MON 87403 that would suggest an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 87403 compared to conventional maize, followed by a risk assessment on detected differences. The plant pest risk assessment in this petition was based on the following lines of evidence: 1) insertion of a single functional copy of the ATHB17Δ113 cassette; 2) characterization and safety of the expressed products; 3) compositional equivalence of MON 87403 forage and grain compared to a conventional control; 4) phenotypic, agronomic, and environmental characteristics demonstrating no increased plant pest potential compared to conventional maize; 5) negligible risk to NTOs, including organisms beneficial to agriculture; 6) familiarity with maize as a cultivated crop and 7) no greater likelihood to impact agronomic practices, cultivation practices, or the management of weeds, diseases and insects, than conventional maize.

Based on the data and information presented in this petition, it is concluded that, like conventional maize and previously deregulated biotechnology-derived maize, MON 87403 is not expected to be a plant pest. Results also support a conclusion of no increased weediness potential of MON 87403 compared to conventional maize. Therefore, Monsanto Company requests a determination from USDA-APHIS that MON 87403 and any progeny derived from crosses between MON 87403 and other commercial maize be granted nonregulated status under 7 CFR Part 340.

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no study results or observations associated with MON 87403 indicating that there would be adverse consequences from its introduction. MON 87403 produces a truncated ATHB17Δ113 protein, which has been fully characterized and its safety has been thoroughly assessed in this submission. As demonstrated by field test results and laboratory tests, the only phenotypic differences between MON 87403 and conventional maize are related to an increased ear biomass phenotype at the R1 growth stage.

The data and information presented in this petition demonstrate that MON 87403 is unlikely to pose an increased plant pest risk compared to conventional maize. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional maize, followed by risk assessment on detected differences. The characterization evaluations included molecular analyses, which confirmed the insertion of one copy of the intended DNA containing the ATHB17Δ113 cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations.

Analysis of key nutrients, anti-nutrients, and secondary metabolites of MON 87403 demonstrate that MON 87403 is compositionally equivalent to conventional maize. The phenotypic evaluations, including an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, pollen characteristics, ecological interaction characteristics, and environmental interactions also indicated MON 87403 is unchanged compared to conventional maize. There is no indication that MON 87403 would have an adverse impact on beneficial or non-target organisms, including threatened or endangered species. Therefore, based on the lack of increased pest potential compared to conventional maize, the risks for humans, animals, and other NTOs from MON 87403 are negligible.

The introduction of MON 87403 will not adversely impact cultivation practices or the management of weeds, diseases, and insects in maize production systems. Farmers familiar with commercial maize hybrids will be advised to continue to employ the same crop rotational practices, weed control practices and/or volunteer control measures currently in place for these products.

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APPENDICES

Appendix A: USDA Notifications and Permits

Field trials of MON 87403 have been conducted in the U.S. since 2007. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the MON 87403 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2012 - 2014 seasons, are still in preparation. A list of trials conducted under USDA notifications or permits and the status of the final reports for these trials are provided in Table A-1.

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Table A-1. USDA Notifications and Permits Approved for MON 87403 and Status of Trials Planted

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2007	07-126-102n	6/8/2007	Submitted	HI	2
2008	07-126-102n	6/8/2007	Submitted	HI	1
	08-036-109n	3/6/2008	Submitted	IA	9
	08-036-112n	3/21/2008	Submitted	IL	4
				IN	2
	08-036-115n	3/20/2008	Submitted	IL	6
	08-036-117n	3/6/2008	Submitted	KS	3
2009	08-106-111rm	6/30/2008	Submitted	HI	1
	08-330-103rm	2/27/2009	Submitted	IL	8
	09-034-101n	3/5/2009	Submitted	IA	8
	09-035-102n	3/25/2009	Submitted	KS	2
				OH	4
	09-035-103n	3/25/2009	Submitted	IA	1
				IL	1
	09-142-101rm	8/31/2009	Submitted	HI	1
	09-266-101n	10/23/2009	Submitted	HI	1
2010	09-317-101rm	2/12/2010	Submitted	CA	5
				IA	12
				IL	11
				IN	3
				KS	10
				OH	6
	10-054-140n	3/24/2010	Submitted	KS	13
	10-056-112n	3/26/2010	Submitted	NE	15
	10-063-105rm	4/17/2010	Submitted	HI	2
	10-067-112n	4/7/2010	Submitted	AR	1
				IL	3
				MO	1
				OH	1
				WI	1
	10-067-113n	4/7/2010	Submitted	IA	9
				IL	6
				IN	1
	10-068-104n	4/8/2010	Submitted	IA	6
2011				IL	7
				OH	5
	10-068-116n	4/8/2010	Submitted	IA	4
				IL	2
				IN	3
				KS	1
				NE	2
	10-068-117n	4/8/2010	Submitted	IL	2
				NE	1
	10-069-121n	4/9/2010	Submitted	IL	1
	10-078-109n	4/18/2010	Submitted	IA	1
	10-123-102n	6/2/2010	Submitted	HI	3
	10-131-103rm	6/29/2010	Submitted	HI	2
	10-264-101n	10/20/2010	Submitted	HI	1

	10-131-103rm	6/29/2010	Submitted	HI	1
	10-264-101n	10/20/2010	Submitted	HI	1
	10-322-103n	12/20/2010	Submitted	HI	2
	10-350-104rm	1/18/2011	Submitted	HI	1
	10-350-109rm	2/15/2011	Submitted	CA	3
				IA	6
				IL	8
				IN	1
				KS	6
				OH	6
				TX	1
	10-351-114rm	3/15/2011	Submitted	CA	2
				IA	9
				IL	7
				IN	1
				KS	5
				OH	2
	11-012-106n	2/6/2011	Submitted	KS	5
				MS	1
2011	11-012-107n	2/6/2011	Submitted	IA	5
	11-014-103n	2/6/2011	Submitted	IL	3
	11-034-115n	3/5/2011	Submitted	CA	5
				KS	5
	11-040-108n	3/11/2011	Submitted	IA	2
				IL	3
				IN	1
	11-040-109n	3/11/2011	Submitted	NE	13
	11-041-101n	3/18/2011	Submitted	KS	14
	11-041-126n	3/11/2011	Submitted	MS	1
	11-045-102n	3/16/2011	Submitted	IL	8
	11-045-104n	3/16/2011	Submitted	OH	6
				TX	1
	11-046-101rm	5/18/2011	Submitted	HI	2
	11-048-102n	3/19/2011	Submitted	IL	11
				IN	1
	11-048-110n	3/19/2011	Submitted	IA	11
	11-066-102n	4/6/2011	Submitted	HI	1
	11-067-109n	4/7/2011	Submitted	IL	2
				OH	5
	11-068-101n	4/7/2011	Submitted	KS	4
	11-068-104n	4/8/2011	Submitted	IL	6
	11-068-106n	4/7/2011	Submitted	IA	6
	11-097-105n	4/20/2011	Submitted	IL	1
	11-123-104rm	9/1/2011	Submitted	HI	2
	11-154-110n	7/3/2011	Submitted	HI	1
	11-154-111n	7/3/2011	Submitted	HI	1
	11-291-108rm	2/15/2012	Submitted	IA	3
				IL	2
				IN	1
2012				KS	21
	11-305-104rm	3/1/2012	Submitted	HI	2
	11-320-102rm	3/15/2012	Submitted	IA	9
				IL	4

				IN	1
				KS	3
				NE	11
				SD	3
	11-322-101n	12/18/2011	Submitted	HI	2
	11-322-102n	12/18/2011	Submitted	HI	2
	11-326-105rm	3/22/2012	Submitted	IA	12
				IL	23
	11-342-104rm	12/23/2011	Submitted	HI	1
				IL	1
	12-032-117rm	6/1/2012	Submitted	HI	1
				PR	1
	12-058-101n	3/28/2012	Submitted	MN	6
				WI	7
	12-059-120n	3/28/2012	Submitted	IL	1
				NE	1
2012	12-061-105n	3/31/2012	Submitted	IA	1
				IL	1
				NC	1
				NE	1
				PA	1
	12-062-111n	4/1/2012	Submitted	AR	1
				IA	1
				IL	3
				IN	1
				KS	1
				MS	1
				OH	1
				PA	1
	12-065-109n	4/4/2012	Submitted	IL	3
				MO	1
				NE	3
	12-072-104n	4/11/2012	Submitted	TX	2
	12-074-110n	4/13/2012	Submitted	IA	1
	12-125-106rm	9/1/2012	Submitted	HI	1
				PR	2
	12-143-104n	6/20/2012	Submitted	HI	4
	12-214-105rm	12/1/2012	Submitted	HI	1
	12-251-101n	10/7/2012	Submitted	AR	1
				IL	1
				KS	1
				NE	1
	12-143-104n	6/20/2012	Submitted	HI	2
	12-214-104rm	12/1/2012	Submitted	HI	1
	12-312-103n	12/7/2012	Submitted	HI	2
	12-312-106rm	3/1/2013	Submitted	HI	1
	12-312-109rm	3/7/2013	Submitted	PR	1
2013	12-320-109rm	3/15/2013	Submitted	IA	19
				SD	4
				TN	1
	12-320-114rm	3/15/2013	In Progress	IL	28
				IN	1
				KS	8

				MS	1
				KS	7
	12-320-125rm	3/15/2013	In Progress	NE	7
	13-037-101rm	6/1/2013	In Progress	HI	2
	13-037-102rm	6/1/2013	In Progress	HI	2
	13-037-104rm	6/1/2013	In Progress	PR	1
	13-037-105rm	6/1/2013	In Progress	HI	1
	13-037-106rm	6/1/2013	In Progress	HI	2
				PR	1
	13-039-102n	3/8/2013	Submitted	HI	3
	13-044-101rm	3/7/2013	Submitted	HI	1
	13-052-105n	3/23/2013	Submitted	IA	2
				MN	9
				SD	1
2013	13-059-103n	3/30/2013	Submitted	IL	1
	13-066-105n	4/6/2013	Submitted	AR	1
				IA	1
				IL	1
				LA	1
	13-119-103n	5/29/2013	In Progress	HI	2
				PR	1
	13-120-102rm	9/1/2013	In Progress	HI	1
				PR	1
	13-120-103rm	9/1/2013	In Progress	HI	1
				PR	1
	13-120-104rm	9/1/2013	In Progress	HI	1
	13-120-105rm	9/1/2013	In Progress	HI	1
	13-120-106rm	9/1/2013	In Progress	HI	1
	13-120-107rm	9/1/2013	In Progress	HI	1
	13-220-103n	9/7/2013	Submitted	PR	1
	13-301-101n	12/4/2013	In Progress	HI	1
	13-213-101rm	12/1/2013	In Progress	HI	1
	13-213-102rm	12/1/2013	In Progress	HI	1
	13-213-104rm	12/1/2013	In Progress	HI	1
	13-213-105rm	12/1/2013	In Progress	HI	1
	13-213-106rm	12/1/2013	In Progress	HI	1
	13-297-103rm	3/1/2014	In Progress	HI	1
	13-297-106rm	3/1/2014	In Progress	HI	1
	13-297-108rm	3/1/2014	In Progress	HI	2
	13-297-109rm	3/1/2014	In Progress	HI	1
	13-301-101n	12/4/2013	In Progress	HI	3
2014	13-305-101rm	3/1/2014	In Progress	IL	1
	13-305-102rm	3/1/2014	In Progress	IL	23
				IN	1
	13-305-103rm	3/1/2014	In Progress	IL	2
	13-305-105rm	3/1/2014	In Progress	IL	17
				IN	1
	13-305-106rm	3/1/2014	In Progress	KS	10
				NE	8
	13-305-108rm	3/1/2014	In Progress	KS	8
				NE	6
	13-305-109rm	3/1/2014	In Progress	KS	8
				NE	6

2014	13-305-113rm	3/1/2014	In Progress	IA	24
				MS	1
				TN	2
	13-305-114rm	3/1/2014	In Progress	IA	15
	13-305-115rm	3/1/2014	In Progress	IL	1
				NE	1
	13-305-116rm	3/1/2014	In Progress	IA	17
	14-031-117rm	6/1/2014	In Progress	HI	1
	14-038-103n	3/9/2014	In Progress	IL	1
				MI	1
				MN	11
				NE	1
	14-065-101n	4/5/2014	In Progress	IL	1
				NE	1
	14-065-102n	4/5/2014	In Progress	IL	1
				NE	1
Grand Total					849

Appendix B: Mode of Action of ATHB17Δ113 Protein in MON 87403

Grain yield results from a sequential growth and development process - first the plant grows in the vegetative phase and produces photosynthetic tissue, followed by flowering, the production of seeds, and finally seed filling and maturation (**Figure B-2**). MON 87403 was produced through insertion of the coding region of the full-length *ATHB17* gene through *Agrobacterium*-mediated transformation into maize. ATHB17 is a member of the HD-Zip family of proteins that are plant transcription factors; i.e. proteins that bind to specific DNA sequences and are essential for regulation of gene expression. This family of proteins is found broadly across plant species (Ariel et al, 2007) and is thought to play an important role in the regulation of plant growth and development. Expression of *ATHB17* in MON 87403 results in increased biomass of the ear at the early reproductive phase which increases yield opportunity in maize. The purpose of this appendix is to describe further details on the mechanism of action of *ATHB17* in MON 87403.

Maize (*Zea mays* subsp. *mays*) is one of the world's most important cereal crops with a global production forecast of ~960 million MT in 2013-14. The United States is the leading maize producing country in the world (USDA-FAS, 2014). In 2013 maize was planted on 35.48 million ha in the United States (USDA-FAS, 2014). Improvements in crop yield have been a primary focus of conventional breeding. Maize breeders have been extremely successful at improving grain yields in the last 70 years (**Figure B-2**). Commercial maize yield in the United States increased nearly six-fold in the last 70 years of genetic improvement, with an approximately 99 kg ha^{-1} increase every year from 1939 (Lee and Tollenaar, 2007). This continuous yield increase was due to many factors including genetic improvement. Yield has increased despite the fact that the ear size per plant has declined (Bruns and Abbas, 2003). Since MON 87403 results in increased ear biomass, it has the potential to address some of the challenges faced by maize breeders in their efforts to continue to improve yield and the efficiency of maize production.

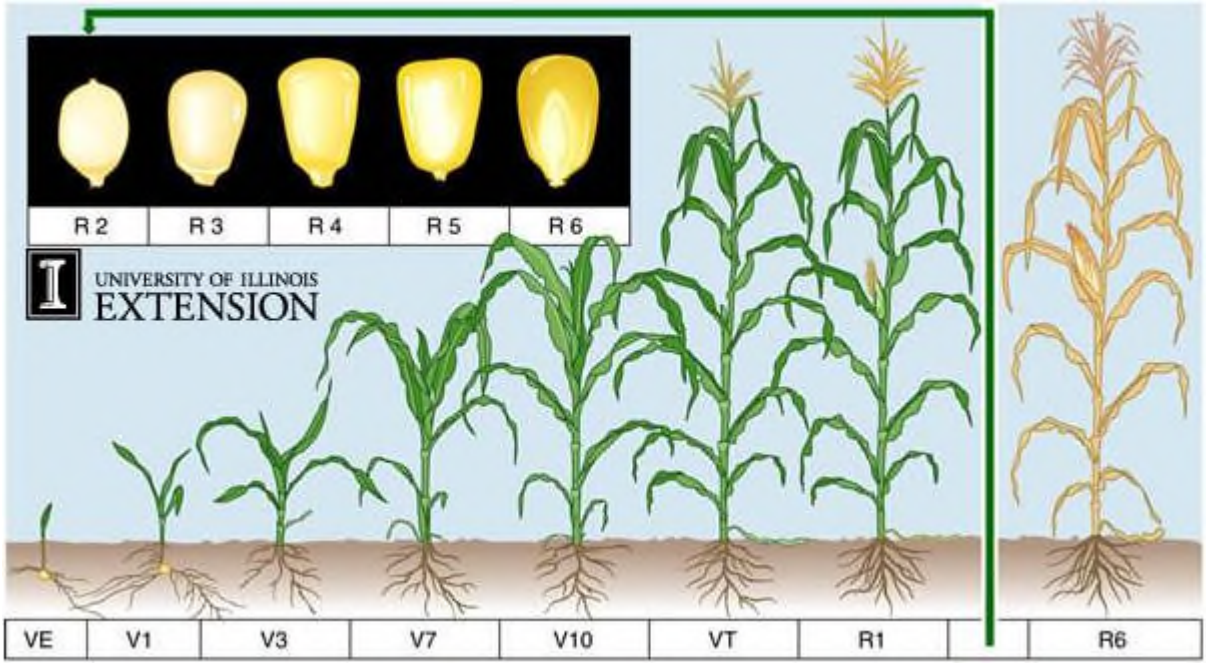

Figure B-1. Maize developmental stages. Vegetative stages are identified based on the number of visible leaf collars. Note the ear with silks at the R1 stage. Reproductive stages are mainly characterized by kernel development as shown in the insert. By the R6 stage, kernels attain physiological maturity. (Nafziger 2012)

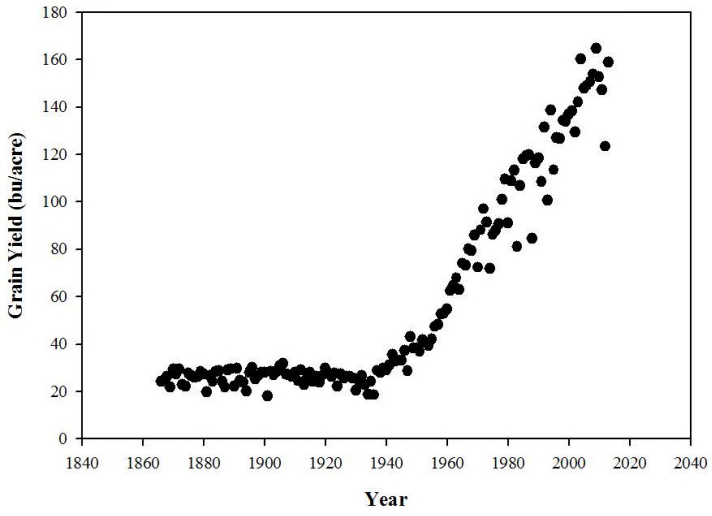

Figure B-2. US maize yields from 1866 to 2013 in bushels per acre (compiled from USDA-NASS, 2014)**.**

B.1. Maize growth stages, source and sink tissues:

In order to understand the mode of action of ATHB17 in maize, a review of maize developmental stages and the impact of partitioning on reproductive tissue development

is needed. Maize vegetative growth begins with germination and seedling emergence (VE, **Figure B-1**) and ends when 'tassel' or male inflorescence has completely emerged from the plant (VT, **Figure B-1**). Pollen from anthers on the tassel is shed normally after the VT stage. Ear tissue is initiated at a node by approximately the V5 growth stage and the ear grows rapidly as the plant approaches the flowering stage. The reproductive growth stage in maize starts with R1 when silks (receptors of pollen) are visible on the ear (female inflorescence), and ends at R6 or physiological maturity (Ritchie et al., 1997). At the R1 stage- ovules on the ear are completely developed, and after pollination they will mature during reproductive growth stages as grain for harvest (**Figure B-1**). Vegetative tissue (mostly leaves) is the primary source tissue for production of reproductive tissue biomass. The ear is considered the major sink tissue during reproductive stages in maize (Ritchie et al., 1997). Sink tissues lack the ability to produce biomass but accumulate the biomass that is produced by the source tissue (Ho, 1988).

B.2. Partitioning of assimilates to sink tissue in maize:

The transport and distribution of photosynthetic assimilates produced by the source tissue to sink tissue is referred to as 'partitioning' (Gifford and Evans, 1981). As the maize ear grows, it generates the driving force needed to transport assimilates produced by the source tissue to the sink tissue (Tang and Boyer, 2013). Increased ear partitioning involves preferential accumulation of biomass in the ear tissue relative to the other growing vegetative organs.

Grain yield in maize is a function of total assimilates (biomass) produced by the source tissue and the fraction of produced assimilates partitioned to the sink or ear tissue (Lee and Tollenaar, 2007). Thus, increased partitioning to the ear can result in higher yield regardless of an associated increase in total assimilates produced by the plant. The increased assimilate partitioning to the ear during the R1 stage has been one of the factors that has contributed to yield increase in North American maize hybrids (Echarte et al., 2004).

B.3. Sink size during early reproductive stage can affect grain yield in maize:

The size of the sink (ear) during the early reproductive stages can have a profound effect on grain yield in maize (Lizaso et al., 2007). In maize, the maximum sink size is determined during early reproductive stages, and is influenced both by the plant's genetics and by prevailing environmental conditions (Jones et al., 1996; Borras and Westgate, 2006). An increase in ear biomass at early reproductive stages can result in larger kernel number at harvest (Fisher and Palmer, 1983; Severini et al, 2011) (Figure B-3) and is considered an important determinant of reproductive success (Zaidi et al, 2003). Thus, the early reproductive stages are crucial phases in maize development, and a greater ear biomass during the early reproductive stages provides increased yield opportunity in maize.

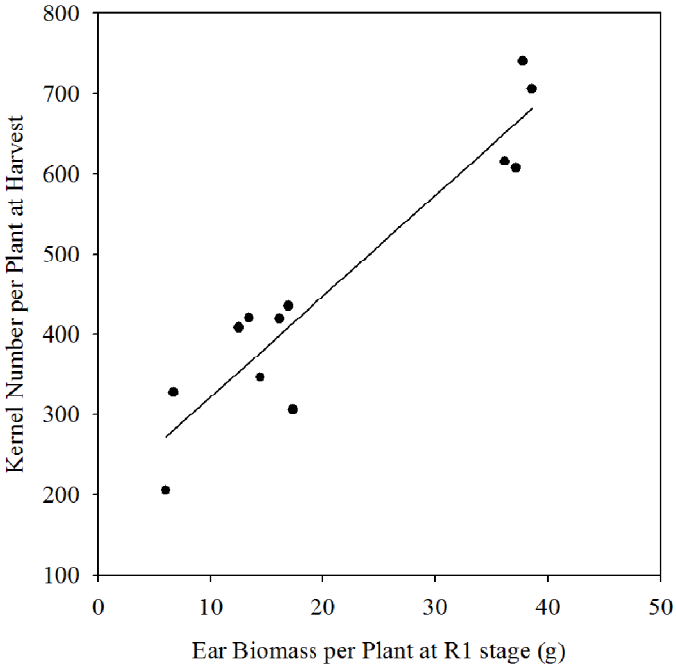

Figure B-3. Relationship between ear biomass during early reproductive stage and kernel number at harvest (graph made from the data on dent corn in Table 1, Severini et al., 2011).

B.4. Grain yield improvement through domestication and breeding has been largely achieved by selection and safe use of transcriptional regulator proteins:

The genetic changes that have resulted in yield improvement through conventional breeding were typically achieved through the selection and safe use of plant genes encoding transcriptional regulator proteins (Doebley et al., 2006). For example, selection for maize plants with single stalk and short branches tipped by ears during the domestication of maize from teosinte indirectly resulted in selection of specific variants of the *tb1* gene, which encodes a transcription factor (Doebley et al., 2006). Other examples of indirect selection for specific variants during conventional breeding can be found in crops like rice, wheat, tomato and cauliflower (Doebley et al., 2006). Thus expression of a transcription factor using agricultural biotechnology is an extension of conventional breeding and provides an opportunity to further enhance crop yields through the introduction of new genetic elements that use or modify existing pathways in the plant.

B.5. ATHB17 is a member of the HD-Zip family of transcription factors:

HD-Zip proteins are a family of plant transcription factors which bind to specific DNA sequences and regulate gene expression. This family of proteins is found broadly across plant species and is thought to play an important role in the regulation of plant growth and development (Ariel et al, 2007). The HD-Zip family comprises four distinct subfamilies, or classes designated as I, II, III, and IV (Ariel et al, 2007). All HD-Zip

proteins are characterized by the presence of a homeodomain (HD) with an immediately adjacent leucine zipper (LZ) domain. HD-Zip proteins have been shown to dimerize with members of their subfamily (as homo- or heterodimers), but not with other subfamilies of HD-Zip proteins to bind to target DNA sequences (Sessa et al., 1993). Many HD-Zip proteins have been shown to function as repressors of gene expression (Sessa et al., 1993; Steindler et al., 1999; Ohgishi et al., 2001; Henriksson et al. 2005), including down regulating the transcription of genes within the HD-Zip family (Ohgishi et al., 2001; Sorin et al., 2009). Transcriptional repressors are proteins that bind to DNA sites to prevent RNA polymerase from initiating transcription (Lewin, 2000).

ATHB17 is a member of the class II subfamily. The protein consists of 275 amino acids and contains five domains; a homeodomain (HD), a leucine zipper (LZ), an N-terminal domain, a repression domain and a C-terminal domain. It has been shown that HD-Zip II proteins in Arabidopsis recognize a 9 bp DNA sequence CAAT(G/C)ATTG (Sessa et al 1993) which is distinct from the consensus sequence for other HD-Zip proteins. In maize 18 HD-Zip II genes have been identified through a systematic bioinformatic analysis (Zhao et al., 2011). Characterization of ATHB17 and of the maize HD-Zip II proteins tested showed that they are all able to function as transcriptional repressors (Rice et al., 2014).

Based on the reported literature, HD-Zip II proteins have diverse functions throughout plant growth and development. For example, overexpression of several HD-Zip II genes has been shown to increase shade avoidance (or avoiding competition for light from neighboring plants) responses (ex: elongation to intercept light) in Arabidopsis (Ciarbelli et al., 2008; Sawa et al., 2002; Sorin et al., 2009; Steindler et al., 1999). In addition, multiple Arabidopsis lines over-expressing ATHB17 suggested a role in regulating photosynthetic capacity (Hymus et al., 2013). Interestingly, some HD-Zip II proteins are involved in regulation of reproductive growth and development (Meijer et al 1997).

In the following sections, the mode-of-action of *ATHB17* expression in MON 87403 is described through an assessment of the molecular and phenotypic characteristics of MON 87403.

B.6. MON 87403 expresses a truncated ATHB17 protein:

MON 87403 was produced through insertion of the coding region of the full-length *ATHB17* gene into maize through *Agrobacterium*-mediated transformation. Western blot analysis of leaf extracts revealed that MON 87403 and other ATHB17-transformed maize line express a truncated ATHB17 protein of ~20 kDa, in comparison to the predicted size ~32 kDa (Rice et al., 2014). Sequence analysis of the *ATHB17* transcript produced in MON 87403 confirms that a truncated transcript is produced, and as a result of splicing is predicted to encode a version of the ATHB17 protein that lacks the first 113 amino acids (ATHB17Δ113) corresponding to an expected molecular weight of ~22kDa. The domains that comprise ATHB17 are shown in Figure B-4, which also depicts the truncation of the repression domain in the ATHB17Δ113 protein produced in MON 87403.

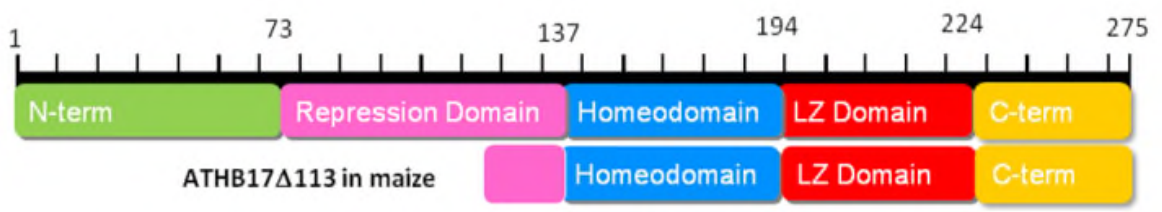

Figure B-4: Domain structure of ATHB17. ATHB17 contains the characteristic Homeodomain (HD) and Leucine Zipper (LZ) domains of HD-Zip family members. HD is required for DNA binding. LZ domain is responsible for homodimerization and hetero-dimerization with other HD-Zip II proteins. A repression domain is present upstream of the HD. ATHB17 protein expressed in MON 87403 lacks the first 113 amino acids resulting in truncation of the repression domain.

B.7. ATHB17Δ113 retains the functional binding properties of ATHB17:

The subcellular localization of ATHB17Δ113 was evaluated in maize protoplasts. The results indicated that ATHB17Δ113 is transported into the nucleus, consistent with activity as a transcription factor (Rice et al., 2014). *In vitro* DNA binding studies showed that like other HD-Zip II proteins, ATHB17Δ113 binds efficiently to consensus DNA targets for class I and class II HD-Zips, with slightly higher affinity for the class II binding sites (Rice et al., 2014). Studies conducted to identify maize proteins that interact with ATHB17 identified several HD-Zip II proteins but did not identify HD-Zip proteins from other classes. (Rice et al., 2014). The results show that ATHB17Δ113 retains DNA binding and protein-protein interaction (ability to form homo- and hetero-dimers) properties that are characteristic of the full-length protein.

B.8. ATHB17Δ113 relieves repression of HD-Zip II proteins:

Since ATHB17Δ113 lacks a large portion of the repression domain of ATHB17, it would not be expected to act as a transcriptional repressor. To determine whether ATHB17Δ113 protein can act as a transcriptional repressor in maize, an *in-vitro* assay system was established that allows for detection of repression of reporter gene expression in maize protoplasts. The results showed that the repression activity observed when full-length ATHB17 was added was not observed when ATHB17Δ113 was added. Based on these results, it was concluded that ATHB17Δ113 lacks the ability to act as a transcriptional repressor (Rice et al., 2014).

Although ATHB17Δ113 does not function as a repressor, the protein retains dimerization and DNA binding properties (Rice et al., 2014). Therefore, the likely action of ATHB17Δ113 expressed in maize is to attenuate the activity of endogenous HD-Zip II proteins through a dominant-negative mechanism. The dominant-negative mechanism can occur either through formation of non-functional homodimers that compete for DNA binding sites, or formation of less active heterodimers through competition for DNA binding (see Figure B-5).

To evaluate the ability of ATHB17Δ113 to act as a dominant negative regulator, the ability of ATHB17Δ113 to relieve repression activity of the full-length ATHB17 protein

was examined using the maize protoplast system described above. The repression of the reporter gene expression caused by the full-length ATHB17 protein was gradually relieved as increasing amounts of ATHB17Δ113 were added (Rice et al., 2014). This suggests that ATHB17Δ113 has the ability to counteract the effect of the full-length protein on gene repression.

Because ATHB17Δ113 can form heterodimers with maize HD-Zip II transcriptional factors and bind to the same DNA sequence, ATHB17Δ113 could be expected to act as a dominant negative regulator of endogenous maize HD-Zip II transcription factors. Experiments were conducted in maize leaf protoplasts to evaluate this possibility (Rice et al., 2014). Dose-dependent relief of the HD-Zip II repression activity by increasing amounts of ATHB17Δ113 was observed for all tested HD-Zip II proteins. These results suggest that ATHB17Δ113 protein can act as a regulator of endogenous maize HD-Zip II proteins, that repress transcription of genes with promoters containing class II binding sites. Thus, it is likely that ATHB17Δ113 has the potential to affect activities and pathways associated with maize HD-Zip II proteins.

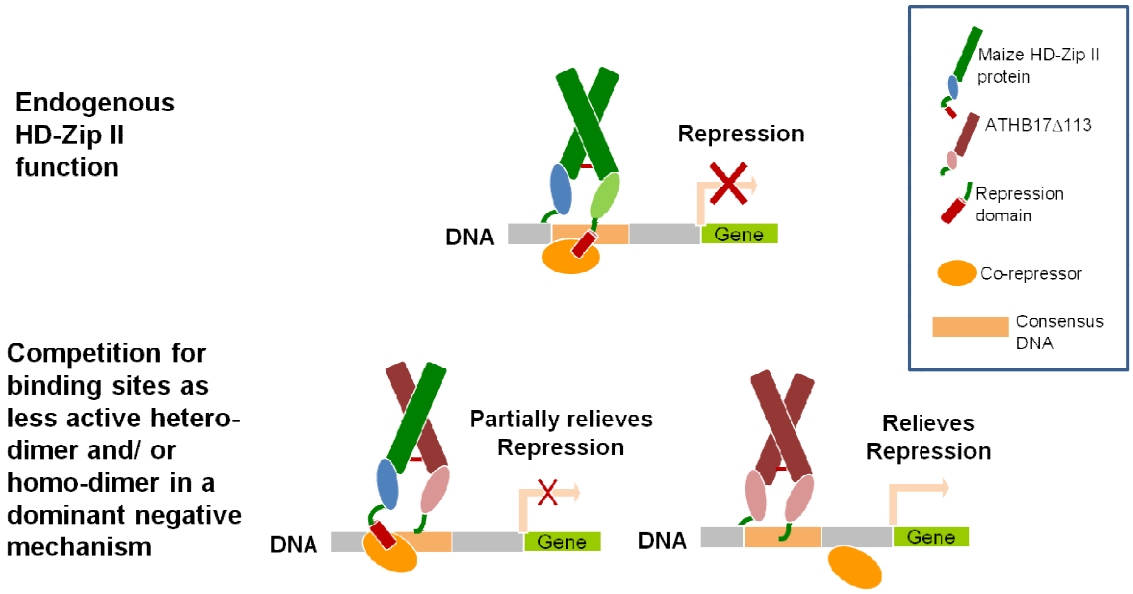

Figure B-5: Model for dominant negative mechanism of ATHB17Δ113
ATHB17 is expressed as a truncated protein in maize lacking part of the repression domain. Maize endogenous HD-Zip IIs function as transcriptional repressors. ATHB17Δ113 can interact with endogenous HD-Zip IIs to form heterodimers of ATHB17Δ113 and endogenous HD-Zip IIs, or ATHB17Δ113 homodimers can compete for DNA binding resulting in altered target expression due to inability to cause active repression. In this view, only one repression domain is on each face of the DNA so that only one of the two repression domains is visible (Rice et al., 2014).

B.9. ATHB17Δ113 likely modulates HD-Zip IIs through a dominant-negative mechanism to impact reproductive growth:

Based on the molecular mechanism proposed above, it is likely that expression of ATHB17Δ113 impacts maize ear growth through interaction with functions of endogenous maize HD-Zip II proteins. HD-Zip II proteins are involved in many processes related to reproductive development (Zuniga-Mayo et al., 2012). For example, a knock-out of a HD-Zip II gene in Arabidopsis showed no altered phenotypes during the vegetative growth phase but showed altered reproductive and fruit development compared to the wild type (Zuniga-Mayo et al., 2012).

To investigate whether the HD-Zip II proteins might be involved in the regulation of ear growth in maize, the expression patterns of HD-Zip II genes were evaluated in two different maize hybrids (Rice et al., 2014). The results showed that HD-Zip II genes were expressed in all sampled maize tissue types, as well as across developmental stages. There were eight HD-Zip II genes that were predominantly expressed in the ear tissue, suggesting that they might be actively involved in the regulation of plant reproductive or ear growth (Rice et al., 2014).

Our analysis indicates that the likely action of ATHB17Δ113 expressed in maize is to attenuate the activity of endogenous HD-Zip II proteins through a dominant-negative mechanism. As several HD-Zip II genes were predominantly expressed in the ear tissue, we believe that ATHB17Δ113 protein in maize likely modulates HD-Zip II regulated pathways in the ear, leading to changes in ear growth.

B.10. MON 87403 maize has increased ear biomass during the early reproductive stages:

Based on the role of specific HD-Zip II proteins in growth and development (Ciarbelli et al., 2008; Zuniga-Mayo et al., 2012; Bou-Torrent et al., 2012), expression patterns of HD-Zip II genes in maize during the V16-R1 stages, and likely action of ATHB17Δ113 through a dominant-negative mechanism, a hypothesis was developed that the MON 87403 event in maize results in increased ear biomass at the R1 stage compared to a conventional maize hybrid. The ear biomass at the R1 growth stage was measured in MON 87403 and conventional control plants which were grown at 13 field locations within the maize production regions of the United States in 2012. Characteristics measured included stover or vegetative biomass and ear biomass at the R1 growth stage (~60-70 days after planting). Stover and ear biomass per 1 meter length of row were determined by cutting plants at the ground level and measuring the dry weight of the vegetative material and top-most ear, respectively. Total biomass per 1 meter length of row was calculated as the sum of stover and ear biomass. From these measurements, biomass partitioning to the ear was calculated as the ratio of ear biomass to total biomass (Marcelis, 1996). Statistical comparisons were made between MON 87403 and the control across all 13 sites (combined-site analyses) for the above characteristics.

Statistical analysis indicated no significant differences in the R1 stover biomass and the R1 total biomass between MON 87403 and the control. There was a statistically

significant increase in R1 ear biomass ($p < 0.05$) in MON 87403 compared to the control in the combined site analysis (Table B-1). The R1 ear biomass in MON 87403 was approximately 12% higher than the control. Moreover, partitioning to the ear was significantly increased in MON 87403 compared to the control. These findings support that ATHB17Δ113 protein in maize regulates ear growth.

Table B-1. Combined Site Analysis of R1 Ear Weight, R1 Stover Weight, Total Plant Weight, and Partitioning in MON 87403 and the Conventional Control from 2012 U.S. Field Trials

Characteristic (units)	MON 87403 (Mean ± SE)	Control (Mean ± SE)	Change (%)	p-value
R1 ear biomass (g)	144.50 (±8.47)	129.30 (±8.13)	11.7	0.004*
R1 stover biomass (g)	768.13 (±20.57)	772.32 (±20.45)	-0.5	0.791
R1 total plant biomass (g)	912.63 (±25.47)	901.62 (±26.02)	1.2	0.563
Partitioning to ear	0.16 (±0.0064)	0.14 (±0.0058)	10.0	0.001*

Locations included in the combined-site analysis: Jackson, Arkansas; Vermilion, Illinois; Warren, Illinois; Boone, Indiana; Greene, Iowa; Jefferson, Iowa; Pawnee, Kansas; (2 sites) Polk, Nebraska; York, Nebraska; Perquimans, North Carolina; Berks, Pennsylvania; Lehigh, Pennsylvania. (Partitioning to ear = R1 ear biomass / R1 total plant biomass). All measurements are dry weight per 1 meter length of row.
*Denotes a statistically significant difference between MON 87403 and the control ($\alpha = 0.05$)

In MON 87403, the expression of the *ATHB17Δ113* transcription factor results in an increase of ear biomass and partitioning to ear at the R1 growth stage. Increased ear biomass and improvement in partitioning to the ear during the R1 stage in maize are traits that have been improved through breeding (Echarte et al., 2004). Thus, increasing ear biomass through introduction of the *ATHB17Δ113* transcription factor in maize is similar to genetic improvements that have been achieved through conventional breeding. The increased ear biomass at the R1 growth stage can provide the opportunity for a yield advantage (Fisher and Palmer, 1983; Zaidi et al., 2003; Severini et al, 2011).

Ear biomass advantage in MON 87403 occurs during early ear development and accumulates through the silking stage

A greenhouse study was conducted to confirm field results and to assess the growth stage when an ear biomass advantage is seen in MON 87403 compared to the control. Plants were grown in pots under optimal growing conditions in a greenhouse. Plants were sampled at 4 stages; V16 (late vegetative), VT (tasseling), early R1, and late R1 (silking). The ear biomass of MON 87403 was approximately 18% greater (29.4 g vs. 25.0 g) than that of the control at the late R1 stage (Figure B-6). Statistical analysis indicated that ear biomass was significantly greater in MON 87403 than the control at early R1 and late R1 stages. Throughout the time course during which biomass was measured (V16 – late R1), ears of MON 87403 plants accumulated approximately 24 % more biomass (26.8 g vs. 21.6 g), on average, than the ears of control plants. In addition to supporting the ear biomass advantage in MON 87403 compared to the control that was observed in the field

trial, these results also suggest that the increased early ear biomass of MON 87403 accumulates gradually over the period from V16 to late R1.

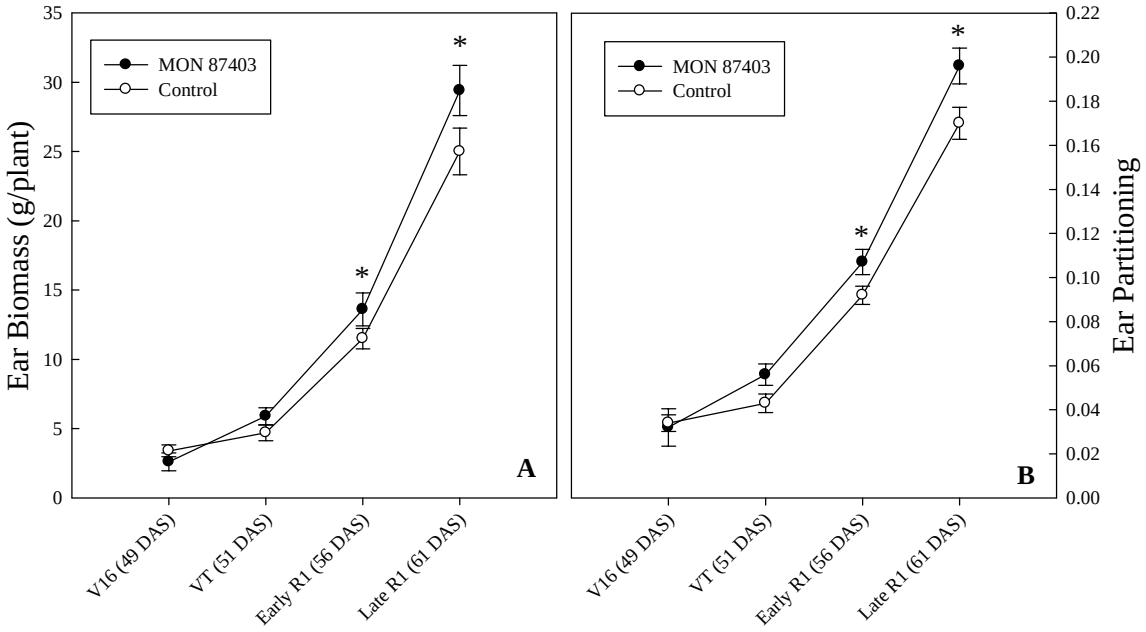

Figure B-6. Ear growth and ear partitioning in MON 87403 and the control at different developmental stages in a greenhouse. Symbol '*' indicates statistical significance at a level of 5% ($\alpha = 0.05$). (A) Ear biomass (dry weight) (B) Ear partitioning (ear dry weight/total dry weight). "DAS" is days after sowing.

Similar to ear biomass advantage, partitioning to ear was significantly higher in MON 87403 compared to the control at the early and the late R1 growth stages (6). These data support the ear biomass advantage in MON 87403 compared to the control was due to increased partitioning to the ear, similar to the findings observed in the field trial.

Conclusion:

Based on the studies described above, the ATHB17Δ113 protein can bind to target DNA sequences but lacks transcriptional repression activity. By a dominant-negative mechanism, ATHB17Δ113 protein in MON 87403 maize can alter the activity of endogenous HD-Zip IIs. HD-Zip IIs have been shown to play a role in reproductive growth in Arabidopsis (Ciarbelli et al., 2008; Zuniga-Mayo et al., 2012; Bou-Torrent et al., 2012). In maize, several HD-Zip IIs are predominantly expressed in ear tissue. The ATHB17Δ113 protein likely modulates HD-Zip II-regulated pathways in the ear, leading to changes in ear growth. The result is an increase in ear partitioning and ear biomass at the R1 growth stage in MON 87403 maize. A larger ear at the R1 growth stage increases

yield opportunity in MON 87403 maize (Leibman et al., 2014). The mode of action of MON 87403 is summarized in Figure B-7.

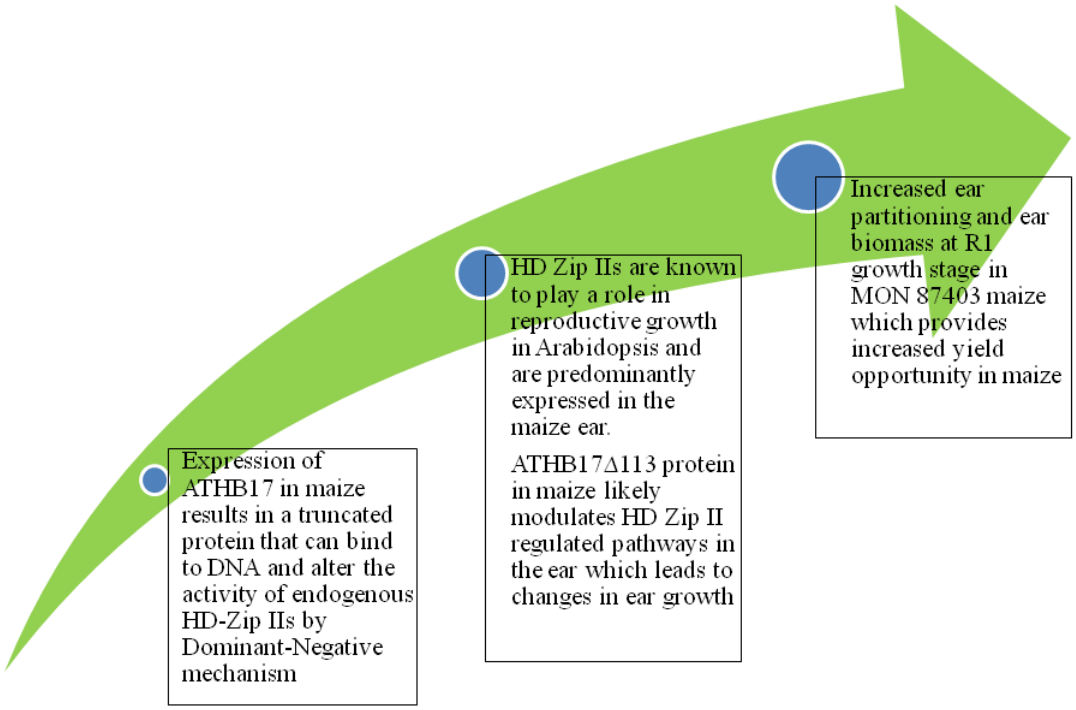

Figure B-7. Summary of mode-of-action of ATHB17Δ113 protein in MON 87403 maize

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Appendix C: Overview, Materials, Methods, and Supplementary Results for Molecular Analyses of MON 87403

C.1. NGS/JSA Overview

Safety assessments of biotechnology-derived crops include a detailed molecular characterization of the inserted DNA sequence and its location within the genome (Codex Alimentarius 2009). Typically, molecular characterization has relied on Southern blot analysis to establish locus and copy number along with targeted sequencing of polymerase chain reaction products spanning any inserted DNA to complete the characterization process. With the advent of next-generation sequencing (Shendure and Ji 2008; Zhang et al. 2011), improvements in sequencing technologies have enabled alternative methods for molecular characterizations which do not require Southern blot analysis. Next-Generation Sequencing and Junction Sequence Analysis bioinformatics (NGS/JSA) utilizes sequencing (both next-generation technologies and traditional methods) and bioinformatics to produce characterizations equivalent to those achieved by current Southern blot-based methods.

There are multiple advantages to using next-generation sequencing and bioinformatics, most notably the robustness, simplicity and consistency of the method compared with Southern blot studies, which require customized experimental design for every transformation event. The new sequencing-based method overcomes many technical challenges inherent in Southern blot analyses (e.g., false positive hybridization bands resulting from incomplete digestion or star activity (Wei et al. 2008)) and the need for radioactive 32P-labeled probes. This new method provides higher reproducibility, because it is less dependent on complex lab based procedures. The method described here is essentially identical for all transformation events and it robustly establishes molecular characteristics of genetically engineered crops (Kovalic et al. 2012). Additionally, similar techniques are being used to characterize transgene integration sites and insert molecular anatomy in mammalian systems (DuBose et al. 2013; Zhang et al. 2012).

Method Synopsis

Molecular characterization of the inserted DNA and associated native flanking sequences consists of a multistep approach to determine:

- the number of insertion sites;
- the presence/absence plasmid backbone;
- insert copy number at each insertion site;
- DNA sequence of each inserted DNA;
- sequence of the native locus at each insertion site.

Additionally, current methods also establish a description of any genetic rearrangements that may have occurred at the insertion site as a consequence of transformation. Generational stability analysis, which demonstrates the stable heritability of inserted DNA sequences over a number of breeding generations, is also routinely conducted.

The first step of the molecular characterization, determination of number of insert sites, is conducted using a combination of next-generation sequencing technologies (NGS) and Junction Sequence Analysis (JSA) bioinformatics (DuBose et al. 2013; Kovalic et al. 2012). A schematic representation of the basis of the characterization, including the NGS/JSA methodology and the directed sequencing, is presented in Figure C-1 (Kovalic et al. 2012).

Genomic DNA from the transformation event and the conventional control are used to generate short (~100 bp) randomly distributed sequence fragments (sequencing reads) in sufficient numbers to ensure comprehensive coverage of the genomes (Shendure and Ji 2008) (Figure C-1, box 1). Sufficient numbers of sequence fragments are obtained (≥75× genome coverage) to comprehensively cover the genomes of the sequenced samples (Ajay et al. 2011; Clarke and Carbon 1976; Wang et al. 2008). A previous study with a variety of transformation events demonstrate that 75× coverage of the genome is adequate to provide comprehensive coverage and ensure detection of inserted DNA, producing results equivalent to Southern blot analysis (Kovalic et al. 2012). Notwithstanding known biases in next-generation sequencing techniques, including the Illumina sequencing by synthesis method employed here (Minoche et al. 2011), it has previously been established experimentally that given deep next-generation sequencing, it is possible to achieve comprehensive coverage of complex genomes that form the foundation for accurate whole genome studies (Ajay et al. 2011; Wang et al. 2008).

To confirm sufficient sequence coverage in both the transformation event and the control, the 100 bp sequence reads are analyzed to determine the coverage of a known single-copy endogenous gene, this analysis demonstrates coverage at ≥75× median depth in each sample. Furthermore, in order to confirm the method's ability to detect any sequences derived from the transformation plasmid, plasmid DNA is spiked into conventional control DNA at a single copy genome equivalent ratio and 1/10 copy genome equivalent ratio. This analysis demonstrates that any portion of the plasmid may be detected at a single copy per genome level and 1/10 copy genome equivalent level, which is adequate sensitivity to observe any inserted fragment.

Also of note is that although the method presented here provides 75× or greater coverage of the genomes under study, accurate assembly of complete genome sequences for the transformation event and conventional control is not technically possible using currently available sequence assembly tools. This is due to the nature of the sequences generated in this study, short reads of a single short insert length (Miller et al. 2010), in addition to limitation on available sequence assembly algorithms (Zhang et al. 2011). The sequences generated with this method represent datasets sufficient for achieving precise molecular characterization of transformed DNA in transformation events where reference to a template sequence (plasmid DNA) is utilized for comparison (Kovalic et al. 2012).

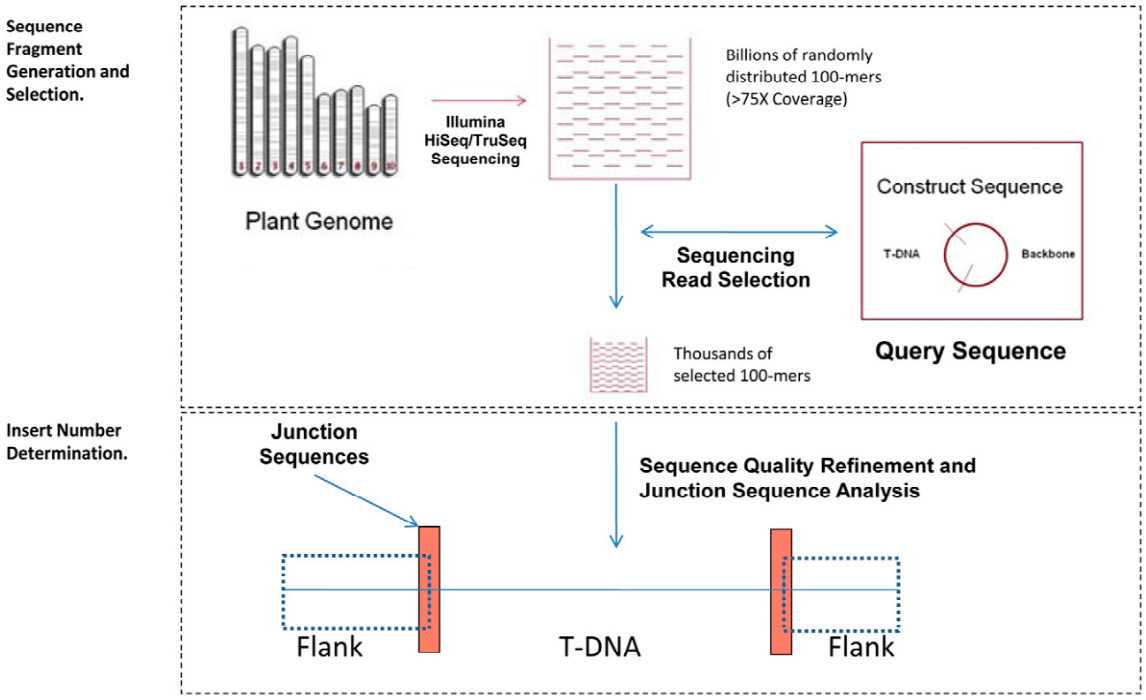

Figure C-1. Sequencing and Sequence Selection
Genomic DNA from the test and control material were sequenced using Illumina HiSeq/TruSeq technology (Illumina, Inc.) that produces large numbers of short sequence reads approximately 100 bp in length. Sufficient numbers of these sequence fragments were obtained to comprehensively cover the genomes of each sample at ≥75× median coverage. Using these genome sequence reads, bioinformatics search tools were used to select all sequence reads that are significantly similar (as defined in the text) to the transformation plasmid. Only the selected sequence reads were used in further bioinformatics analysis to determine the insert number by detecting and characterizing all junction sequences and the presence or absence of the plasmid backbone sequences by lack of detectable sequences, including the use of suitable controls for experimental comprehensiveness and sensitivity.

Using bioinformatics tools, the sequence reads that are derived from the plasmid vector are selected for further analysis out of the comprehensive genomic sequence dataset produced from the transformation event. To determine the insert number, the known sequence of the transformation vector plasmid is used as a query sequence in the bioinformatics analysis to search for and select the sequences that contain any portion of sequence of the plasmid. The DNA sequencing reads with a match to the query sequence having an e-value of 1×10^{-5} or less and having a match length of at least 30 bases with at least 96.7% sequence identity are collected. The results of a parameter optimization study that systematically evaluated many different potential parameter sets established these selection criteria as providing the best possible combination of sensitivity and specificity.

The number of DNA inserts is determined by analyzing the selected sequences for novel junctions. The junctions of the DNA insert and flanking DNA are unique for each

insertion and an example is shown in Figure C-2 below (Kovalic et al. 2012). Therefore, insertion sites can be recognized by analyzing for sequence reads containing such junctions. Each insertion will produce two unique junction sequence classes characteristic of the genomic locus, with one at the 5' end of the insert, in this case named Junction Sequence Class A (JSC-A), and similarly one at the 3' end of the insert, JSC-B (as illustrated in Figure 3 from Kovalic et al., 2012). By evaluating the number and the sequences of all unique junction classes detected, the T-DNA copy number and the number of insertion sites of the plasmid sequence can be determined. For a single insert, two junction sequence classes are expected, one each originating from either end of the insert, both containing portions of T-DNA and flanking sequence.

The identity of inserted DNA was confirmed by mapping of sequence reads. The selected sequences, a subset of which are junction sequences, represent plasmid sequence integrated into the genome of the transformation event. These sequences were compared to the transformation plasmid to determine which region(s), T-DNA or backbone, of the transformation plasmid was (were) integrated during transformation.

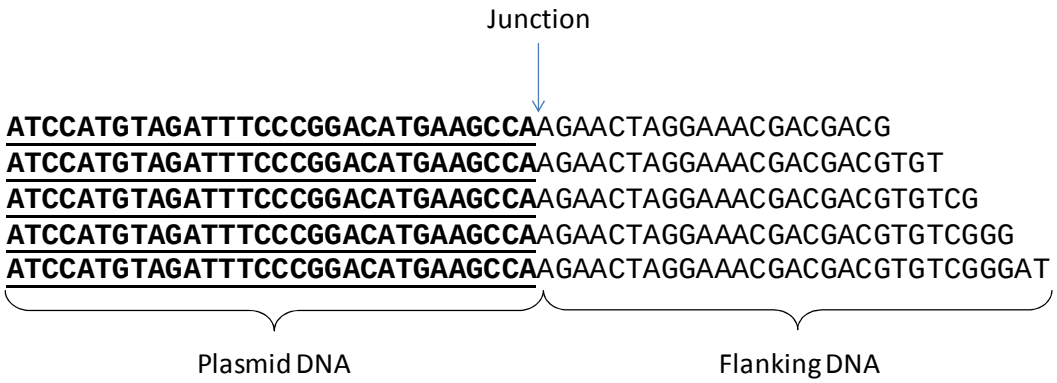

Figure C-2. Junctions and Junction Sequences
Depicted above are five example junction sequences formatted and labeled to indicate the plasmid/flanking DNA portions of the sequences and with the junction point indicated (plasmid DNA is shown in bold, underlined text and flank DNA is shown in plain text). Junctions are detected by examining the NGS data for sequences having portions of plasmid sequences that span less than the full read. Detected junctions are typically characteristic of plasmid insertions in the genome. A group of junction sequences which share the same junction point and common flanking sequence (as shown above) is called a Junction Sequence Class (or JSC).

The next step in the molecular characterization is confirmation of the integrity of the insert and flanking sequence of the native locus at the insertion site. This analysis is conducted using directed sequencing, locus-specific PCR and DNA sequencing analyses, which complements the NGS/JSA analyses, and is common to both the Southern-based and the NGS/JSA characterization methods. Directed sequencing (locus-specific PCR and DNA sequencing analyses) of the transformation event determines the complete sequence of the insert and flanks. This confirms that the sequence of the insert is identical to the corresponding sequence in the plasmid vector and if each genetic element in the insert is intact. Furthermore, the genomic organization at the insertion site is

assessed by comparing the insert and flanking sequence to the sequence of the insertion site in conventional control genome.

Finally, the stability of the T-DNA across multiple generations is evaluated by NGS/JSA analyses. Genomic DNA from multiple generations of the transformation event is assayed for the number and sequences of all unique junction classes, as well as the identity of the inserted sequence, as described above. This information is used to determine the number and identity of insertion sites. For a single T-DNA insert, two junction sequence classes are expected, both containing portions of T-DNA and flanking sequence (Figure C-2), with one each originating from either end of the insert (Figure C-3). All the integrated sequences align to the T-DNA portion of the plasmid. In the case of an event where a single locus is stably inherited over multiple generations, two identical junction sequence classes are expected in all the generations tested and all the integrated sequences align to the T-DNA portion of the plasmid.

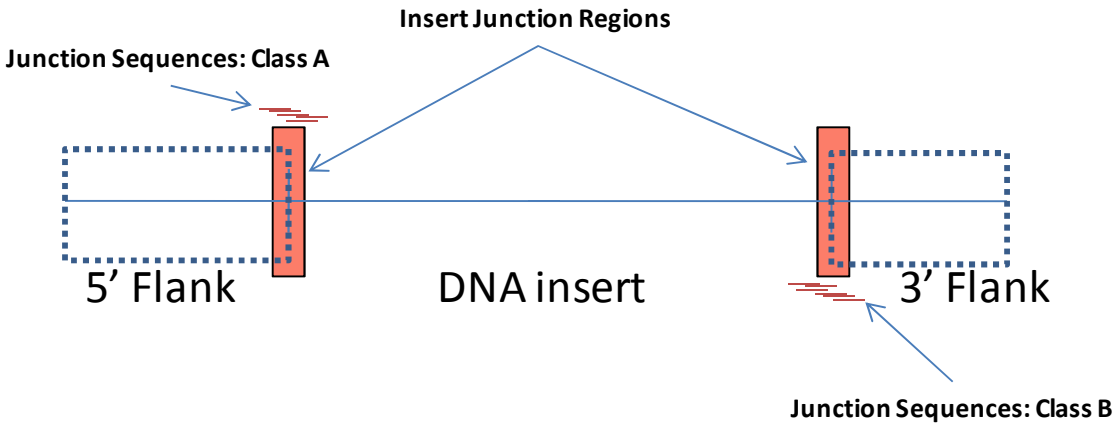

Figure C-3. Two Unique Junction Sequence Classes are Produced by the Insertion of a Single Plasmid Region

A schematic representation of a single DNA insertion within the genome showing the inserted DNA, the 5' and 3' flanks (depicted as areas bounded by dotted lines), and the two distinct regions spanning the junctions between inserted DNA and flanking DNA (shaded boxes). The group of ~100-mer sequences in which each read contains sequences from both the DNA insert and the adjacent flanking DNA at a given junction is called a Junction Sequence Class. In this example, two distinct junction sequence classes (in this case: Class A at the 5’ end and Class B at the 3’ end) are represented.

C.2. Materials and Methods

C.2.1. Test Substance

The test substance in this study was MON 87403. Genomic DNA for use in this study was extracted from tissue listed in the table below.

Generation	Seed ORION[6] ID
R_3	11346584
R_4	11346578
R_5	11346585
R_4F_1	11346581
R_5F_1	11346575

[6] ORION is a proprietary database used at Monsanto Company to track Regulatory plant samples.

C.2.2. Control Substance

The control substance is the conventional maize variety LH244, which has similar genetic background as the test substances. Genomic DNA for use in this study was extracted from tissue listed in the table below.

Control Substance	Generations	Seed ORION ID
LH244	R_3, R_4, R_5	11264747
LH244 x LH295	R_4F_1 (hybrid)	11266870
LH244 x LH287	R_5F_1 (hybrid)	11333170

C.2.3. Reference Substance

The reference substance was plasmid vector PV-ZMAP5714, which was used to develop MON 87403. Whole plasmid served as a positive control for sequencing and bioinformatic analyses. The identity of the reference plasmid was confirmed by restriction enzyme digestion prior to the study. Documentation of the confirmation of the plasmid vector identity was archived with the raw data. Appropriate molecular size markers from commercial sources were used for size estimations on agarose gels. The unique identity of the molecular weight markers was documented in the raw data.

C.2.4. Characterization of Test, Control, and Reference Substances

The seed for the test and control substances used in this study were obtained from Monsanto Trait Development. The synthesis records for these materials are located in the MIDAS7 system. The identities of the test substance and the conventional control substance were confirmed by the sequencing in the study. No certificates of analysis (COA) or verification of identity (VOI) certificates were generated for these materials. The Study Director reviewed the chain of custody documentation to confirm the identity of the test and control substances prior to the use of these materials in the study.

Test, control and reference DNA substance were considered stable during storage if they yielded interpretable signals in sequencing experiments and/or did not appear visibly degraded on the stained gels.

[7] MIDAS is a proprietary database used at Monsanto Company to track plant synthesis records.

C.2.5. Genomic DNA Isolation

For sequencing library construction and PCR reactions, genomic DNA was isolated from seed tissues of the test and control substances. First the seeds were decontaminated by vigorously agitating them by hand for 30 seconds with 0.05% (v/v) Tween-20, followed by a tap water rinse. The seeds were then vigorously agitated with 0.5% (w/v) NaOCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The seeds were then vigorously agitated with 1% (v/v) HCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The 1% (v/v) HCl rinse was repeated one time, and then the seeds were rinsed with distilled water and placed in a drying oven at 80°C to dry. The dried seeds were ground to a fine powder in a Harbil paint shaker. Genomic DNA was extracted using a hexadecyltrimethylammonium bromide (CTAB) extraction protocol. Briefly, 16 ml CTAB buffer (1.5% (w/v) CTAB, 75 mM Tris HCl (pH 8.0), 100 mM EDTA (pH 8.0), 1.05 M NaCl, and 0.75% (w/v) PVP) and RNase A was added to ground seed tissue. The samples were incubated at ~65° for 60 minutes with intermittent mixing. The samples were cooled to room temperature and subjected to three rounds of chloroform:isoamyl alcohol (24:1) extraction. Approximately 1.6 ml of 10% CTAB solution (10% (w/v) CTAB and 0.7 M NaCl) was added to the samples, mixed by inversion and extracted once with chloroform:isoamyl alcohol (24:1). Approximately 15 ml of CTAB precipitation buffer (1% (w/v) CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA (pH 8.0)) was added to the samples, mixed by inversion, and allowed to stand at room temperature for 50-70 minutes. Following centrifugation to pellet the DNA, the samples were dissolved in high salt TE buffer (10 mM Tris HCl (pH 8.0), 1 mM EDTA (pH 8.0), 1 M NaCl). The DNA was precipitated with 3 M sodium acetate (pH 5.2) and 100% (v/v) ethanol. The DNA pellets were washed with 70% ethanol, air dried and resuspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). All extracted DNA was stored in a 4°C refrigerator.

C.2.6. DNA Quantification

PV-ZMAP5714 DNA and extracted genomic DNA were quantified using a Qubit™ Fluorometer (Invitrogen, Carlsbad, CA). For directed sequencing, genomic DNA was quantified using a Nanodrop™ Spectrophotometer (Thermo Scientific, Wilmington, DE) according to manufacturer's instructions.

C.2.7. Agarose Gel Electrophoresis

After quantification, approximately 0.5-1 µg of the extracted DNA for NGS/JSA sequencing library construction was run on a 1% (w/v) agarose gel to check the quality.

C.2.8. Shearing of DNA

Approximately 1 µg of DNA from the test, control and reference substances were sheared using a Covaris S-220 ultrasonicator. The DNA was diluted to ~ 20 ng/µl in Buffer EB (Qiagen Inc., Valencia, CA) and fragmented using the following settings to create approximately 325 bp fragments with 3' or 5' overhangs: duty cycle of 10; peak incident

power of 175; intensity of 5.0, 200 bursts per cycle, in the frequency sweeping mode at ~ 6°C for 80 seconds for test and control DNA or 60 seconds for reference DNA.

C.2.9. Bioanalyzer Analysis

One microliter of sheared genomic DNA was diluted 1:10 in Buffer EB and run on a DNA High Sensitivity chip on an Agilent 2100 Bioanalyzer to check the quality of the shearing. After preparing the chip according to the manufacturer's instructions, 1 µl of each diluted DNA sample or water was added to individual wells and the chip was run on the Bioanalyzer using the dsDNA, High Sensitivity Assay reagents.

C.2.10. Paired End Library Preparation

Paired end genomic DNA libraries were prepared for the test, control, and reference substances using the Illumina TruSeq DNA Sample Preparation Kit (Illumina, San Diego, CA) according to the manufacturer's instructions for the low-throughput procedure with the following exception: a Sage Science Pippin Prep DNA Size Selection system (Sage Science Inc., Beverly, MA) was used to size select the DNA fragments instead of agarose gel electrophoresis.

First, the 3' and 5' overhangs of the DNA fragments generated by the shearing process were converted into blunt ends by adding 10 µl of Illumina Resuspension Buffer and 40 µl of Illumina End Repair mix to each sample and mixing thoroughly by pipette.

Then the libraries were incubated for 30 minutes at 30°C. The end-repaired samples were purified using AMPure XP beads (Beckman Coulter, Inc., Brea, CA) and resuspended in 17.5 µl of Illumina Resuspension Buffer.

Fifteen microliters of each library was transferred to a new tube for adenylation, which adds a single adenosine nucleotide to the 3' ends of the blunt fragments. Then 2.5 µl of Illumina Resuspension Buffer and 12.5 µl of Illumina A-Tailing Mix were added to each library and mixed thoroughly by pipetting. The libraries were incubated for 30 minutes at 37°C. After incubation, 2.5 µl each of individual adapter index, Illumina Resuspension Buffer, and Illumina DNA Ligase Mix was immediately added to each tube, and mixed thoroughly by pipetting to begin ligation of each library. The libraries were incubated for 10 minutes at 30°C. Then 5 µl of Illumina Stop Ligase Buffer was added to each tube and mixed thoroughly by pipetting to stop the ligation reaction. Next, another AMPure XP bead cleanup was performed on the libraries which were then resuspended in 32.5 µl of Illumina Resuspension Buffer prior to size selection.

The libraries were run on the Sage Science Pippin Prep Size Selection system using 2% gel cassettes according to the manufacturer's instructions. Ten microliters of loading solution were added to 30 µl of each of the purified libraries and mixed thoroughly by pipetting. Forty microliters of Marker B was loaded in the cassette well designated for the reference sample, and 40 µl of each DNA library was loaded in the remaining wells for analysis. After elution of the desired size range (~445 bp) of DNA fragments, the DNA sample in the elution chamber of the cassette was removed from the cassette by pipette and transferred into PCR strip tubes.

After removal from the Pippin Prep, the libraries were again put through the AMPure XP bead cleanup procedure and resuspended in 22.5 µl of Illumina Resuspension Buffer. Twenty microliters of the resuspended library was added to five microliters of Illumina PCR Master Mix and 25 µl of Illumina PCR Primer Cocktail and mixed thoroughly by pipetting. The DNA fragments were enriched through PCR using the following cycling conditions: 1 cycle at 98°C for 30 seconds; 10 cycles at 98°C for 10 seconds, 60°C for 30 seconds, 72°C for 30 seconds; 1 cycle at 72°C for 5 minutes. Following PCR amplification, a final AMPure XP bead cleanup was performed on the libraries which were resuspended in 32.5 µl of Illumina Resuspension Buffer. Finally, 1 µl of each DNA library was diluted 1:10 in Buffer EB for running in a DNA High Sensitivity chip on an Agilent 2100 Bioanalyzer as described above. All purified library DNA was stored in a -20°C freezer.

C.2.11. Next-Generation Sequencing

The library samples described above were sequenced by The Genome Analysis Center (TGAC, Monsanto) using Illumina HiSeq technology that produces short sequence reads (~100 bp long). Sufficient numbers of these sequence fragments were obtained (≥75x genome coverage) to comprehensively cover the entire genomes of the test event, the conventional control and the spiked-in control (Kovalic et al. 2012). Sequencing runs performed by the TGAC passed standard QC criteria. No sequence data in this study failed to meet these QC acceptance criteria.

C.2.12. Junction Sequence Analysis Bioinformatics

High-throughput sequence reads were enriched by mapping to the PV-ZMAP5714 transformation plasmid sequence using the local alignment software BlastAll (V2.2.21) in order to collect all reads that were sourced from the plasmid as well as reads with sequences representing integration point. All collected reads were further refined by removing sequencing artefacts of sequencing adapters, redundant reads and low quality read ends. All quality refined reads were then used to identify junction points with custom developed bioinformatics tools as detailed below. All significant junctions are reported for both the test and the control samples. All software versions were documented in the archived data package and the software versions which were used in this study have been archived.

Sequencing Read Enrichment

The transformation plasmid PV-ZMAP5714 sequence was used as reference to find all reads that were either fully matched to the insert plasmid fragments or partially matched as junction sequences. The sequence used was obtained from the MEGA[8] system. A junction sequence is characterized by a combination of transformation plasmid sequence

[8] MEGA is a proprietary database used at Monsanto Company to track sequences and annotations.

and flanking sequence that is likely to be host genome flanking sequence or any other co-inserted sequence, or a discontinuity in plasmid sequence caused by duplication or deletion. Local alignment with BlastAll (V2.2.21) was performed to collect all sequencing reads with an E-score of less than 1e-5 and at least 30 bases match of greater than 96.7% identity to the transformation plasmid (Kovalic et al. 2012). Both reads of the paired-end sequences were collected in all cases.

Read Quality Refinement

In order to identify all duplicate read pairs, a high quality segment (bases 3-42) of all collected pairs was compared to all others with short sequence alignment software (Bowtie v.0.12.3) allowing up to 1 mismatch. If multiple read pairs were matched at both paired reads, such read pairs were deemed redundant and only the best quality pair of reads was kept for further analysis.

Computer software Novoalign (v.2.06.09) was used to remove any adapter sequences at either end of the sequencing reads. Low quality read ends (with phred scores of 12 or lower) were trimmed. Only reads of 30 bases or longer after adapter and quality trimming were collected. A custom developed Perl script "farm_gen_sm_bucket.pl" was used to perform read enrichment and read quality refinement as described above.

Junction Detection

Enriched and quality refined reads of both test and control samples were aligned against the whole PV-ZMAP5714 transformation plasmid sequence in order to detect junction sequences using custom developed Perl script "farm_blast_map.pl". Reads with partial match to the transformation plasmid of at least 30 bases match and 96.7% identity were collected as potential junction sequences and their match cutoff position on the plasmid were noted (Kovalic et al. 2012). The collected reads were also aligned against the genomic sequence collection of the host genome in order to remove junction reads sourced from the plant endogenous homologues. Custom developed Perl script “junctions_by_bn.pl” was used to map reads and to identify the junction position on the transformation plasmid and their supporting junction reads. For each junction position, all supporting junction reads were aligned at the 30 plasmid bases proximal to the junction position. The remaining bases of these reads were sorted to show the alignment and the consensus of the flanking junction sequences past the junction point.

Effective Sequencing Depth Determination

A single copy locus (*Zea mays* pyruvate decarboxylase (*pdc3*), GenBank accession version: AF370006.2) was selected from the *Zea mays* genome and used to determine the effective sequence depth coverage. All reads with at least 30 bases match and 96.7% identity were considered as reads sourced from this locus. A custom developed Perl script “farm_match_reads.pl” was used to perform such alignment and calculate the actual depth distribution at this locus. The analysis showed that *pdc3* displayed 75× or greater median coverage for each sample, as listed in Table C-1.

Table C-1. Sequencing (NGS) Conducted for MON 87403 and Control Genomic DNA

Sample	Total Nucleotides (Gb)	Effective Median Depth of Coverage (x-fold)
LH244	264.5	106x
LH244 x LH295	296.1	108x
LH244 x LH287	294.7	115x
R_3	246.4	113x
R_4	280.7	110x
R_4F_1	310.1	120x
R_5	281.5	113x
R_5F_1	311.0	121x

For each sample the raw data produced are presented in terms of total nucleotide number. Effective depth of coverage is determined by mapping and alignment of all raw data to a well known single copy locus within the maize genome (*pdc3*). The median effective depths of coverage are shown for all samples.

Positive Spike-in Controls and Experimental Limit of Detection

To produce "spike-in" positive control samples for sequencing, plasmid DNA libraries were created as described above and then diluted to 1 and 1/10 maize genome equivalents (representation of the plasmid DNA at concentrations equivalent to single copy or 1/10 copy per genome) before pooling with samples produced from the control materials (as described above). At 1 genome equivalent, 100% nucleotide identity was observed over 100% of PV-ZMAP5714 (Table C-2). This result demonstrates that all nucleotides of the transformation plasmid are observed by the sequencing and bioinformatic assessments performed. Also, observed coverage was adequate (Clarke and Carbon 1976) at a level of at least 1/10th genomic equivalent (98.83% coverage at 99.97% identity for the 1/10th genome equivalent spiked control sample) and, hence, a detection level of at most 1/10th genome equivalent was achieved for the plasmid DNA sequence assessment.

Table C-2. Summary of NGS Data for the Conventional Control DNA Sample Spiked with PV-ZMAP5714

	$1/10^{th}$ copy Spike	1 copy Spike
Extent of coverage[1] of PV-ZMAP5714	98.83%	100%
Percent identity of coverage[2] of PV-ZMAP5714	99.97%	100%

[1] Extent of coverage is calculated as the percent of all PV-ZMAP5714 bases observed in the sequencing of the spike-in samples:

$$\text{Extent of coverage} = \frac{\text{number of spike in bases detected}}{\text{total length (bp) of spike in plasmid}} \times 100$$

[2] Percent identity of coverage is calculated as the percent of all PV-ZMAP5714 bases observed in the sequencing of the spike-in samples:

$$\text{Percent identity of coverage} = \frac{\text{number of identical bases (spike in vs. plasmid sequence) detected}}{\text{total length (bp) of spike in plasmid detected}} \times 100$$

C.2.13. PCR and DNA Sequence Analyses to Examine the Insert and Flanking Sequences in MON 87403

Overlapping PCR products, denoted as Product A, Product B, and Product C (Figure IV-7) were generated that span the insert and adjacent 5′ and 3′ flanking DNA sequences in MON 87403. For each fragment generation experimental conditions were chosen to successfully produce on-target amplifications. These products were analyzed to determine the nucleotide sequence of the insert in MON 87403, as well as that of the DNA flanking the 5′ and 3′ ends of the insert.

The PCR analyses for Product A, Product B, and Product C were each conducted using 84 ng of genomic DNA template in a 50 µl reaction volume. The reaction contained a final concentration of 0.5 µM of each primer and 1x concentration of Phusion High Fidelity PCR Master Mix with HF Buffer (Thermo Scientific).

The amplification of Product A and Product B were performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 35 cycles at 98°C for 30 seconds, 65°C for 30 seconds, 72°C for 2 minutes 30 seconds; 1 cycle at 72°C for 5 minutes.

The amplification of Product C was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 35 cycles at 98°C for 30 seconds, 72°C for 30 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

Aliquots of each PCR product were separated on an agarose gel and visualized by ethidium bromide staining to verify that the products were the expected size. Prior to sequencing, each verified PCR product was purified using Exo-SAP IT (Affymetrix, Santa Clara, CA) and a MinElute PCR Purification Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions and quantified using the Nanodrop spectrophotometer according to the manufacturer's instructions. The purified PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Monsanto TGAC (The Genome Analysis Center) using BigDye terminator chemistry (Applied Biosystems, Foster City, CA).

A consensus sequence was generated by compiling sequences from multiple sequencing reactions performed on the overlapping PCR products. This consensus sequence was aligned to the PV-ZMAP5714 sequence to determine the integrity and organization of the integrated DNA and the 5′ and 3′ insert-to-flank DNA junctions in MON 87403.

C.2.14. PCR and DNA Sequence Analyses to Examine the Integrity of the DNA Insertion Site in MON 87403.

To examine the MON 87403 T-DNA insertion site in control maize, PCR and sequence analyses were performed on genomic DNA from the conventional control maize LH244. The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87403. A forward primer specific to the DNA sequence flanking the 5′ end of the insert was paired with a reverse primer specific to the DNA sequence flanking the 3′ end of the insert.

The PCR reactions were conducted using 84 ng of genomic DNA template in a 50 µl reaction volume. The reaction contained a final concentration of 0.5 µM of each primer and 1x concentration of Phusion High Fidelity PCR Master Mix with HF Buffer (Thermo Scientific). The amplification was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 35 cycles at 98°C for 20 seconds, 71°C for 20 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

A small aliquot of each PCR product was separated on an agarose gel and visualized by ethidium bromide staining to verify that the PCR products were the expected size prior to sequencing. Only the verified PCR product from the conventional control LH244 was purified using Exo-SAP IT (Affymetrix, Santa Clara, CA) and a MinElute PCR Purification Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions and quantified using the Nanodrop spectrophotometer according to the manufacturer's instructions. The purified PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by TGAC using BigDye terminator chemistry (Applied Biosystems).

A consensus sequence was generated by compiling sequences from multiple sequencing reactions performed on the verified PCR product. This consensus sequence was aligned to the 5′ and 3′ sequences flanking the MON 87403 insert to determine the integrity and organization of the insertion site.

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Appendix D: Materials, Methods and Results for Characterization of ATHB17Δ113 Protein Produced in MON 87403

D.1. Characterization of ATHB17Δ113 Protein in MON 87403

D.1.1. Materials

The MON 87403-produced ATHB17Δ113 protein was purified from lyophilized leaf (lot 11347255) of MON 87403. The MON 87403-produced ATHB17Δ113 protein was stored in a -80 ºC freezer in a buffer solution containing 20 mM NaPO4, pH 7.4, 0.5 M NaCl, 1 mM dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic (EDTA), 2 µM leupeptin, 2 µM E-64 and 1% IGEPAL CA-630.

The *E. coli*-produced ATHB17Δ113 protein (lot 11380003) was used as the reference substance. The ATHB17Δ113 protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON109241 expression plasmid. The coding sequence for *ATHB17* contained on the expression plasmid (pMON109241) was confirmed prior to and after fermentation. Records pertaining to the production of the *E. coli*-produced ATHB17Δ113 protein are archived under Orion lot 11359538 in the Monsanto Regulatory Archives.

D.1.2. ATHB17Δ113 Protein Purification

The ATHB17Δ113 protein was purified from lyophilized leaf (lot# 11347255) of MON 87403. The purification procedure was not performed under a Good Laboratory Practice (GLP) protocol, however, all procedures were documented and, where applicable, Standard Operation Procedures were followed. The ATHB17Δ113 was purified from an extract of lyophilized leaf using a combination of techniques, including anionic exchange and immunoaffinity chromatography. The purification procedure is briefly described below.

ATHB17Δ113 was enriched from lyophilized MON 87403 leaf tissue. Approximately 1 kg of lyophilized tissue was resuspended in a total of 44 L of chilled extraction buffer (100 mM $NaPO_4$, pH 7.4, 100 mM NaCl, 5 mM DTT, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM benzamidine-HCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 µM leupeptin, 2 µM pepstatin A, 2 µM E-64, 2 µM bestatin, 10U/ml benzonase and 1% IGEPAL CA-630) and homogenized with an Ultra-Turrax T-50 dispersing instrument (IKA Work Inc., Wilmington, NC) equipped with a medium grind probe at 6400 rpm for 30 seconds. The suspension was then transferred to 4°C and ~560 g of poly(vinylpolypyrrolidone) equilibrated in 4 L of extraction buffer, and 1.25 kg of Amberlite IRA-402 resin equilibrated in 4 L of extraction buffer were added yielding concentrations of ~1% (w/v) and ~2.5 (w/v), respectively, in a final volume of 52 L (approximately 1:50 tissue to buffer ratio). The extraction slurry was incubated with stirring at 4°C for 2 hours before the addition of 1 kg of Celpure P100 filter agent (Imerys Filtration Minerals, San Jose, CA). The extraction slurry was then filtered using a 14-inch plate and frame filter press (Ertel Alsop, Kingston, NY) with MicroMedia cellulose and diatomaceous earth filter sheets (Ertel Alsop). The filtrate was chased through the filter press with 75 L of chase

buffer (1 mM DTT, 1 mM EDTA, 1 mM PMSF, 2 µM leupeptin, 2 µM pepstatin A, 2 µM E-64, 2 µM bestatin and 1% IGEPAL CA-630). The eluting chase filtrate was combined with the extraction filtrate until a total volume of 100 L was reached and the remaining chase filtrate was discarded.

The resulting clarified extract solution was loaded onto a 2.2 L (7 cm x 20 cm) Sulfopropyl-Sepharose Fast Flow cation exchange resin (GE Healthcare, Piscataway, NJ) column pre-equilibrated with Equilibration Buffer (20 mM $NaPO_4$, pH 7.4, 1 mM DTT, 1 mM EDTA, 1 mM $MgCl_2$ and 1% IGEPAL CA-630). The loaded column was washed with Buffer A (20 mM $NaPO_4$, pH 7.4, 1 mM DTT, 1 mM EDTA, 2 µM leupeptin and 2 µM E-64) and the bound ATHB17Δ113 was eluted using a 4-step salt gradient of 50 mM, 500 mM, 600 mM and 1.0 M NaCl in Buffer A. Elution fractions containing the MON 87403-produced ATHB17Δ113 protein were identified by western blot analysis and pooled. The ATHB17Δ113 in these fractions was precipitated by the addition of $(NH_4)_2SO_4$ at a final saturation of 60% followed by overnight incubation on an orbital shaker at 4°C. Following overnight incubation, the precipitated ATHB17Δ113 was pelleted by centrifugation and the resulting supernatants were discarded.

The resulting precipitated MON 87403-produced ATHB17Δ113 protein was immediately resuspended in 240 ml of Immuno Equilibration Buffer (20 mM $NaPO_4$, pH 7.4, 0.5 M NaCl, 1 mM DTT, 1 mM EDTA, 2 µM leupeptin, 2 µM E-64 and 1% IGEPAL CA-630). The resuspended solution was clarified by centrifugation and the resulting supernatant was loaded onto a 1.0 ml (1.3 cm x 1.0 cm) Protein G Fast Flow (GE Healthcare) column to which a monoclonal anti-ATHB17 antibody had been conjugated. The loaded column was washed with successive 10 column volume washes of Immuno Wash Buffer (20 mM $NaPO_4$, pH 7.4, 0.1 M NaCl, 1 mM DTT, 1 mM EDTA, 2 µM leupeptin and 2 µM E-64), Immuno Wash Buffer with 1.0 M NaCl and Immuno Wash Buffer with 10% propylene glycol. The bound ATHB17Δ113 was then eluted with IgG Elution Buffer (Thermo Scientific, Rockford, IL) containing 2 µM leupeptin and 2 µM E-64. Elution fractions containing the MON 87403-produced ATHB17Δ113 protein were identified by western blot analysis. The ATHB17Δ113 in these fractions was further concentrated by the addition of 25 µl of UNOsphere S cation exchange support resin (Bio-Rad, Hercules, CA), pre-equilibrated in IgG Elution Buffer, to each fraction. Following overnight incubation at 4°C with shaking, the ATHB17Δ113 bound resin was pelleted by centrifugation and the resulting supernatant was discarded. The resulting resin pellets were washed with 15 ml of Immuno Wash Buffer followed by centrifugation and combined in a filter paper spin cup microfuge tube (Thermo Scientific). The bound ATHB17Δ113 was then eluted with Immuno Equilibration Buffer. Elution fractions containing the MON 87403-produced ATHB17Δ113 protein were identified by western blot analysis and pooled.

The final buffer composition of the sample was 20 mM $NaPO_4$, pH 7.4, 0.5 M NaCl, 1 mM DTT, 1 mM EDTA, 2 µM leupeptin, 2 µM E-64 and 1% IGEPAL CA-630. The purified MON 87403-produced ATHB17Δ113 protein was aliquoted, assigned lot 11361368 and stored at in a -80°C freezer.

D.1.3. MALDI-TOF Tryptic Mass Map Analysis

D.1.3.1. Methods

MALDI-TOF tryptic mass fingerprint analysis was used to confirm the identity of the MON 87403-produced ATHB17Δ113 protein. The MON 87403-produced ATHB17Δ113 protein was diluted in 1× loading buffer (LB; 62 mM Tris-HCl, 5% (v/v) 2-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate (SDS), 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol, pH 6.8). The sample was heated to 99°C for 5 minutes and loaded at ~150 ng in four lanes of a pre-cast Tris-glycine (4-20 %) polyacrylamide gradient mini-gel (Invitrogen). Pre-stained MW standards (Precision Plus Protein Standards, Bio-Rad) were loaded on the gel for molecular weight reference. Following electrophoresis, proteins were briefly fixed in 40% (v/v) methanol, 7% (v/v) acetic acid and stained for 16 hours with Brilliant Blue G-Colloidal stain (Sigma-Aldrich). Gels were briefly destained in 10% (v/v) acetic acid, 25% (v/v) methanol followed by 3 hours in 25% (v/v) methanol. Each ~22 kDa band was excised and destained with 40% (v/v) methanol/10% (v/v) acetic acid. The excised bands were incubated in 100 mM ammonium bicarbonate for 1 hour and treated with 10 mM DTT at 37°C for 2 hours followed by incubation for 20 minutes with 10 mM iodoacetic acid in the dark. The excised bands were then washed 3 times with 25 mM ammonium bicarbonate, dried using vacuum centrifugation and rehydrated with 20 µl of 20 µg/ml trypsin (Promega, Madison, WI). After 1 hour, excess liquid was removed and the excised bands were incubated overnight at 37°C in 40 µl of 10% (v/v) acetonitrile in 25 mM ammonium bicarbonate. The excised bands were then sonicated for 5 minutes and the resulting extract was transferred to new microcentrifuge tube labeled Extract 1. The excised bands were then extracted two more times, each with 30 µl of a 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid (TFA) solution and sonicated for 5 min. These two extracts were combined with the first extract and dried using vacuum centrifugation. The extracts were solubilized in 5 µl of 50% (v/v) acetonitrile, 0.1% TFA and sonicated for 5 min. Extract was spotted to wells on an analysis plate and mixed with α-cyano-4-hydroxycinnamic acid (α-Cyano, Thermo Scientific). Test samples were analyzed using AB Sciex TOF/TOF 5800 MS in reflector positive ion mode. Signal-to-Noise was set to ≥10. Only monoisotopic ions were assigned in a mass list. A plate model/default calibration was performed using TOF/TOF calibration mixture standards (AB Sciex, Foster City, CA). The samples in α-Cyano matrix were analyzed in the 500 to 5000 Da range. The mass spectra were searched against the ATHB17Δ113 protein sequence using Mascot and ProteinPilot protein identification tools. Search parameter criteria included: Peptide Mass Tolerance ± 0.5 Da, Fixed Modifications- Carboxymethyl (C). Peptide mass fingerprint was generated by Mascot and ProteinPilot. All matching masses were tallied and a coverage map was generated for the mass fingerprint.

D.1.3.2. Results of MALDI-TOF Tryptic Mass Map Analysis

Peptide mass fingerprint analysis is a standard technique used for confirming the identity of proteins. The identity of the MON 87403-produced ATHB17Δ113 protein was confirmed by MALDI-TOF MS analysis of peptide fragments produced by the trypsin digestion of the MON 87403-produced ATHB17Δ113 protein.

There were 8 unique peptides identified that corresponded to the expected masses (Table D-1). The identified masses were used to assemble a peptide map of the ATHB17Δ113 protein (Figure D-1). The experimentally determined coverage of the ATHB17Δ113 protein was 50% (81 out of 162 amino acids). This analysis confirms the identity of MON 87403-produced ATHB17Δ113 protein.

Table D-1. Summary of the Tryptic Masses Identified for the MON 87403 - produced ATHB17Δ113 using MALDI-TOF MS

Experimental Mass[1]	Calculated Mass[2]	Diff.[3]	Fragment[4]	Sequence[5]
873.46	873.43	0.03	154-160	TFPPQER
878.47	878.45	0.02	37-43	LLEDSFR
1001.56	1001.53	0.03	153-160	KTFPPQER
1218.65	1218.61	0.04	66-74	QIEVWFQNR
1374.67	1374.63	0.04	93-103	WFGS…ENHR
1378.68	1378.68	0.00	33-43	EQSR…DSFR
2224.17	2223.99	0.18	116-135	VGPT…RCER
2358.02	2357.95	0.07	4-25	LPSS…APPR

[1] Only experimental masses that matched calculated masses are listed in the table.
[2] The calculated mass is the relative molecular mass calculated from the matched peptide sequence.
[3] The calculated difference between the experimental mass and the calculated mass.
[4] Fragment numbering is based on the predicted N-terminus of the protein.
[5] For peptide matches greater than nine amino acids in length the first 4 residues and last 4 residues are shown separated by dots (…).

001 MNRLPSSEDG DDEEFSHDDG SAPPRKKLRL TREQSRLLED SFRQNHTLNP

051 KQKEVLAKHL MLRPRQIEVW FQNRRARSKL KQTEMECEYL KRWFGSLTEE

101 NHRLHREVEE LRAIKVGPTT VNSASSLTMC PRCERVTPAA SPSRAVVPVP

151 AKKTFPPQER DR

Figure D-1. MALDI-TOF MS Coverage Map of the MON 87403-produced ATHB17Δ113

The amino acid sequence of the MON 87403-produced ATHB17Δ113 protein was deduced from the *ATHB17* gene present in MON 87403 and RT-PCR analysis of MON 87403 *ATHB17* RNA. Boxed regions correspond to peptides that were identified from the MON 87403-produced ATHB17Δ113 protein sample using MALDI-TOF MS. In total, 50% (81 out of 162 amino acids) of the expected protein sequence was identified.

D.1.4. Western Blot Analysis-Immunoreactivity

D.1.4.1. Methods

Quantitative western blot analysis was used to confirm the identity of the MON 87403 produced ATHB17Δ113 protein, determine the concentration of MON 87403 produced ATHB17Δ113 in the enriched sample, and to assess the equivalence of the immunoreactivity of the MON 87403 produced and *E. coli*-produced ATHB17Δ113 proteins.

MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins were diluted into 1× LB and heated to 99°C for 7 minutes. The *E. coli*-produced ATHB17Δ113 was used to prepare a standard curve ranging between ~0.5 and ~3.0 ng (purity corrected). The *E. coli*-produced ATHB17Δ113 protein and MON 87403-produced ATHB17Δ113 protein (two dilutions of 1:40 and 1:80 in duplicate) were loaded onto a pre-cast Tris-glycine (4-20%) polyacrylamide mini-gel (Invitrogen). Precision Plus Protein™ Molecular Dual color Standards (Bio-Rad) were loaded on the gel in parallel for molecular weight reference and to verify electrotransfer of the proteins to the membrane. Following electrophoresis at a constant voltage, proteins were electrotransferred to a nitrocellulose membrane (Invitrogen, Grand Island, NY).

The membrane was blocked with 5% non-fat dry milk (NFDM) in 1× phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST) and incubated with a polyclonal anti-ATHB17Δ113 antibody (lot G858502B) at a dilution of 1:4000 in 1% NFDM in PBST for 1 hour at RT. After washing with PBST, the membrane was next incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (H+L) (Thermo Scientific) at a dilution of 1:8,000 in 1% NFDM in PBST for 30 minutes at RT and washed again, with PBST. Immunoreactive bands were detected using the ECL™ detection system (GE Healthcare) and Amersham Hyperfilm (GE Healthcare). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta Medical & Graphic, Inc., Tokyo, Japan).

The prominent immunoreactive band in each lane, representing ATHB17Δ113, was quantified. Quantification of the bands on the blot was performed on a GS 800 densitometer with the supplied Quantity One® software (Bio-Rad). The concentration of MON 87403-produced ATHB17Δ113 in the sample was calculated as the mean of the concentrations of MON 87403-produced ATHB17Δ113 in each lane relative to the standard curve.

D.1.4.2. Results of ATHB17Δ113 Protein Immunoreactivity Equivalence

Western blot analysis was conducted using rabbit anti-ATHB17Δ113 polyclonal antibodies to 1) assess the identity of MON 87403-produced ATHB17Δ113, 2) determine the concentration of the MON 87403-produced ATHB17Δ113 in the enriched sample from the leaf tissue of MON 87403; and 3) assess the relative immunoreactivity of the MON 87403 produced and the *E. coli*-produced ATHB17Δ113 proteins.

The results demonstrated that immunoreactive bands with the same electrophoretic mobility were present in lanes loaded with the MON 87403-produced (Figure D-2, lanes 8-11) or the *E. coli*-produced (Figure D-2, lanes 2-7) ATHB17Δ113 protein. As expected, the signal intensity increased with increasing load amounts of the MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins, supporting identification of MON 87403-produced ATHB17Δ113 protein. Quantitative western blot analysis was conducted to determine the concentration of MON 87403-produced ATHB17Δ113 in the enriched sample. The concentration of the MON 87403-produced ATHB17Δ113 protein was determined to be 0.008 mg/ml. Because the MON 87403-produced and the *E. coli*-produced ATHB17Δ113 proteins were both immunoreactive to an anti-ATHB17Δ113 antibody, the MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins were determined to have equivalent immunoreactivity.

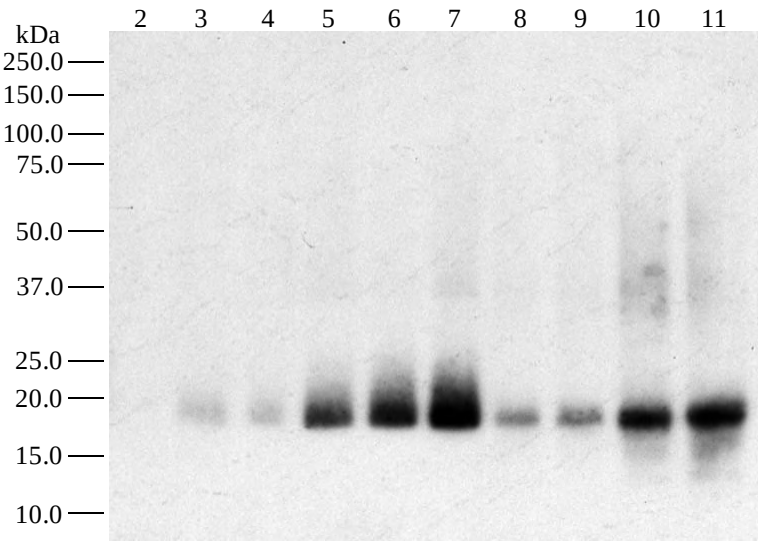

Figure D-2. Western Blot Analysis of MON 87403- and E. coli-produced ATHB17Δ113 Proteins

Aliquots of the MON 87403-produced ATHB17Δ113 and the *E. coli*-produced ATHB17Δ113 proteins were subjected to SDS-PAGE and electrotransferred to a nitrocellulose membrane. Proteins were detected using anti- ATHB17Δ113 antibodies as the primary antibodies. Immunoreactive bands were visualized using HRP-conjugated secondary antibodies and an ECL system. The molecular weights (kDa) of the standards are shown on the left. The 10 minute exposure is shown. Lanes 1 and 12 were cropped from the image. Lane designations are as follows.

Lane	Sample	Amount (ng)	Dilution
1	Precision Plus Protein™ Standards	-	-
2	*E. coli*-Produced ATHB17Δ113	0.4	-
3	*E. coli*-Produced ATHB17Δ113	0.7	-
4	*E. coli*-Produced ATHB17Δ113	1.1	-
5	*E. coli*-Produced ATHB17Δ113	1.4	-
6	*E. coli*-Produced ATHB17Δ113	1.8	-
7	*E. coli*-Produced ATHB17Δ113	2.1	-
8	MON 87403-produced ATHB17Δ113	-	1:80
9	MON 87403-produced ATHB17Δ113	-	1:80
10	MON 87403-produced ATHB17Δ113	-	1:40
11	MON 87403-produced ATHB17Δ113	-	1:40
12	Precision Plus Protein™ Standards	-	-

D.1.5. Apparent Molecular Weight and Purity Estimation using SDS-PAGE

D.1.5.1. Methods

MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins were diluted into 1× LB and heated to 95°C for 5 min. The MON 87403-produced ATHB17Δ113 protein was loaded in duplicate at ~8.0, ~12.0, and ~16.0 ng, based on the ATHB17Δ113 protein concentration, onto a pre-cast (4-20%) polyacrylamide mini-gel (Invitrogen). The *E. coli*-produced ATHB17Δ113 protein was loaded at ~4 ng, based on the purity corrected ATHB17Δ113 protein concentration, in a single lane. Broad Range Molecular Weight Standards (Bio-Rad) were prepared and loaded on the gel at ~90 ng. Following electrophoresis at a constant voltage, proteins were stained using a ProteoSilver™ Silver Stain Kit (Sigma-Aldrich, St. Louis, MO) according to manufacturer's recommended protocol. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer supplied with Quantity One software (version 4.6.7). Apparent MW and purity were reported as an average of all six lanes containing the MON 87403-produced ATHB17Δ113 protein.

D.1.5.2. Results of ATHB17Δ113 Protein Molecular Weight Equivalence

The MON 87403-produced ATHB17Δ113 protein (Figure D-3, lanes 3-8) migrated to the same position on the gel as the *E. coli*-produced ATHB17Δ113protein (Figure D-3, lane 2) and the apparent MW was calculated to be 22.4 kDa (Table D-2). Because the experimentally determined apparent MW of the MON 87403-produced ATHB17Δ113 protein was within the pre-set acceptance limits for equivalence (Table D-2), the MON 87403-produced and *E. coli*-produced ATHB17Δ113 proteins were determined to have equivalent apparent molecular weights.

The purity of the MON 87403-produced ATHB17Δ113 protein was calculated based on the six loads on the gel (Figure D-3, lanes 3-8). The average purity was determined to be 3%.

Table D-2. Molecular Weight Comparison Between the MON 87403- and *E. coli*-produced ATHB17Δ113 Proteins

Apparent MW of MON 87403-produced ATHB17Δ113 Protein (kDa)	Apparent MW of *E. coli*-produced ATHB17Δ113 Protein[1] (kDa)	Preset Acceptance Limits for the MON 87403-produced ATHB17Δ113 Protein[2] (kDa)
22.4	22.2	22.2 – 23.5

[1]As reported on the Certificate of Analysis for lot 11380003

[2] Calculated lower and upper bounds for one future assay based on two-tailed 95% prediction interval derived from apparent MW determinations for *E. coli*-produced ATHB17Δ113.

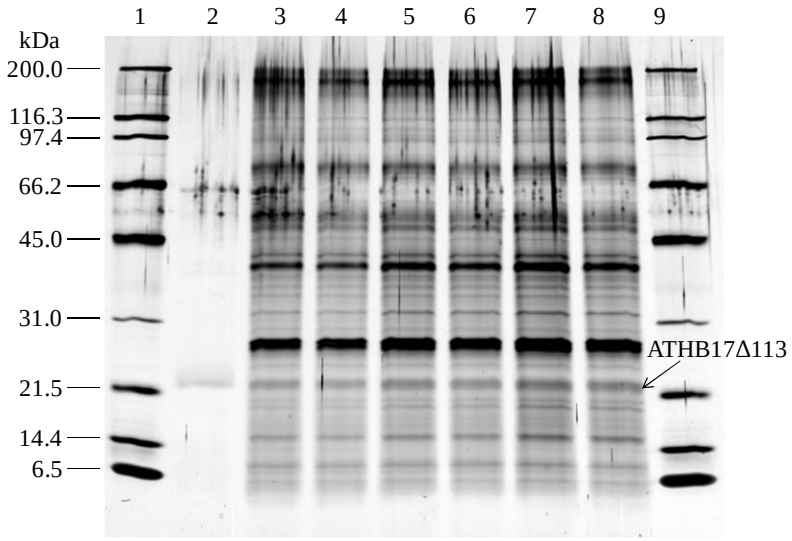

Figure D-3. Molecular Weight and Purity Analysis of the MON 87403-produced ATHB17Δ113 Protein

Aliquots of the MON 87403-produced ATHB17Δ113 and the *E. coli*-produced ATHB17Δ113 proteins were subjected to SDS-PAGE and the gel was stained with silver stain. The molecular weights (kDa) of the standards are shown on the left. Lane 10 was cropped from the image. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Broad Range MW Standards	90
2	*E. coli*-produced ATHB17Δ113	4
3	MON 87403-produced ATHB17Δ113	8
4	MON 87403-produced ATHB17Δ113	8
5	MON 87403-produced ATHB17Δ113	12
6	MON 87403-produced ATHB17Δ113	12
7	MON 87403-produced ATHB17Δ113	16
8	MON 87403-produced ATHB17Δ113	16
9	Broad Range MW Standards	90
10	Blank	-

Appendix E: Materials and Methods Used for the Analysis of the Levels of ATHB17Δ113 Protein in MON 87403

E.1. Materials

Over season leaf (OSL1), over season root (OSR1), forage, and grain tissue samples from MON 87403 were harvested from five field sites in The United States during the 2012 growing season from starting seed lot 11332602. *E coli*-produced ATHB17Δ113 (lot 11280374) was used as the analytical reference standard.

E.2. Characterization of the Materials

The identity of MON 87403 was confirmed by conducting MON 87403 event-specific polymerase chain reaction (PCR) analyses on the starting seed.

E.3. Field Design and Tissue Collection

Field trials were initiated during the 2012 planting season to generate tissues of MON 87403 at various maize growing locations in The United States. OSL1, OSR1, forage, and grain, tissue samples from the following field sites were analyzed: Jackson County, Arkansas (site code ARNE); Story County, Iowa (site code IALL); Jefferson County, Iowa (site code IARL); Pawnee County, Kansas (site code KSLA) and Lehigh County, Pennsylvania (site code PAGR). At each site, four replicated plots of plants containing MON 87403 were planted using a randomized complete block field design. Tissue samples were collected from each replicated plot at all field sites. See Table V-1 for detailed descriptions of when the samples were collected.

E.4. Tissue Processing and Protein Extraction

All tissue samples harvested were shipped to Monsanto's processing facility and were ground by the Monsanto Sample Management Team to facilitate protein extraction. All ground tissue samples were then lyophilized by the Monsanto Sample Management Team. The lyophilized tissue samples were stored in a -80°C freezer until transferred on dry ice to the analytical facility.

The ATHB17Δ113 protein was extracted from maize tissues as described in Table E-1. The protein extracts were aliquoted and stored frozen in a -80°C freezer until analysis.

Table E-1. ATHB17Δ113 Extraction Methods[1] for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf[2]/Grain/Root[3]/Forage	1:100	1 × TB + 0.1% BSA[4]

[1]ATHB17Δ113 protein was extracted from each tissue by adding the appropriate volume of extraction buffer, beads, and shaking in a Harbil mixer (Harbil Industries Inc., Compton, CA). The extracted samples were clarified using centrifugation and a serum filter.
[2]Over season leaf (OSL1).
[3]Over season root (OSR1).
[4]0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.005 M $MgCl_2$, 0.05% (v/v) Tween-20 and 0.1 % (w/v) BSA.

E.5. ATHB17Δ113 Antibodies

Mouse monoclonal antibody (Lot G-869055) specific for the ATHB17Δ113 protein was purified using Protein G column. The concentration of the purified antibody was determined to be 3.79 mg/ml by spectrophotometric methods. The purified antibody was stored in a phosphate buffered saline (0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.0027 M KCl and 0.05 % NaN_3).

Goat polyclonal antibodies specific for the ATHB17Δ113 protein were purified using affinity chromatography with a Protein G column. The concentration of the purified antibodies was determined to be 10.72 mg/ml by spectrophotometric methods. The purified antibodies (lot G-869057) were stored in a phosphate buffered saline (0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.0027 M KCl and 0.05% NaN_3).

The goat polyclonal antibodies were coupled with biotin (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions and assigned lot G-869059. The detection reagent was Poly-HRP conjugate (Thermo Fisher Scientific).

E.6. ATHB17Δ113 ELISA Method

Mouse anti-ATHB17Δ113 capture antibody was diluted into a coating buffer (0.015 M Na_2CO_3 and 0.035 M $NaHCO_3$) and immobilized onto 96-well microtiter plates at 1 µg/ml followed by incubation in a 4°C refrigerator for ≥12 hours. Prior to each step in the assay, plates were washed with 1 × phosphate buffered saline containing 0.05 % (v/v) Tween 20. Plates were blocked with the addition of 300 µl per well of Blocker Casein in PBS (Thermo Fisher Scientific, catalog number 37528) for 60 to 90 minutes at 37°C. ATHB17Δ113 protein standard or sample extract was added at 200 µl per well and incubated for 60 to 65 minutes at 37°C. Biotinylated goat anti-ATHB17Δ113 antibodies were added at 200 µl per well and incubated for 60 to 65 minutes at 37°C. Poly-HRP conjugate was added at 200 µl per well and

incubated for 30 to 35 minutes at 37°C. Plates were developed by adding 200 µl per well of horseradish peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantification of the ATHB17Δ113 protein was accomplished by interpolation from an ATHB17Δ113 protein standard curve that ranged from 0 - 0.5 ng/ml.

E.7. Data Analyses

ELISA plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. Protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP version 5.4 software. Absorbance readings and protein standard concentrations were fitted with a five-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 dwt" basis for data that were greater than or equal to the LOQ. This conversion utilized a sample dilution factor and a tissue-to-buffer ratio. Microsoft Excel 2007 (Microsoft, Redmond, WA) was used to calculate the protein levels in tissues. The sample means, standard deviations (SDs), and ranges were also calculated using Microsoft Excel 2007. All protein expression levels were rounded to two significant figures.

Any test substance extract that resulted in unexpectedly negative or positive results by ELISA analysis were re-extracted twice for the protein of interest and re-analyzed by ELISA to confirm the results. Samples with confirmed unexpected results were omitted from all calculations. Samples that were not confirmed to be either positive or negative were reported as inconclusive and omitted from all calculations.

Appendix F: Materials and Methods for Compositional Analysis of MON 87403 Maize Grain and Forage

Compositional comparisons between MON 87403 and the conventional control maize hybrid were performed using the principles and analytes outlined in the OECD consensus documents for maize composition (OECD 2002). These principles are accepted globally and have been employed previously in assessments of maize products derived through biotechnology. The compositional assessment was conducted on grain and forage samples harvested from a single growing season conducted in the United States during 2012 under typical agronomic practices.

F.1. Materials

Harvested grain and forage from MON 87403, a conventional control (MPA640B) that has similar genetic background to that of MON 87403, and conventional, commercial reference maize hybrids were compositionally assessed. The reference hybrids are listed in Table F-1.

Table F-1. Conventional Commercial Reference Maize Hybrids

Material Name	Seed Lot #	Field Site Codes
Burrus 645	11227210	ILMN, NESH
Gateway 4148	11273005	ILMN, PAGR
Gateway 6158	11273006	ARNE, INSH
H-9180	11226704	IARL
Lewis 6442	11226558	NESH
Lewis 7007	11226559	IALL, IARL, KSLA
LG2540	11226898	PAGR
	11266730	ARNE, IARL, ILMN, INSH
LG2620	11226861	KSLA
Midland Phillips 799	11226703	ARNE, ILMN, INSH, NESH, PAGR
Mycogen 2M746	11226705	IALL
NC+ 4443	11226700	ARNE
NC+ 5220	11226701	IALL, KSLA
Phillips 713	11300072	KSLA
Phillips 717	11300073	IALL, NESH
Stewart S588	11226918	PAGR
Stewart S602	11226919	IARL
Stine 9724	11298951	INSH

F.2. Characterization of the Materials

The identities of MON 87403, the conventional control, and reference hybrids were confirmed prior to use in the compositional assessment.

F.3. Field Production of the Samples

Grain and forage samples from MON 87403, the conventional control, and the reference hybrids were collected from eight replicated sites in United States during the 2012 growing season. The field sites were located in: Jackson, Arkansas (ARNE); Story, Iowa (IALL); Jefferson, Iowa (IARL); Warren, Illinois (ILMN); Boone, Indiana (INSH); Pawnee, Kansas (KSLA); Polk, Nebraska (NESH); and Lehigh, Pennsylvania (PAGR). Starting seeds were planted in a randomized complete block design with four plots for each of MON 87403, the conventional control, and the reference hybrids. The production was conducted under normal agronomic field conditions for their respective geographic regions that are typical areas for maize production in the United States.

Forage was collected at early dent (R5) and grain was collected at physiological maturity. Forage samples were shipped on dry ice and grain was shipped at ambient temperature from the field sites to Monsanto Company (Saint Louis, Missouri). Subsamples were ground to a powder, stored in a freezer set to maintain -20°C located at Monsanto Company (Saint Louis, Missouri). Subsamples were shipped on dry ice to Covance Laboratories Inc. (Madison, Wisconsin) for compositional analysis.

F.4. Summary of Analytical Methods

Nutrients analyzed in this study included moisture, ash, protein, total fat, carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), amino acids (18 components), fatty acids (22 components), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) and vitamins [β-carotene (referred to as vitamin A), B1, B2, B6, E (α-tocopherol), niacin, and folic acid], in the grain, and moisture, ash, protein, total fat, carbohydrates by calculation, ADF, NDF, calcium and phosphorus in the forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric 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.

F.4.1. 2-Furaldehyde

The ground samples were extracted with 4% trichloroacetic acid and injected directly on a high-performance liquid chromatography system for quantitation of free furfurals by ultraviolet detection (Albala-Hurtado, et al. 1997). The limit of quantitation was 0.500 ppm.

Reference Standard:

ACROS Organics, 2 Furaldehyde, 99.5%, Lot Number A0296679

F.4.2. Acid Detergent Fiber

The ANKOM2000 Fiber Analyzer automated the process of removal of proteins, carbohydrates, and ash. Fats and pigments were removed with an acetone wash prior to analysis. The fibrous residue that was primarily cellulose and lignin and insoluble protein complexes remained in the Ankom filter bag, and was determined gravimetrically (Goering and Van Soest 1970; Komarek, et al. 1993). The limit of quantitation was 0.100%.

F.4.3. Amino Acid Composition

The following 18 amino acids were analyzed:

- Total alanine
- Total arginine
- Total aspartic acid (including asparagine)
- Total cystine (including cysteine)
- Total glutamic acid (including glutamine)
- Total glycine
- Total histidine
- Total isoleucine
- Total leucine
- Total lysine
- Total methionine
- Total phenylalanine
- Total proline
- Total serine
- Total threonine
- Total tryptophan
- Total tyrosine
- Total valine

The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 hours at approximately 106-118ºC. Phenol was added to the 6N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. Tryptophan was hydrolyzed from proteins by heating at approximately 110ºC in 4.2N sodium hydroxide for approximately 20 hours.

The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acids are derivatized with fluorenylmethyl chloroformate (FMOC) before injection (AOAC 2012d; Barkholt and Jensen 1989; Henderson and Brooks 2010; Henderson, et al. 2000; Schuster 1988). The limit of quantitation for this study was 0.100 mg/g.

Reference Standards:

Component	**Manufacturer**	**Lot No.**	**Purity** (%)
L-Alanine	Sigma-Aldrich	BCBC5470	99.8
L-Arginine Monohydrochloride	Sigma-Aldrich	1361811	100
L-Aspartic Acid	Sigma-Aldrich	BCBB9274	100.6
L-Cystine	Sigma-Aldrich	1451329	100
L-Glutamic Acid	Sigma-Aldrich	1423805	100.2
Glycine	Sigma-Aldrich	1119375	100
L-Histidine Monohydrochloride Monohydrate	Sigma-Aldrich	BCBB1348	99.9
L-Isoleucine	Sigma-Aldrich	1423806	100
L-Leucine	Sigma-Aldrich	BCBC6907	99.9
L-Lysine Monohydrochloride	Sigma-Aldrich	1362380	100.2
L-Methionine	Sigma-Aldrich	1423807	99.9
L-Phenylalanine	Sigma-Aldrich	BCBC5774	100
L-Proline	Sigma-Aldrich	1414414	99.7
L-Serine	Sigma-Aldrich	1336081	99.9
L-Threonine	Sigma-Aldrich	1402329	100
L-Tyrosine	Sigma-Aldrich	BCBC2417	100
L-Valine	Sigma-Aldrich	1352709	100
L-Tryptophan	Sigma-Aldrich	BCBB1284	99.8

F.4.4. Ash

All organic matter was driven off when the samples were ignited at approximately 550ºC in a muffle furnace for at least 5 hours. The remaining inorganic material was determined gravimetrically and referred to as ash (AOAC 2012h). The limit of quantitation was 0.100%.

F.4.5. Vitamin A (Beta Carotene)

The samples were saponified and extracted with hexane. The samples were then injected on a reverse phase high-performance liquid chromatography (HPLC) system with ultraviolet light detection. Quantitation was achieved with a linear regression analysis (AOAC 2012a; Quackenbush 1987). The limit of quantitation was 0.0200 mg/100g.

Reference Standard:

Manufacturer	**Analyte**	**Lot No.**	**Purity (%)**
Sigma-Aldrich	Beta Carotene	091M1417V	98.2*

* Based on E1%=2280 for Lambda Maximum of 478 to 479 nm in Hexane.

F.4.6. Carbohydrate

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation (USDA 1973):

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The limit of quantitation was calculated as 0.100%.

F.4.7. Fat by Acid Hydrolysis

The samples were hydrolyzed with hydrochloric acid. The fat was extracted using ether and hexane. The extracts were dried down and filtered through a sodium sulfate column. The remaining extracts were then evaporated, dried, and weighed (AOAC 2012k; l). The limit of quantitation was 0.100%.

F.4.8. Fat by Soxhlet Extraction

The samples were weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the samples to remove the fat. The extract was then evaporated, dried, and weighed (AOAC 2012m; n). The limit of quantitation was 0.100%.

F.4.9. Fatty Acids

The following 22 fatty acids were analyzed:

- 8:0 Caprylic
- 10:0 Capric
- 12:0 Lauric
- 14:0 Myristic
- 14:1 Myristoleic
- 15:0 Pentadecanoic
- 15:1 Pentadecenoic
- 16:0 Palmitic
- 16:1 Palmitoleic
- 17:0 Heptadecanoic
- 17:1 Heptadecenoic
- 18:0 Stearic
- 18:1 Oleic
- 18:2 Linoleic
- 18:3 gamma-Linolenic
- 18:3 Linolenic
- 20:0 Arachidic
- 20:1 Eicosenoic
- 20:2 Eicosadienoic
- 20:3 Eicosatrienoic
- 20:4 Arachidonic
- 22:0 Behenic

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 (AOCS 2009b; a). The limit of quantitation was 0.00400%.

Reference Standards:

Manufacturer	Lot No.	Component	Weight (%)		Purity (%)
			JY10-W	**MA7-W**	
Nu-Chek Prep GLC Reference Standard Covance 1 Covance 2	JY10-W MA7-W	Methyl Octanoate	3.0	1.25	99.7
		Methyl Decanoate	3.25	1.25	99.6
		Methyl Laurate	3.25	1.25	99.8
		Methyl Myristate	3.25	1.25	99.8
		Methyl Myristoleate	1.0	1.25	99.5
		Methyl Pentadecanoate	1.0	1.25	99.6
		Methyl Pentadecenoate	1.0	1.25	99.4
		Methyl Palmitate	10.0	15.75	99.8
		Methyl Palmitoleate	3.0	1.25	99.7
		Methyl Heptadecanoate	1.0	1.25	99.6
		Methyl 10-Heptadecenoate	1.0	1.25	99.5
		Methyl Stearate	7.0	14.00	99.8
		Methyl Oleate	10.0	15.75	99.8
		Methyl Linoleate	10.0	15.75	99.8
		Methyl Gamma Linolenate	1.0	1.25	99.4
		Methyl Linolenate	3.0	1.25	99.5
		Methyl Arachidate	2.0	1.25	99.8
		Methyl 11-Eicosenoate	2.0	1.25	99.6
		Methyl 11-14 Eicosadienoate	1.0	1.25	99.5
		Methyl 11-14-17 Eicosatrienoate	1.0	1.25	99.5
		Methyl Arachidonate	1.0	1.25	99.4
		Methyl Behenate	1.0	1.25	99.8

F.4.10. Vitamin B9 (Folic Acid)

The samples were hydrolyzed in a potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis by autoclaving, the samples were 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 samples, using the bacteria *Lactobacillus casei*, with the growth response of a folic acid standard. This response was measured turbidimetrically (AOAC 2012p; q; Infant Formula Council 1985). The limit of quantitation was 0.0600 µg/g.

Reference Standard:

USP, Folic acid, 98.9%, Lot Number Q0G151

F.4.11. Minerals / ICP Emission Spectrometry

The following nine minerals were analyzed:

- Calcium
- Copper
- Iron
- Magnesium
- Manganese
- Phosphorus
- Potassium
- Sodium
- Zinc

The samples were dried, precharred, and ashed overnight in a muffle furnace set to maintain 500°C. The ashed samples were 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 samples, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions (AOAC 2012s; o). The limits of quantitation were as follows:

Inorganic Ventures Reference Standards and Limits of Quantitation:

Mineral	Lot Numbers	Concentration (µg/mL)	LOQ (ppm)
Calcium	F2-MEB453071MCA, F2-MEB453073	200, 1000	20.0
Copper	F2-MEB453071MCA, F2-MEB453072MCA	2.00, 10.0	0.500
Iron	F2-MEB453071MCA, F2-MEB453074	10.0, 50.0	2.00
Magnesium	F2-MEB453071MCA, F2-MEB453072MCA	50.0, 250	20.0
Manganese	F2-MEB453071MCA, F2-MEB453072MCA	2.00, 10.0	0.300
Phosphorus	F2-MEB453071MCA, F2-MEB453073	200, 1000	20.0
Potassium	F2-MEB453071MCA, F2-MEB453073	200, 1000	100
Sodium	F2-MEB453071MCA, F2-MEB453073	200, 1000	100
Zinc	F2-MEB453071MCA, F2-MEB453072MCA	10.0, 50.0	0.400

F.4.12. Moisture

The samples were dried in a vacuum oven at approximately 100°C. The moisture weight loss was determined and converted to percent moisture (AOAC 2012r; j). The limit of quantitation was 0.100%.

F.4.13. Neutral Detergent Fiber

The ANKOM2000 Fiber Analyzer automated the process of the removal of protein, carbohydrate, and ash. Fats and pigments were removed with an acetone wash prior to analysis. Hemicellulose, cellulose, lignin and insoluble protein fraction were left in the

filter bag and determined gravimetrically (AACC 1998; Komarek, et al. 1994; USDA 1970). The limit of quantitation was 0.100%.

F.4.14. Vitamin B3 (Niacin)

The samples were 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 samples, using the bacteria *Lactobacillus plantarum*, with the growth response of a niacin standard. This response was measured turbidimetrically (AOAC 2012i; p). The limit of quantitation was 0.300 µg/g.

Reference Standard:

USP, Niacin, 99.8%, Lot Number J0J235

F.4.15. p-Coumaric Acid and Ferulic Acid

The ground samples were extracted with methanol followed by alkaline hydrolysis and buffering prior to injection on an analytical HPLC system for quantification of p-coumaric acid and ferulic acid by ultra violet (UV) detection (Hagerman and Nicholson 1982). The limit of quantitation for the p-coumaric acid and ferulic acid was 33.3 ppm.

Reference Standards:

Component	Manufacturer	Lot No.	Purity (%)
p-Hydroxycinnamic Acid (p-Coumaric Acid)	Sigma-Aldrich	091M1197V	99.6
4-Hydroxy-3-methoxycinnamic Acid (Ferulic Acid)	ACROS Organics	A0261354	99.4
4-Hydroxy-3-methoxycinnamic Acid (Ferulic Acid)	ACROS Organics	A0294716	99.4

F.4.16. Phytic Acid

The samples were extracted using hydrochloric acid and sonication, purified using a silica based anion exchange column, concentrated and injected onto a high-performance liquid chromatography (HPLC) system with a refractive index detector (Lehrfeld 1989; Lehrfeld 1994). The limit of quantitation was 0.100%.

Reference Standard:

Sigma-Aldrich, Phytic Acid Sodium Salt Hydrate, 97.9%, Lot Number BCBH8701V

F.4.17. Protein

The protein and other organic nitrogen in the samples were converted to ammonia by digesting the samples with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. Instrumentation was used to automate the digestion, distillation and titration processes. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC 2012g; f). The limit of quantitation was 0.100%.

F.4.18. Raffinose

Sugars in the samples were extracted with a 50:50 water:methanol solution. Aliquots were taken, dried under inert gas, and then reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucopyranoside as the internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid treatment, and then analyzed by gas chromatography using a flame ionization detector (Brobst 1972; Mason and Slover 1971). The limit of quantitation was 0.0500%**.**

Reference Standard:

Sigma-Aldrich, D-(+)-Raffinose pentahydrate, 99.6%, Lot Number 019K1156

F.4.19. 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 sample was 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 protein and ash values (AOAC 2012c). The limit of quantitation was 1.00%.

F.4.20. Vitamin B1 (Thiamine Hydrochloride)

The samples were autoclaved under weak acid conditions to extract the thiamine. The resulting solutions were incubated with a buffered enzyme solution to release any bound thiamine. The solutions were purified on a cation-exchange column. Aliquots were 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 (AOAC 2012b; t; u). The limit of quantitation was 0.010 mg/100g.

Reference Standard:

USP, Thiamine Hydrochloride, 99.7%, Lot Number P0K366

F.4.21. Vitamin B2 (Riboflavin)

The samples were 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 samples, using the bacteria *Lactobacillus rhamnosus*, with the growth response of multipoint riboflavin standards. The growth response was measured turbidimetrically (AOAC 2012e; p; c). The limit of quantitation was 0.200 µg/g.

Reference Standard:

USP, Riboflavin, 99.7%, Lot Number N1J079

The United States Pharmacopeia, Twenty-Ninth Revision, p. 1913, United States Pharmacopeial Convention, Inc., Rockville, MD (2005).

F.4.22. Vitamin B6 (Pyridoxine Hydrochloride)

The samples were 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 samples, using the yeast *Saccharomyces cerevisiae,* with the growth response of a pyridoxine standard. The response was measured turbidimetrically. Results were reported as pyridoxine hydrochloride (AOAC 2012v; Atkins, et al. 1943). The limit of quantitation was 0.0700 µg/g.

Reference Standard:

USP, Pyridoxine hydrochloride, 99.8%, Lot Number Q0G409

F.4.23. Vitamin E (α-Tocopherol)

The samples were saponified to break down any fat and release vitamin E. The saponified mixtures were extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column (Cort, et al. 1983; McMurray, et al. 1980; Speek, et al. 1985). The limit of quantitation was 0.00500 mg/g.

Note: Alpha tocopherol is part of a mixed standard which also includes beta, delta, and gamma isomers. The reference standard material for those isomers may contain small amounts of alpha tocopherol. All reference standards that contributed to the alpha tocopherol concentration are listed below.

Reference Standard:

Manufacturer	**Component**	**Lot No.**	**Purity** (%)
USP	Alpha Tocopherol	O0K291	99
Acros Organics	D-gamma-Tocopherol	A0083534	99.3
Sigma-Aldrich	(+)-δ-Tocopherol	090M1916V	92

F.5. Data Processing and Statistical Analysis

After compositional analyses were performed, data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International, Inc., where they were converted into the appropriate units and statistically analyzed. The following formulas were used for re-expression of composition data for statistical analysis (Table F-2):

Table F-2. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Anti-nutrients	% fwt	% dw	X/d
Amino Acids (AA)	mg/g fwt	% dw	X/(10d)
Secondary Metabolites	ppm fwt	µg/g dw	X/d
Copper, Iron, Manganese, Zinc	ppm fwt	mg/kg dw	X/d
Calcium, Magnesium, Phosphorus, Potassium	ppm fwt	% dw	$X/(10^4 d)$
Vitamins B2, B3, B6, B9	µg/g fwt	mg/kg dw	X/d
Vitamin A, Vitamin B1	mg/100g fwt	mg/kg dw	10X/d
Vitamin E	mg/g fwt	mg/kg dw	$10^3 X/d$
Fatty Acids (FA)	% fwt	% Total FA	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA

[1]'X' is the individual sample value; d is the fraction of the sample that is dry matter.

In order to complete a statistical analysis for a compositional constituent in this compositional assessment, at least 50% of all the values for an analyte in grain or forage had to be greater than the assay limit of quantitation (LOQ). Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 16 analytes in grain 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, individual results below the LOQ were assigned a value equal to one-half the quantitation limit. Forty-five observations for 22:0 behenic acid and one value for calcium were assigned a value equal to one-half of the LOQ (0.002% fwt and 10.00 ppm fwt, respectively).

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the data 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 ranges are considered for exclusion, as outliers, from the final analyses. Eleven results had PRESS residual values outside of the ± 6 range.

Of the eleven flagged values, only the copper values from two conventional references were removed from further analysis as outliers. The remaining values were not removed because they were not extreme values or they were deemed sufficiently close to neighboring values to lack sufficient evidence for removal.

The outlier test procedure was reapplied to the remaining copper data to detect potential outliers that were masked in the first analysis. One copper value from a commercial reference was identified as a potential outlier, but the value was not an extreme value and was not removed as an outlier.

Maize compositional components were statistically analyzed using a mixed-model analysis of variance with the SAS MIXED procedure.

Analyses of the combined replicated sites were performed using model (1).

(1) $Yijk = U + Ti + Lj + B(L)jk + LTij + eijk,$

where Yijk = unique individual observation, U = overall mean, Ti = substance effect, Lj = random site effect, B(L)jk = random block within site effect, LTij = random site by substance interaction effect, and eijk = residual error.

For each component analysis, individual mean comparison tests of MON 87403 vs. conventional control were conducted.

A range of observed values from the reference hybrids was determined for each analytical component. Additionally, data from the reference hybrids were used to develop 99% 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 analyzed, two-sided 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of reference hybrids. Each estimate was based upon the average of all observations per unique reference hybrid. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

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Appendix G: Materials, Methods, and Individual Site Results for Seed Dormancy and Germination Assessment of MON 87403

G.1. Materials

Seed germination and dormancy characteristics were assessed on seed from MON 87403, the conventional control, and reference hybrids produced in replicated field trials during 2012 at the following sites: Story County, Iowa (IALL); Warren County, Illinois (ILMN); and Lehigh County, Pennsylvania (PAGR).

G.2. Characterization of the Materials

The identities of the MON 87403 and the conventional control starting seed were verified by event-specific polymerase chain reaction (PCR) analyses. During the growing season, the field planting order of MON 87403 and the conventional control plots was confirmed by event-specific PCR analyses. Chain-of-custody documentation for all starting seed for this dormancy and germination study was maintained from harvest through shipment to the performing laboratory with the use of packaging labels and plant sample transfer forms.

G.3. Germination Testing Facility and Experimental Methods

Germination and dormancy evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was qualified to conduct seed germination and dormancy testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association and Society of Commercial Seed Technologists (SCST) (AOSA/SCST 2010; AOSA, 2012a; 2012b).

The seed lots (Selfed F_2 grain) of MON 87403, the conventional control, and four reference hybrids from each location were tested under seven different temperature regimes. Seven germination chambers were used in the study and each chamber was maintained dark under one of the following seven temperature regimes: constant temperature of approximately 5, 10, 20, or 30 °C or alternating temperatures of approximately 10/20, 10/30, or 20/30° C. The alternating temperature regimes were maintained at the lower temperature for 16 hours and the higher temperature for 8 hours. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Approximately 100 seeds each of MON 87403, the conventional control, and the reference hybrids were placed on pre-moistened germination towels. Additional pre-moistened germination towels were placed on top of the seed. All rolled germination towels were labeled and placed into an appropriately labeled bucket. Each bucket within a temperature regime represented a replicate per site. There were 4 reps per site for a total of 12 buckets for each temperature regime. Each bucket contained 1 towel per entry. Buckets were then placed in the appropriate germination chambers. Each temperature regime constituted a separate split-plot experiment with four replications. A description of each germination characteristic evaluated and the timing of evaluations are

presented in Table VII-1. The types of data collected depended on the temperature regime. Each rolled germination towel in the AOSA-recommended temperature regime (*i.e.*, alternating 20/30 °C) was assessed periodically during the study for normally germinated, abnormally germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed as defined by AOSA guidelines (AOSA, 2012a; 2012b). AOSA only provides guidelines for testing seed under optimal temperatures, whereas additional temperature regimes were included to test diverse environmental conditions. Therefore, each rolled germination towel in the additional temperature regimes (*i.e.*, 5, 10, 20, 30, alternating 10/20, and 10/30 °C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed. Because temperature extremes could affect the development of seedlings, AOSA standards were not applied and no distinction was made between normal or abnormal germinated seed. Therefore, any seedling with a radical of 1 mm or more was classified as germinated.

The calculation of percent seed in each assessment category was based on the actual number of seeds evaluated (*e.g.*, 99 or 100). Across temperature regimes, the total number of seeds evaluated from each germination towel was approximately 100.

Within both AOSA and the additional temperature regimes, hard and firm-swollen seeds remaining at the final evaluation date were subjected to a tetrazolium (Tz) test for evaluation of viability according to AOSA standards (AOSA/SCST 2010). The number of nonviable hard and nonviable firm-swollen seed was added to the number of dead seed counted on all collection dates to determine the total percent dead seed. Total counts for percent viable hard and viable firm-swollen seed were determined from the Tz test.

G.4. Statistical Analysis

An analysis of variance was conducted using SAS® version 9.3 (SAS 2010) according to a split-plot design (production site as the whole plot and starting seed material as the sub-plot) with four replications. MON 87403 was compared to the conventional control for dormancy and germination characteristics of seed produced within each site (*i.e.*, individual-site analysis) and in a combined-site analysis in which the data were pooled across all three sites. The seed dormancy and germination characteristics analyzed included percent germinated seed, percent viable hard seed, percent dead seed, and percent viable firm swollen seed. The percent germinated seed were categorized as either normal germinated or abnormal germinated for the AOSA temperature regime. The level of statistical significance was predetermined to be 5% (α=0.05). MON 87403 was not statistically compared to the reference hybrids, nor were comparisons made across temperature regimes. The minimum and maximum mean values were determined from the reference materials across the study sites (*i.e.*, reference range) representative of maize hybrids. Results from the combined-site analysis are presented in Table VII-2.

G.5. Individual-Site Seed Dormancy and Germination Analysis

In the individual site analyses, no statistically significant differences were detected at sites IALL and ILMN. Two statistically significant differences were detected between

MON 87403 and the conventional control at the PAGR site for the measured characteristics abnormal germinated seed and viable firm-swollen seed. MON 87403 had significantly fewer abnormal germinated seed (0.3% vs. 1.3%) and more viable firm-swollen seed (0.3% vs. 0.0%) than the conventional control at the 20/30 °C. Statistically significant differences between MON 87403 and the conventional control for both germination characteristics in the individual site analyses were not detected in the combined site analysis (Table VII-2) and are unlikely to be biologically meaningful in terms of increased pest/weed potential (See Figure VII-1, Step 2, answer "no").

Table G-1. Starting Seed of MON 87403, Conventional Control and Commercial Maize Reference Hybrids Used in Dormancy Assessment

Site[1]	Material Type	Material Name	Phenotype	Material ID
IALL	Control	MPA640B	Conventional	11354876
IALL	Reference	Lewis 7007	Conventional	11354877
IALL	Reference	Mycogen 2M746	Conventional	11354878
IALL	Reference	NC+ 5220	Conventional	11354879
IALL	Reference	Phillips 717	Conventional	11354880
IALL	Test	MON 87403	Increased Ear Biomass Maize	11354881
ILMN	Control	MPA640B	Conventional	11354882
ILMN	Reference	Gateway 4148	Conventional	11354883
ILMN	Reference	Midland Phillips 799	Conventional	11354884
ILMN	Reference	Burrus 645	Conventional	11354885
ILMN	Reference	LG2540	Conventional	11354886
ILMN	Test	MON 87403	Increased Ear Biomass Maize	11354887
PAGR	Control	MPA640B	Conventional	11354888
PAGR	Reference	Gateway 4148	Conventional	11354889
PAGR	Reference	Midland Phillips 799	Conventional	11354890
PAGR	Reference	Stewart S588	Conventional	11354891
PAGR	Reference	LG2540	Conventional	11354892
PAGR	Test	MON 87403	Increased Ear Biomass Maize	11354893

[1]IALL = Story County, Iowa, ILMN = Warren County, Illinois, and PAGR = Lehigh County, Pennsylvania.

Table G-2. Dormancy and Germination Characteristics of MON 87403 and the Conventional Control Seed Produced at each of the Three Field Sites

Temperature	Assessment	IALL[1] Mean % (S.E.)[2]		ILMN[1] Mean % (S.E.)[2]		PAGR[1] Mean % (S.E.)[2]	
(°C)	Category	MON 87403	Control	MON 87403	Control	MON 87403	Control
5	Germinated	8.8 (0.95)	10.8 (1.80)	4.5 (1.04)	5.0 (1.29)	3.8 (1.44)	2.8 (0.85)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	3.8 (0.48)	2.3 (1.11)	4.0 (0.71)	3.3 (0.75)	4.5 (1.76)	5.0 (1.47)
	Viable Firm Swollen	87.5 (1.32)	87.0 (2.12)	91.5 (1.55)	91.8 (1.31)	91.8 (2.87)	92.3 (1.65)
10	Germinated	93.8 (1.18)	92.5 (1.94)	97.0 (0.58)	95.5 (0.87)	94.3 (1.44)	94.0 (0.91)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	1.5 (1.50)	1.8 (1.11)	1.0 (0.41)	1.8 (0.85)	3.0 (0.71)	2.5 (0.87)
	Viable Firm Swollen	4.8 (1.38)	5.8 (2.63)	2.0 (0.71)	2.8 (1.18)	2.8 (1.03)	3.5 (0.87)
20[3]	Germinated	100.0 (0.00)	99.8 (0.25)	99.3 (0.25)	99.0 (0.41)	98.8 (0.63)	98.5 (0.29)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	0.0 (0.00)	0.3 (0.25)	0.8 (0.25)	1.0 (0.41)	1.3 (0.63)	1.5 (0.29)
	Viable Firm Swollen	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0(0.00) †	0.0 (0.00)
30	Germinated	99.8 (0.25)	99.8 (0.25)	99.8 (0.25)	99.5 (0.50)	98.8 (0.48)	99.5 (0.29)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	0.3 (0.25)	0.3 (0.25)	0.3 (0.25)	0.5 (0.50)	1.3 (0.48)	0.5 (0.29)
	Viable Firm Swollen	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)

Table G-2 (continued). Dormancy and Germination Characteristics of MON 87403 and the Conventional Control Seed Produced at each of the Three Field Sites

Temperature	Assessment	IALL[1] Mean % (S.E.)[2]		ILMN[1] Mean % (S.E.)[2]		PAGR[1] Mean % (S.E.)[2]	
(°C)	Category	MON 87403	Control	MON 87403	Control	MON 87403	Control
10/20	Germinated	99.5 (0.29)	100.0 (0.00)	99.5 (0.50)	99.8 (0.25)	99.3 (0.25)	98.8 (0.25)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	0.3 (0.25)	0.0 (0.00)	0.3 (0.25)	0.3 (0.25)	0.8 (0.25)	1.3 (0.25)
	Viable Firm Swollen	0.3 (0.25)	0.0 (0.00)	0.3 (0.25)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
10/30	Germinated	99.5 (0.29)	99.5 (0.29)	99.8 (0.25)	99.8 (0.25)	99.5 (0.50)	99.3 (0.48)
	Viable Hard	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	0.5 (0.29)	0.5 (0.29)	0.3 (0.25)	0.3 (0.25)	0.5 (0.50)	0.8 (0.48)
	Viable Firm Swollen	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
20/30	Normal Germinated	99.5 (0.50)	100.0 (0.00)	98.0 (0.41)	98.3 (0.25)	99.3 (0.48)	98.3 (0.63)
(AOSA)	Abnormal Germinated	0.5 (0.50)	0.0 (0.00)	1.5 (0.29)	0.8 (0.25)	0.3 (0.25)*	1.3 (0.48)
	Viable Hard	0.0 (0.00)†	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
	Dead	0.0 (0.00)	0.0 (0.00)	0.5 (0.29)	1.0 (0.41)	0.3 (0.25)	0.5 (0.50)
	Viable Firm- Swollen	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.3 (0.25)*	0.0 (0.00)

Note: The experimental design was a split-plot with four replications.
*Indicates a statistically significant differences between MON 87403 and the conventional control (α=0.05) using ANOVA.
[1]Site codes are as follows: IALL = Story County, Iowa, ILMN = Warren County, Illinois and PAGR = Lehigh County, Pennsylvania.
[2]MON 87403 and the conventional control values represent means with standard error (S.E.) in parentheses. N = 4. In some instances, the total percentage of both MON 87403 and the conventional control did not equal 100% due to numerical rounding of the means.
[3]Three replicates of the reference Midland Phillips 799 were used from sites ILMN and PAGR.
†No statistical comparison could be made due to lack of variability in the data.

References for Appendix G

AOSA/SCST (2010). Tetrazolium testing handbook. Ithaca, New York, Association of Official Seed Analysts and the Society of Commercial Seed Technologists.

Association of Official Seed Analysts. 2012a. AOSA Rules for testing seeds. Volume 1. Principles and Procedures. Association of Official Seed Analysts, Ithaca, NY.

Association of Official Seed Analysts. 2012b. AOSA Rules for testing seeds. Volume 4. Seedling Evaluation. Association of Official Seed Analysts, Ithaca, NY.

SAS. (2010). SAS/STAT software version 9.3. SAS Institute, Inc., Cary, North Carolina.

Appendix H: Materials, Methods, and Individual Site Results from Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87403 under Field Conditions

H.1. Materials

Agronomic, phenotypic, and environmental interaction characteristics were assessed for MON 87403, the conventional control, and 21 reference hybrids grown under similar agronomic conditions. Four reference hybrids were planted per site (Table H-1).

H.2. Characterization of the Materials

The presence or absence of the MON 87403 event in the starting seed of MON 87403 and the conventional control was verified by event-specific polymerase chain reaction (PCR) analyses. No molecular analyses were performed on the reference starting seed.

H.3. Field Sites and Plot Design

Field trials were established in 2012 at 13 sites that provided a range of environmental and agronomic conditions representative of U.S. maize growing regions (Table VII-3). The Principal Investigator at each site was familiar with the growth, production, and evaluation of maize characteristics.

At all sites, seed of MON 87403, the conventional control, and four reference hybrids were planted in a randomized complete block design with four replications. At all sites except IABG, ILCX, NEDC, NEYO, NCBD, and PAHM, each replicated plot consisted of 8 rows of maize spaced approximately 0.76 m apart and approximately 6 m long. Phenotypic and qualitative environmental interaction data were targeted to be collected from rows 4 and 5. The remaining rows were used for other purposes.

At sites: IABG, NEYO, NCBD, and PAHM each replicated plot consisted of 16 rows of maize spaced approximately 0.76 - 0.96 m apart and approximately 6 m long. Phenotypic and qualitative environmental interaction data were targeted to be collected from rows 2 and 3. Rows 6 and 8 were targeted to collect arthropod samples using sticky traps (rows 8 and 10 at NCBD site). Rows 9, 10, 11, and 12 were targeted for visual counts of arthropod abundance. Rows 13 and 14 were targeted to assess plant damage caused by corn earworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis*). The remaining rows were used for other purposes.

At sites: ILCX, and NEDC, each replicated plot consisted of 6 rows of maize spaced approximately 0.76 m apart and approximately 6 m long. Phenotypic and qualitative environmental interaction data were collected from rows 2 and 3. The remaining rows were used for other purposes.

H.4. Planting and Field Operations

Planting information, soil description, and cropping history of the trial area are listed in Table H-2. Prior to planting, the Principal Investigator at each site prepared the plot area

with a proper seed bed according to local agronomic practices, including tillage, fertilization, and pest management. During the growing season, all plots were assessed for agronomic conditions and pest populations, including pest arthropods, diseases and weeds. Fertilizer, irrigation, agricultural chemicals, and other management practices were applied as necessary. Maintenance operations were performed uniformly across all plots.

Table H-1. Starting Seed for Phenotypic, Agronomic, and Environmental Interaction Assessment

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype	T/C/R[2]
All	MON 87403	11332602	Increased Ear Biomass Maize	T
	MPA640B[3]	11332601	Conventional	C
ARNE	Gateway 6158	11273006	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	NC+ 4443	11226700	Conventional	R
	LG2540	11266730	Conventional	R
IABG	Gateway 6158	11273006	Conventional	R
	LG2620	11226861	Conventional	R
	Burrus 645	11227210	Conventional	R
	Legacy L7671	11226598	Conventional	R
IARL	Lewis 7007	11226559	Conventional	R
	H-9180	11226704	Conventional	R
	Stewart S602	11226919	Conventional	R
	LG2540	11266730	Conventional	R
ILCX	Gateway 4148	11273005	Conventional	R
	Mycogen 2M746	11226705	Conventional	R
	NC+ 4443	11226700	Conventional	R
	LG2548	11266731	Conventional	R
ILMN	Gateway 4148	11273005	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	Burrus 645	11227210	Conventional	R
	LG2540	11266730	Conventional	R
INSH	Gateway 6158	11273006	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	Stine 9724	11298951	Conventional	R
	LG2540	11266730	Conventional	R
KSLA	Lewis 7007	11226559	Conventional	R
	LG2620	11226861	Conventional	R
	NC+ 5220	11226701	Conventional	R
	Phillips 713	11300072	Conventional	R
NCBD	Pioneer 32T16	11226579	Conventional	R
	Mycogen 2M746	11226705	Conventional	R
	Stewart S588	11226918	Conventional	R
	Legacy L7671	11226598	Conventional	R

Table H-1 (continued). Test, Control, and Reference Starting Seed

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype	T/C/R[2]
NEDC	Gateway 4148	11273005	Conventional	R
	LG2620	11226861	Conventional	R
	Stewart S588	11226918	Conventional	R
	Phillips 717	11300073	Conventional	R
NESH	Lewis 6442	11226558	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	Burrus 645	11227210	Conventional	R
	Phillips 717	11300073	Conventional	R
NEYO	Pioneer 32B81	11226578	Conventional	R
	Mycogen 2M746	11226705	Conventional	R
	Burrus 645	11227210	Conventional	R
	LG2540	11266730	Conventional	R
PAGR	Gateway 4148	11273005	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	Stewart S588	11226918	Conventional	R
	LG2540	11226898	Conventional	R
PAHM	Gateway 4148	11273005	Conventional	R
	Midland Phillips 799	11226703	Conventional	R
	Stewart S588	11226918	Conventional	R
	LG2540	11226898	Conventional	R

[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.
[2] T/C/R = Test/Control/Reference.
[3] MPA640B = LH244+LH287.

Table H-2. Field and Planting Information

Site[1]	Planting Date[2]	Harvest Date[2]	Approximate Planting Rate (seeds/m)	Approximate Plot Size (m × m)	Rows per Plot	Soil Type	% OM[3]	Previous Crop 2011
ARNE	05/15/2012	9/20/2012	7.2	6.1 × 6.1	8	Sandy Loam	1.3	Soybean
IABG	05/09/2012	10/05/2012	7.2	6.1 × 12.2	16	Loam	4.0	Soybean
IARL	05/11/2012	10/11/2012	7.2	6.1 × 6.1	8	Silt Clay Loam	3.4	Soybean
ILCX	05/11/2012	09/24/2012	7.2	6.2 × 4.6	6	Silt Clay Loam	3.1	Soybean
ILMN	05/10/2012	10/01/2012	6.9	6.2 × 6.3	8	Silt Clay Loam	4.5	Soybean
INSH	05/16/2012	11/01/2012	7.2	6.1 × 6.1	8	Silt Loam	1.8	Soybean
KSLA	05/11/2012	09/20/2012	8.2	6.1 × 6.1	8	Silt Loam	2.6	Sorghum
NCBD	05/11/2012	09/20/2012	6.6	6.1 × 15.4	16	Sandy Loam	2.6	Cotton
NEDC	05/05/2012	09/10/2012	7.2	6.2 × 4.6	6	Silt Loam	2.6	Soybean
NESH	05/09/2012	09/25/2012	7.2	6.2 × 6.1	8	Silt Loam	1.5	Wheat
NEYO	05/09/2012	10/09/2012	7.2	6.1 × 12.2	16	Silt Loam	3.0	Soybean
PAGR	05/18/2012	10/10/2012	7.2	6.1 × 6.1	8	Loam	2.4	Soybeans
PAHM	05/19/2012	10/22/2012	8.2	6.1 × 12.2	16	Sandy Loam	1.6	Vegetables[4]

[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.
[2] Planting and Harvest Date = mm/dd/yyyy.
[3] % OM = Percent Organic Matter.
[4] Vegetables = peppers, tomatoes, potatoes, cabbage, maize.

H.5. Phenotypic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in Table VII-1.

H.6. Environmental Observations

Environmental interactions (*i.e.*, interactions between the crop plants and their receiving environment) were used to characterize MON 87403 by evaluating plant response to abiotic stressors, disease damage, and arthropod-related damage using qualitative methods described in Section H.7. In addition, specific arthropod damage (corn earworm damage and European corn borer) and arthropod abundance were evaluated using the quantitative methods described in Section H.8.

H.7. Plant Response to Abiotic Stress, Disease Damage, and Arthropod-Related Damage

MON 87403 and the conventional control were evaluated at all sites for plant response to abiotic stressors, disease damage, and arthropod damage. A target of three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the following four crop developmental stages: V6–V8; V12-VT; R1-R3; and R5-R6.

Abiotic stressor, disease damage and arthropod damage observations were collected from each plot using the categorical scale of increasing severity listed below:

Category	Severity of plant damage
None	No symptoms observed
Slight	Symptoms not damaging to plant development (*e.g.*, minor feeding or minor lesions); mitigation likely not required
Moderate	Intermediate between slight and severe; likely requires mitigation
Severe	Symptoms damaging to plant development (*e.g.*, stunting or death); mitigation unlikely to be effective

Method used for selecting stressors at each field site:

1. Prior to each data collection, maize was surveyed in proximity to the study area or the border rows of the study for abiotic stressors (*e.g.*, drought), diseases (*e.g.*, gray leaf spot), and arthropod damage (*e.g.*, corn flea beetle).
2. The Principal Investigator chose three abiotic stressors, three diseases, and three arthropod species that are actively causing damage for subsequent evaluation in the study plots. The Principal Investigators were requested to select additional stressors if present.
3. If fewer than three abiotic stressors, diseases, or arthropod species were present, the cooperator chose additional abiotic stressors, diseases, and arthropod species that are known to commonly occur in that geographical region and cause damage at the study site at that time.

4. All plots at a site were rated for the same abiotic stressors, diseases, and arthropod pests at a given observation, even if that selected stressor was not present in some or all of the plots.
5. If a selected stressor was not present, the cooperator recorded the rating as "none".

As indicated above, the Principal Investigator at each field site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in maize during the given observation period. Therefore, the type of abiotic stressors, diseases, and arthropod pests assessed varied between observations at a site and between sites.

In addition, ear and kernel rot disease and stalk rot disease were evaluated at harvest (R6 growth stage) using the above categorical scale. Ear and kernel rot disease data were collected by evaluating five non-systematically selected ears (one per plant) from each plot. The husks were pulled back and each ear was examined for disease. To evaluate stalk rot, five non-systematically selected stalks in each plot were cut longitudinally. The stalks were then examined for disease.

H.8. Arthropod Abundance

Specific arthropod (corn earworm and European corn borer) damage and arthropod abundance were assessed quantitatively from observations/collections performed at IABG, NEYO, NCBD, and PAHM sites.

Corn earworm damage was evaluated at R5 growth stage by examining ears from ten plants (5 consecutive plants per row) in each plot. The husks were pulled back and each ear was examined for corn earworm damage using a plastic film grid (size of each grid 0.5 cm^2). Damage (cm^2) per plant was calculated as the total number of grid cells matching the damage area multiplied by 0.5 (each grid cell = 0.5 cm^2).

European corn borer damage was evaluated at R6 growth stage by examining ten plants (5 consecutive plants per row) in each plot. Damage was assessed by splitting each of ten plants and counting the number of feeding galleries per plant and length of feeding gallery (cm.) in each stalk.

Arthropods were collected using yellow sticky traps five times during the growing season at the following intervals: late vegetative – VT, R1, R2, R3, and R4 growth stage. Sticky traps (two per plot) were deployed in rows 6 and 8 of each plot at the approximate midpoint between the ground level and the top of the plant canopy for all arthropod collections. At each specified collection, traps were deployed for approximately 7 days. Sticky traps were then sent to the Kansas State University, Manhattan, Kansas, for arthropod identification and enumeration. For pre-processing, four sticky traps from each sampling date at each site were non-systematically selected to prepare a list of pest and beneficial arthropods for enumeration. A maximum of twelve arthropods were enumerated for each collection. From the selected samples, up to six pest and six beneficial arthropods were counted based on their abundance and likelihood of their presence in maize. Thus, the suite of arthropods assessed often varied between

collections from a site and between sites due to differences in temporal activity and geographical distribution of arthropod taxa.

Five visual counts were conducted during the growing season at approximately VT-R1, R1, R2, R3, and R4-R5 from five non-systematically selected plants per plot to collect abundance data per plot. Visual counts were made by examining the stalk, the leaf blade, the leaf collar, the ear tip, the silk, and the tassel of each plant.

H.9. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the trials that would impact the trial objectives were noted. Data were then subjected to data summarization or statistical analysis as indicated Section H-10.

H.10. Statistical Analysis

Plant Growth and Development

Plant vigor data were summarized but not subjected to an analysis of variance (ANOVA); due to the qualitative and subjective nature of the rating. MON 87403 was considered different from the conventional control in vigor if the ranges of vigor of MON 87403 did not overlap with the range of vigor of the conventional control across all replications. Any observed differences between the MON 87403 and conventional control were further assessed in the context of the range of the commercial reference materials, and for consistency at other sites.

An ANOVA was conducted according to a randomized complete block design using SAS® (SAS Software Release 9.3 (TS1M0), 2010) to compare MON 87403 and the conventional control for the phenotypic characteristics listed in Table VII-1, with the exception of plant vigor. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). Comparisons of MON 87403 and the conventional control were conducted within site (individual site analysis) and in a combined-site analysis, in which the data were pooled across sites. MON 87403 and the conventional control materials were not statistically compared to the commercial reference materials. The reference range for each measured phenotypic characteristic was determined from the minimum and maximum mean values from the 21 conventional commercial reference maize hybrids planted among the sites. Data excluded from the study and the reasons for their exclusion are listed in Table H-3.

H.11. Environmental Interaction Data

The environmental interaction data (*i.e.*, plant response to abiotic stressors, disease damage, and arthropod damage) are categorical and were not subjected to ANOVA. MON 87403 and conventional control were considered different in susceptibility or tolerance if the range of injury symptoms of each did not overlap across all four replications. Any observed differences were further assessed in the context of the range of the reference materials, and for consistency at other sites.

An ANOVA was conducted according to a randomized complete block design using SAS® (SAS Software Release 9.3 (TS1M0), 2010) for corn earworm damage, European corn borer damage, and arthropod abundance. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 87403 was compared to the conventional control at each site (individual-site analysis) for corn earworm damage, European corn borer damage, and the arthropod abundance. Additionally, corn earworm damage and European corn borer damage data were pooled across sites (combined-site analysis) for statistical comparison between MON 87403 and the conventional control. Minimum and maximum mean values were calculated for corn earworm damage and European corn borer damage from 11 conventional commercial reference maize hybrids that were included at IABG, NEYO, NCBD, and PAHM sites. The reference range for arthropod abundance evaluated from a given collection and site was determined from the minimum and maximum mean values collected from the conventional commercial reference maize hybrids at the site.

For the arthropod abundance data, statistical analyses and significance testing of differences between MON 87403 and the conventional control materials were only performed for the arthropods present in sufficient numbers to estimate the material mean arthropod counts and the variation of the means. An inclusion criterion was established where a given arthropod must have an average count per plot per collection time (across all materials) of ≥ 1.

Data excluded from the study and the reasons for their exclusion are listed in Table H-3.

H.12. Individual Field Site Plant Growth, Development, and Environmental Interactions Results and Discussion

H.12.1. Plant Growth Development

In the individual-site analysis, a total of 14 statistically significant differences were detected out of 155 comparisons between MON 87403 and the conventional control (Table H-4). These differences were distributed among eight of the 13 phenotypic characteristics. MON 87403 had higher early stand count than the conventional control at the ILMN site (82.8 vs. 79.8 plants). MON 87403 reached days to 50% pollen shed later than the conventional control at the PAGR site (68.8 vs. 65.8 days). MON 87403 reached days to 50% silking earlier than the conventional control at the NESH site (68.8 vs. 71.5 days) but reached days to 50% silking later than the conventional control at the PAGR site (69.3 vs. 66.8 days). MON 87403 had higher stay green (more green tissue)

than the conventional control at the NESH site (9.0 vs. 8.0 rating). MON 87403 had higher final stand count than the conventional control at the NESH site (77.5 vs. 74.8 plants). MON 87403 had lower test weight than the conventional control at the NCBD site (70.2 vs. 71.9 kg/hL). MON 87403 had a lower yield than the conventional control at the ILMN site (15.5 vs. 17.0 Mg/ha), at the NCBD site (8.7 vs. 10.0 Mg/ha), and at the PAGR site (12.1 vs. 13.9 Mg/ha). The statistical differences between MON 87403 and the conventional control detected in the individual-site analyses for early stand count, days to 50% pollen shed, days to 50% silking, stay green, final stand count, test weight, and yield were not detected in the combined-site analysis. Thus, the differences detected for these phenotypic characteristics at individual sites do not indicate a consistent response associated with the trait and are unlikely to be biologically meaningful, in terms of increased pest/weed potential of MON 87403 compared to conventional control (Figure VII-1, step 2, "no" answer). MON 87403 had increased ear height compared to the conventional control at the ILMN site (125.3 vs. 119.9 cm), NEDC site (121.8 vs. 111.4 cm), NESH site (124.3 vs. 118.2 cm) and PAHM site (89.3 vs. 78.0 cm). While statistical differences were detected for ear height in both the individual site and the combined-site analyses, the mean values of MON 87403 for ear height in the combined-site analysis were within the range of values for commercial maize reference hybrids. Therefore, these differences in ear height were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased plant pest/weed potential of MON 87403 compared to conventional control (Figure VII-1, step 3, "no" answer).

In individual site assessments of plant vigor, MON 87403 and the conventional control were considered different if the range of values did not overlap between MON 87403 and the conventional control across all four replications. There were no differences observed between MON 87403 and the conventional control in plant vigor (Table H-4).

Plant Response to Abiotic Stressor, Disease Damage, and Arthropod-related Damage:

In the individual-site assessment, no differences were observed between MON 87403 and the conventional control for any of the 143 comparisons for the assessed abiotic stressors, including cold, drought, flood, frost, hail, heat, nutrient deficiency, soil compaction, sunscald, and wind (Table H-5).

In the individual-site assessment, no differences were observed between MON 87403 and the conventional control for any of the 176 comparisons for the assessed diseases, including anthracnose, bacterial leaf spot, ear rot, eyespot, Fusarium sp., Goss's bacterial wilt, gray leaf spot, leaf blight, maize rough dwarf virus, northern leaf spot, Pythium sp., Rhizoctonia sp., rust, seedling blight, smut, stalk rot, and Stewart's bacterial wilt (Table H-6).

In the individual-site assessment, no differences were observed between MON 87403 and the conventional control for any of the 150 comparisons for the assessed arthropods, including aphids, armyworms, bean leaf beetles, billbugs, cutworms, corn earworms, corn flea beetles, corn rootworm beetles, European corn borers, grasshoppers, Japanese

beetles, June beetles, sap beetles, slugs, spider mites, stink bugs, and wireworm beetles (Table H-7).

Corn Earworm and European Corn Borer Damage:

In the individual-site analysis, no statistically significant difference was detected out of 12 comparisons between MON 87403 and the conventional control for corn earworm and European corn borer among all observations at all four sites (Table H-8).

Sticky Trap:

A total of 144 statistical comparisons were made between MON 87403 and the conventional control for arthropod abundance involving the following arthropods: aphids, billbugs, corn flea beetles, corn rootworm beetles, delphacid planthoppers, grasshoppers, lacewings, ladybird beetles, leafhoppers, macro-parasitic hymenoptera, micro-parasitic hymenoptera, minute pirate bugs, damsel bugs, sap beetles, seedcorn beetles, spiders, syrphid flies, tachinid flies, tarnished plant bugs, thrips and click beetles (Table H-9). Lack of sufficient arthropod abundance precluded statistical comparisons between MON 87403 and the conventional control for 92 additional comparisons; however, descriptive statistics were provided for these comparisons.

No statistically significant differences were detected between MON 87403 and the conventional control for 130 out of 144 comparisons. The mean abundance of lacewings was higher in MON 87403 than the conventional control in Collection 1 (2.0 vs. 0.3 per plot) at the IABG site. The mean abundance of lacewings was lower in MON 87403 than the conventional control in Collection 3 (2.5 vs. 8.0 per plot) and higher than the conventional control in Collection 5 (3.0 vs. 0.8 per plot), at the NEYO site. The mean abundance of macro-parasitic hymenoptera was lower in MON 87403 than the conventional control in Collection 4 (0.3 vs. 3.3 per plot) at the PAHM site. The mean abundance of micro-parasitic hymenoptera was higher in MON 87403 than the control in Collection 2 (21.0 vs. 8.3 per plot) at the NCBD site. The mean abundance of aphids was higher in MON 87403 than the conventional control in Collection 5 (22.5 vs. 13.8 per plot) at the NEYO site. The mean abundance of corn flea beetles was lower in MON 87403 than the conventional control in Collection 3 (0.0 vs. 1.5 per plot) at the IABG site and higher in MON 87403 than the conventional control in Collection 5 (1.3 vs. 0.3 per plot) at the IABG site. The mean abundance of corn rootworm beetles was lower in MON 87403 than the conventional control in Collection 3 (23.0 vs. 34.3 per plot) at the NEYO site. The mean abundance of thrips was higher in MON 87403 than the conventional control in Collection 2 (32.5 vs. 18.5 per plot) at the NCBD site, in Collection 2 (183.0 vs. 71.5 per plot), Collection 4 (134.3 vs. 60.3 per plot), and Collection 5 (138.0 vs. 62.3 per plot) at the NEYO site and Collection 3 (9.7 vs. 2.8 per plot) at the PAHM site.

The mean abundance values for MON 87403 were within the reference ranges for all differences detected in arthropod abundance with the exception of the difference detected for lacewing abundance in Collection 5 at the NEYO site (MON 87403 mean = 3.0 per plot; reference range = 0.5 – 2.8 per plot), macro-parasitic hymenoptera abundance in

Collection 4 at the PAHM site (MON 87403 mean = 0.3 per plot; reference range = 1.5 – 2.3 per plot), corn flea beetles abundance in Collection 3 (MON 87403 mean = 0.0 per plot; reference range = 1.8 – 2.0 per plot) and Collection 5 (MON 87403 mean = 1.3 per plot; reference range = 2.0 – 3.8 per plot) at the IABG site, thrips abundance in Collection 2 (MON 87403 mean = 183.0 per plot; reference range = 66.8 – 159.0 per plot) and Collection 4 (MON 87403 mean = 134.3 per plot; reference range = 56.3 – 125.0 per plot) at the NEYO site, and in Collection 3 at the PAHM site (MON 87403 mean = 9.7 per plot; reference range = 2.3 – 4.8 per plot). However, these differences were not consistently detected across collections or sites.

Thus, these differences in lacewings, macro-parasitic hymenoptera, micro-parasitic hymenoptera, aphids, corn flea beetles, corn rootworms, and thrips were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of increased pest potential of MON 87403 compared to conventional maize (Section VII.B.2).

Visual Counts:

A total of 66 statistical comparisons were made between MON 87403 and the conventional control for arthropod abundance involving the following arthropods: ant-like flower beetles, corn flea beetles, Japanese beetles, lacewing adults, lacewing larvae, ladybird beetle adults, ladybird beetle larvae, minute pirate bugs, corn rootworm beetles, sap beetles, shining flower beetles, spiders, stink bugs and click beetles (Table H-10). Lack of sufficient arthropod abundance precluded statistical comparisons between MON 87403 and the conventional control for 171 additional comparisons; however, the descriptive statistics were provided for these comparisons.

No statistically significant differences were detected between MON 87403 and the conventional control for 61 out of 66 comparisons. The mean abundance of minute pirate bugs was lower in MON 87403 than the conventional control in Collection 4 (0.0 vs. 1.0 per plot) at the PAHM site. The mean abundance of corn rootworm beetles was lower in MON 87403 than the conventional control in Collection 1 (0.3 vs. 2.0 per plot) at the PAHM site. The mean abundance of sap beetles was lower in MON 87403 than the conventional control in Collection 5 (1.3 vs. 4.5 per plot) at the NCBD site and Collection 3 (1.0 vs. 4.5 per plot) at the NEYO site. The mean abundance of shining flower beetles was lower in MON 87403 than the conventional control in Collection 2 (7.0 vs. 12.3 per plot) at the NCBD site.

The mean abundance values for MON 87403 were within the reference ranges for all differences detected in arthropod abundance with the exception of the difference detected for minute pirate bug abundance in Collection 4 at the PAHM site (MON 87403 mean = 0.0 per plot; reference range = 0.5 – 3.7 per plot) and corn rootworm beetle abundance in Collection 1 at the PAHM site (MON 87403 mean = 0.3 per plot; reference range = 1.0 – 2.0 per plot). However, these differences were not consistently detected across collections or sites.

Thus these differences in minute pirate bugs, corn rootworm beetles, sap beetles and shining flower beetles were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of increased pest potential of MON 87403 compared to conventional maize (Section VII.B.2).

Table H-3. Data Missing or Excluded from Analysis

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
All	All	All	All	Data was both collected & analyzed for Anthesis Silking Interval, but not reported for combined site or individual site analysis.	Not relevant to the mode of action for this trait.
IALL, ILCY, ILPH, ILRD, OHTR, MSST, ILWY	All	All	All	All phenotypic and environmental interactions data.	Extreme weather conditions (strong wind or drought) that caused extensive damage.
ILCX	All	All	401thru 407	All phenotypic and environmental interactions data.	Drought stress
PAHM	Midland Phillips 799 MON 87403 Gateway 4148 Midland Phillips 799	Reference Test Reference Reference	106, 206, 306, 406	Arthropod data collection	Planting error
PAHM NEDC IABG	MON 87403 Burrus 645	Test Reference	206, 407, 405, 401	First sub-sample of ear height and/or plant height are missing.	Electronic data collection error

Table H-3 (continued). Data Missing or Excluded from Study

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
NESH	Midland Phillips 799	Reference	203	Test Weight	Data collection error
PAGR	MON 87403	Test	204	Early Stand Count	Statistical outlier
NCBD	Legacy L7671	Reference	207	Moisture & Yield	Data collection error
ARNE NEYO	LG2540 Mycogen 2M746	Reference	301, 103, 203, 204, 304, 403	Days to 50% Pollen	Fewer than 10 plants shed pollen.
PAGR INSH	Stewart S588 LG2540	Reference	102, 202, 206	Phenotypic data, stalk rot and ear rot	Plants were 10% below final stand count for site
IABG	All	All	All	Environmental interaction evaluation # 4 (Other)	Improper selection of stressor
NEYO	All	All	All	Environmental interaction evaluation # 3 (2 arthropod, 1 abiotic and1 disease stressor)	Missing data for five stressors
INSH	All	All	All	Environmental interaction evaluation # 3 (Drought)	Missing data for 8 stressors
KSLA	All	All	All	Insect stressor observation 2 (velvet bean caterpillar)	Improper selection of stressor

Note: The study also included additional test entry not relevant to the objectives of this report, which was included in the statistical analysis input file. However, no statistical comparisons were made using this additional test entry.

[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.

Table H-4. Individual Site Analysis of Phenotypic Characteristics of MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant Vigor (1-9 scale)[2]		Early stand count (#/plot)		Days to 50% pollen shed	
	Range		Mean (S.E.)[3]		Mean (S.E.)[3]	
Site Code[1]	MON 87403	Control	MON 87403	Control	MON 87403	Control
ARNE	3 – 4	3 – 4	83.5 (0.96)	83.5 (0.87)	54.5 (0.87)	53.5 (0.65)
IABG	1 – 2	2 – 3	82.5 (2.87)	84.5 (0.87)	65.0 (0.00)	65.0 (0.00)
IARL	3	3 – 5	83.3 (2.43)	79.0 (2.68)	61.0 (0.00)	61.3 (0.48)
ILCX	1	1	82.7 (1.33)	83.0 (0.58)	60.3 (0.33)	60.7 (0.33)
ILMN	1 – 2	1 – 3	82.8 (0.63)*	79.8 (0.48)	63.0 (0.00) †	63.0 (0.00)
INSH	2 – 4	3 – 5	81.3 (0.48)	82.0 (1.08)	65.0 (1.08)	63.5 (0.87)
KSLA	2	2	94.0 (1.22)	95.0 (1.63)	57.5 (0.96)	57.0 (0.58)
NCBD	3 – 4	3	72.3 (2.50)	72.8 (1.70)	59.3 (0.25)	59.8 (0.25)
NEDC	2	2	89.0 (1.35)	86.5 (0.65)	71.0 (0.00)	70.5 (0.50)
NESH	2	2	88.5 (1.19)	92.8 (2.59)	68.0 (0.00)	68.0 (0.00)
NEYO	1	1	78.3 (1.55)	79.3 (1.11)	68.0 (0.00)	68.0 (0.00)
PAGR	3 – 4	1 – 8	73.0 (1.00)	80.0 (1.83)	68.8 (0.75)*	65.8 (0.75)
PAHM	2 – 4	2 – 4	86.0 (2.20)	88.5 (0.87)	63.0 (0.71)	63.0 (0.41)

Table H-4 (continued). Individual Site Analysis of Phenotypic Characteristics of MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Days to 50% silking		Stay-green rating (1-9 scale)		Ear height (cm)	
	Mean (S.E.)[3]		Mean (S.E.)[3]		Mean (S.E.)[3]	
Site Code[1]	MON 87403	Control	MON 87403	Control	MON 87403	Control
ARNE	55.3 (1.18)	54.0 (0.58)	4.3 (0.25)	4.5 (0.50)	136.1 (3.99)	133.1 (3.66)
IABG	63.8 (0.63)	63.8 (0.25)	8.0 (0.41)	7.8 (0.75)	98.7 (1.66)	104.6 (2.52)
IARL	60.5 (0.50)	60.5 (0.50)	3.5 (0.29)	4.0 (0.41)	96.4 (4.00)	91.7 (4.85)
ILCX	64.0 (1.00)	66.0 (0.00)	3.3 (1.45)	3.3 (0.67)	108.6 (0.92)	105.1 (3.64)
ILMN	63.0 (0.00) †	63.0 (0.00)	4.3 (0.25)	4.3 (0.25)	125.3 (1.28)*	119.9 (1.26)
INSH	66.0 (1.35)	64.5 (1.19)	4.0 (0.00)	4.0 (0.00)	107.2 (6.34)	108.9 (3.69)
KSLA	58.5 (0.50)	59.0 (0.58)	1.0 (0.00)	1.0 (0.00)	100.5 (3.36)	95.8 (3.40)
NCBD	59.3 (0.25)	59.5 (0.29)	6.3 (0.25)	6.3 (0.25)	127.1 (5.15)	128.7 (3.14)
NEDC	70.0 (0.58)	71.0 (0.00)	7.8 (0.48)	8.0 (0.41)	121.8 (3.99)*	111.4 (2.40)
NESH	68.8 (0.75)*	71.5 (1.66)	9.0 (0.00)*	8.0 (0.00)	124.3 (1.17)*	118.2 (1.74)
NEYO	68.0 (0.00)	68.3 (0.25)	6.0 (0.00)	5.5 (0.29)	113.2 (3.21)	103.6 (1.90)
PAGR	69.3 (0.75)*	66.8 (0.75)	2.0 (0.41)	1.5 (0.29)	95.9 (6.04)	98.8 (7.60)
PAHM	64.0 (0.71)	64.3 (0.63)	8.5 (0.29)	8.5 (0.29)	89.3 (5.39)*	78.0 (1.95)

Table H-4 (continued). Individual Site Analysis of Phenotypic Characteristics of MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant height (cm)		Dropped ears (#/plot)		Stalk lodged plants (#/plot)	
	Mean (S.E.)[3]		Mean (S.E.)[3]		Mean (S.E.)[3]	
Site Code[1]	MON 87403	Control	MON 87403	Control	MON 87403	Control
ARNE	265.0 (2.39)	268.6 (0.83)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
IABG	257.1 (3.96)	257.3 (3.51)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
IARL	213.5 (4.32)	205.7 (3.93)	0.3 (0.25)	0.0 (0.00)	1.8 (1.75)	0.0 (0.00)
ILCX	209.5 (9.34)	205.3 (5.74)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
ILMN	265.4 (2.74)	269.5 (3.41)	0.0 (0.00)	0.0 (0.00)	0.5 (0.29)	1.0 (0.58)
INSH	223.4 (8.86)	230.2 (5.59)	3.8 (0.63)	2.3 (0.48)	10.3 (3.64)	11.3 (2.02)
KSLA	213.1 (8.54)	212.8 (4.04)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
NCBD	264.7 (6.83)	261.1 (3.03)	8.5 (4.33)	7.3 (3.20)	0.5 (0.29)	0.3 (0.25)
NEDC	257.8 (4.28)	251.8 (2.14)	0.0 (0.00)	0.5 (0.29)	3.5 (1.55)	3.8 (1.31)
NESH	258.8 (2.49)	249.2 (4.03)	0.0 (0.00) †	0.0 (0.00)	0.3 (0.25)	0.8 (0.25)
NEYO	265.3 (2.60)	260.1 (0.87)	0.0 (0.00) †	0.0 (0.00)	0.3 (0.25)	0.3 (0.25)
PAGR	214.4 (6.66)	224.4 (4.12)	2.0 (0.00)	1.0 (0.41)	7.5 (1.85)	6.3 (2.39)
PAHM	210.8 (7.96)	209.3 (5.87)	1.0 (0.71)	1.0 (0.41)	6.0 (1.78)	4.8 (1.03)

Table H-4 (continued). Individual Site Analysis of Phenotypic Characteristics of MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Root lodged plants (#/plot)		Final stand count (#/plot)		Grain moisture (%)	
	Mean (S.E.)[3]		Mean (S.E.)[3]		Mean (S.E.)[3]	
Site Code[1]	MON 87403	Control	MON 87403	Control	MON 87403	Control
ARNE	0.3 (0.25)	0.0 (0.00)	75.3 (0.25)	75.0 (0.00)	16.1 (0.32)	16.1 (0.14)
IABG	0.8 (0.75)	1.3 (0.75)	72.3 (1.55)	72.3 (1.11)	16.6 (0.10)	17.5 (0.66)
IARL	4.3 (1.89)	1.8 (1.03)	74.8 (0.75)	73.5 (0.96)	17.4 (0.13)	17.7 (0.17)
ILCX	0.3 (0.33)	0.3 (0.33)	76.0 (0.00) †	76.0 (0.00)	25.9 (0.58)	26.4 (1.22)
ILMN	2.3 (1.93)	1.8 (1.44)	75.3 (0.25)	75.5 (0.29)	20.9 (1.55)	19.1 (1.18)
INSH	0.8 (0.48)	1.0 (0.71)	71.5 (1.04)	70.8 (1.89)	19.3 (0.27)	19.5 (0.48)
KSLA	0.0 (0.00) †	0.0 (0.00)	76.8 (1.60)	76.3 (0.25)	16.4 (0.34)	16.0 (0.32)
NCBD	0.0 (0.00) †	0.0 (0.00)	68.8 (1.60)	68.8 (1.89)	19.5 (0.25)	19.6 (0.21)
NEDC	0.0 (0.00) †	0.0 (0.00)	75.8 (0.63)	73.8 (1.31)	15.5 (0.27)	15.4 (0.55)
NESH	0.0 (0.00)	0.8 (0.48)	77.5 (1.26)*	74.8 (1.44)	16.7 (0.60)	16.4 (0.27)
NEYO	0.0 (0.00) †	0.0 (0.00)	61.5 (0.65)	60.0 (1.41)	15.7 (0.13)	15.7 (0.14)
PAGR	0.0 (0.00) †	0.0 (0.00)	63.8 (0.63)	65.8 (0.75)	30.6 (0.46)	29.4 (1.09)
PAHM	0.0 (0.00) †	0.0 (0.00)	73.8 (0.25)	74.5 (0.65)	19.9 (0.52)	20.2 (0.46)

Table H-4 (continued). Individual Site Analysis of Phenotypic Characteristics of MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

	Phenotypic Characteristics (units)			
	Test weight (kg/hl)		Yield (Mg/ha)	
	Mean (S.E.)[3]		Mean (S.E.)[3]	
Site Code[1]	MON 87403	Control	MON 87403	Control
ARNE	74.6 (0.69)	74.5 (0.27)	10.2 (0.17)	10.7 (0.17)
IABG	75.8 (0.36)	77.4 (0.46)	7.5 (1.35)	8.8 (1.19)
IARL	75.4 (0.95)	75.7 (0.84)	7.6 (0.51)	6.4 (0.75)
ILCX	72.7 (0.66)	71.8 (2.82)	7.9 (1.38)	4.9 (1.41)
ILMN	70.1 (0.35)	71.4 (0.61)	15.5 (0.44)*	17.0 (0.44)
INSH	71.1 (0.34)	70.6 (0.86)	10.5 (0.88)	11.1 (0.53)
KSLA	78.4 (0.33)	77.8 (0.57)	14.0 (0.41)	13.5 (0.46)
NCBD	70.2 (0.64)*	71.9 (0.73)	8.7 (0.64)*	10.0 (0.54)
NEDC	74.8 (0.53)	74.7 (0.37)	7.0 (0.12)	7.7 (0.11)
NESH	76.4 (0.38)	75.3 (0.13)	5.0 (0.16)	4.6 (0.56)
NEYO	76.0 (0.40)	75.8 (0.27)	13.3 (0.47)	12.9 (0.40)
PAGR	59.3 (0.38)	60.3 (0.98)	12.1 (0.73)*	13.9 (0.86)
PAHM	68.6 (0.50)	68.2 (0.26)	11.7 (0.44)	11.0 (0.34)

Note: The experimental design was a randomized complete block with four replicates per site.
***** Indicates statistically significant difference between MON 87403 and the conventional control (α =0.05) using ANOVA.
† Indicates p-values could not be generated due to lack of variability in the data.
[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.
[2] Data were not subjected to statistical analysis. Plant vigor rating range (minimum - maximum); the range of plant vigor ratings for the references is as follows: ARNE 1– 4; IABG 1 – 3; IARL 2 – 4; ILCX 1; ILMN 1 – 3; INSH 3 – 4; KSLA 2; NCBD 3 – 4; NEDC 2; NESH 2; NEYO 1; PAGR 1 – 8; PAHM 2 – 5
[3] MON 87403 and the conventional control values represent means with standard error in parentheses. N = 4, except where noted in Table H-5.

Table H-5. Abiotic Stressor Evaluations Using a Categorical Scale for MON 87403 and the Conventional Control in 2012 U.S. Field Trials

Abiotic Stressor	Number of Observations across Sites[1]	Number of Observations where No Differences were Observed between MON 87403 and the Conventional Control
Total	143	143
Cold	2	2
Drought[2]	36	36
Flood[3]	7	7
Frost	2	2
Hail	9	9
Heat	27	27
Nutrient deficiency	24	24
Soil compaction	4	4
Sunscald	4	4
Wind	28	28

Note: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 87403 and the conventional control during any observation for damage caused by any of the assessed abiotic stressors. Data were not subjected to statistical analysis.
Observational data collected at four crop development stages: V6–V8; V12-VT; R1-R3; and R5-R6.

[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.

[2] Includes dryness, dry/heat.

[3] Includes wet soil.

Table H-6. Disease Damage Evaluations Using a Categorical Scale for MON 87403 and the Conventional Control in 2012 U.S. Field Trials

Disease	Number of Observations across Sites[1]	Number of Observations where No Differences were Observed between MON 87403 and the Conventional Control
Total	176	176
Anthracnose	8	8
Bacterial leaf spot	2	2
Ear rot[2]	19	19
Eyespot	6	6
Fusarium sp.	8	8
Goss's bacterial wilt	11	11
Gray leaf spot	23	23
Leaf blight[3]	24	24
Maize rough dwarf virus	3	3
Northern leaf spot	7	7
Pythium sp.	2	2
Rhizoctonia sp.	2	2
Rust[4]	12	12
Seedling blight	2	2
Smut	15	15
Stalk rot[5]	17	17
Stewart's bacterial wilt	15	15

Note: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 87403 and the conventional control during any observation for damage caused by any of the assessed diseases. Data were not subjected to statistical analysis.
Observational data collected at four crop development stages: V6–V8; V12-VT; R1-R3; and R5-R6.
Additional assessments of ear rot disease and stalk rot disease were made on 5 plant/plots at harvest.
[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.
[2] Assessed on 5 non-systematically selected plants. At ARNE, ILCX, ILMN, INSH, NEDC, and NESH ear rot data were collected both on a per plot basis and on 5 plant/plots.
[3] Includes northern and southern.
[4] Includes common and southern.
[5] Assessed on 5 non-systematically selected plants. At ILCX, NEDC, NESH, and PAGR stalk rot data were collected both on a per plot basis and on 5 plant/plots.

Table H-7. Arthropod Damage Evaluations Using a Categorical Scale for MON 87403 and the Conventional Control in 2012 U.S. Field Trials

Arthropod	Number of Observations across Sites[1]	Number of Observations where No Differences were Observed between MON 87403 and the Conventional Control
Total	150	150
Aphids (Aphididae)	5	5
Armyworms (Noctuidae)[2]	24	24
Bean leaf beetles (*Cerotoma trifurcata*)	2	2
Billbugs (*Sphenophorus parvulus*)	2	2
Cutworms (Noctuidae)[3]	10	10
Corn earworms (*Helicoverpa zea*)	13	13
Corn flea beetles (*Chaetocnema pulicaria*)[4]	7	7
Corn rootworm beetles (*Diabrotica* sp.*)*	14	14
European corn borers (*Ostrinia nubilalis*)	17	17
Grasshoppers (*Melanoplus* spp.)	20	20
Japanese beetles (*Popillia japonica*)	7	7
June beetles (Scarabaeidae)	2	2
Sap beetles (Nitidulidae)	6	6
Slugs[5] (Gastropoda)	1	1
Southwestern corn borer (*Diatraea grandiosella*)	2	2
Spider mites (*Tetranychus* sp.)	5	5
Stink bugs (Pentatomidae)	11	11
Western bean cutworm (*Richia albicosta*)	1	1
Click beetles (Elateridae)[6]	1	1

Note: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 87403 and the conventional control during any observation for damage caused by any of the assessed arthropods. Data were not subjected to statistical analysis.
Observational data collected at four crop development stages: V6–V8; V12-VT; R1-R3; and R5-R6.
[1] Site code: ARNE = Jackson County, AR; IABG = Greene County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; KSLA = Pawnee County, KS; NCBD = Perquimans County, NC; NEDC = Butler County, NE; NESH = Polk County, NE; NEYO = York County, NE; PAGR = Lehigh County, PA; PAHM = Berks County, PA.
[2] Includes beet armyworm and fall armyworm
[3] Includes black cutworm
[4] Includes flea beetle
[5] Slugs are not arthropods but are one of the common stressor in Maize.
[6] Wireworm adults

Table H-8. Individual Site Analysis: Corn Earworm Damage and European Corn Borer Damage to MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

Pest	Damage assessment	Site[1]	Mean (S.E.)[2]	
			MON 87403	Control
Corn earworm (*H. zea*)[3]	Damage area of 10 plants per plot (cm^2)	IABG	0.6 (0.12)	1.2 (0.25)
		NCBD	3.4 (0.85)	6.4 (2.01)
		NEYO	2.9 (0.38)	3.3 (0.44)
		PAHM	0.4 (0.19)	0.1 (0.06)
European corn borer (*O. nubilalis*)[4]	Number of stalk galleries of 10 plants per plot	IABG	0.0 (0.00)	0.0 (0.03)
		NCBD	0.1 (0.08)	0.2 (0.09)
		NEYO	0.0 (0.03)	0.1 (0.03)
		PAHM	1.0 (0.27)	1.0 (0.25)
European corn borer (*O. nubilalis*)[4]	Stalk gallery length (cm) of 10 plants per plot	IABG	0.0 (0.00)	0.1 (0.08)
		NCBD	0.6 (0.39)	1.3 (0.64)
		NEYO	0.1 (0.10)	0.2 (0.10)
		PAHM	4.6 (1.27)	4.0 (1.04)

Note: The experimental design was a randomized complete block with four replicates per site.
[1] Site code: IABG = Greene, IA; NCBD = Perquimans, NC; NEYO = York, NE; PAHM = Berks, PA.
[2] MON 87403 and the conventional control values represent means with standard error in parentheses.
[3] Damage assessment for *H. zea* was conducted at R5 growth stage.
[4] Damage assessments for *O. nubilalis* were conducted at R6 growth stage.

Table H-9. Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Spiders (Araneae)			Ladybird beetles (Coccinellidae)			Lacewings (Chrysopidae)		
		Beneficial Arthropod			Beneficial Arthropod			Beneficial Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	—	—	—	0.0 (0.00)†	0.3 (0.25)	0.0 – 3.0	2.0 (0.41)*	0.3 (0.25)	1.0 – 3.3
	NCBD	0.3 (0.25)†	1.0 (1.00)	0.5 – 1.0	1.0 (0.41)	1.5 (0.87)	1.0 – 2.0	—	—	—
	NEYO	0.0 (0.00)†	0.3 (0.25)	0.3 – 0.5	0.3 (0.25)†	0.0 (0.00)	0.3 – 2.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	1.3 (0.48)	0.0 – 0.7	12.3 (2.40)	14.5 (0.87)	11.3 – 25.5	—	—	—
2	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5	0.0 (0.00)†	0.3 (0.25)	0.3 – 1.5	1.8 (0.48)	2.3 (1.11)	2.5 – 4.0
	NCBD	0.3 (0.25)†	0.5 (0.29)	0.3 – 1.3	5.0 (1.08)	5.3 (1.65)	5.5 – 6.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.5	0.3 (0.25)†	0.0 (0.00)	0.3 – 1.0	0.8 (0.75)†	0.5 (0.29)	0.0 – 1.0
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	7.3 (4.06)	4.3 (1.84)	2.5 – 11.5	—	—	—
3	IABG	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3	—	—	—	0.0 (0.00)†	0.3 (0.25)	0.0 – 1.0
	NCBD	0.5 (0.29)†	0.5 (0.29)	0.0 – 0.8	4.5 (0.96)	3.0 (0.71)	2.8 – 4.5	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3
	NEYO	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.8	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	2.5 (1.04)*	8.0 (1.73)	1.0 – 3.3
	PAHM	2.0 (0.58)†	0.3 (0.25)	0.0 – 0.5	1.7 (0.67)	2.8 (1.38)	2.5 – 4.3	—	—	—
4	IABG	—	—	—	0.0 (0.00)†	0.3 (0.25)	0.3 – 1.5	2.3 (0.85)	0.5 (0.29)	3.5 – 6.3
	NCBD	0.5 (0.29)†	0.5 (0.29)	0.0 – 0.8	6.0 (1.47)	6.0 (0.82)	3.0 – 7.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NEYO	—	—	—	—	—	—	1.5 (0.65)	3.3 (1.97)	0.5 – 2.8
	PAHM	1.3 (0.88)†	0.5 (0.29)	0.0 – 0.8	2.3 (1.20)	0.8 (0.25)	0.3 – 2.7	—	—	—
5	IABG	—	—	—	0.3 (0.25)	0.5 (0.50)	0.3 – 2.8	5.3 (2.29)	6.5 (2.53)	5.5 – 9.3
	NCBD	1.0 (0.41)†	1.0 (0.41)	0.3 – 1.8	2.0 (1.41)	1.5 (0.65)	0.5 – 3.5	—	—	—
	NEYO	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.5	—	—	—	3.0 (0.41)*	0.8 (0.48)	0.5 – 2.8
	PAHM	—	—	—	0.0 (0.00)†	0.5(0.50)	0.5 – 2.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Macro-parasitic hymenoptera			Micro-parasitic hymenoptera			Damsel bugs[5] (Nabidae)		
		Beneficial Arthropod			Beneficial Arthropod			Beneficial Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	6.5 (1.19)	4.5 (1.04)	6.0 – 9.0	15.0 (2.12)	12.0 (3.58)	13.3 – 18.0	—	—	—
	NCBD	0.5 (0.50)†	0.0 (0.00)	0.0 – 0.3	33.3 (1.93)	44.5 (1.32)	50.8 – 61.8	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.8
	NEYO	2.0 (0.91)	0.8 (0.25)	1.5 – 1.8	15.3 (2.14)	13.3 (2.06)	14.0 – 19.8	—	—	—
	PAHM	0.7 (0.33)†	1.0 (0.41)	0.0 – 1.8	24.7 (2.33)	27.0 (4.80)	22.0 – 37.5	—	—	—
2	IABG	7.3 (3.20)	4.8 (0.85)	5.3 – 11.0	20.8 (1.44)	17.8 (4.11)	15.5 – 20.8	—	—	—
	NCBD	0.8 (0.48)†	0.3 (0.25)	0.0 – 1.5	21.0 (6.47)*	8.3 (1.80)	14.3 – 25.8	—	—	—
	NEYO	0.3 (0.25)	1.0 (0.58)	0.5 – 1.8	15.5 (2.60)	12.3 (2.50)	15.5 – 24.0	—	—	—
	PAHM	1.0 (1.00)	1.3 (0.95)	1.0 – 2.3	19.3 (1.67)	25.5 (6.64)	16.0 – 29.8	—	—	—
3	IABG	3.8 (0.75)	3.3 (1.11)	3.3 – 5.3	11.3 (2.10)	10.5 (5.12)	12.8 – 15.5	—	—	—
	NCBD	—	—	—	18.8 (2.95)	19.8 (2.78)	13.5 – 26.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NEYO	2.3 (1.11)	2.3 (0.75)	1.8 – 3.0	15.5 (1.85)	13.5 (1.19)	12.5 – 25.3	—	—	—
	PAHM	1.3 (0.33)	2.0 (0.41)	0.3 – 2.8	25.3 (5.49)	23.5 (6.22)	21.0 – 29.3	—	—	—
4	IABG	6.0 (0.91)	5.3 (0.95)	3.0 – 6.5	19.5 (1.50)	15.0 (7.75)	10.5 – 21.5	—	—	—
	NCBD	—	—	—	17.8 (1.49)	17.0 (0.91)	9.0 – 22.8	—	—	—
	NEYO	1.8 (0.75)	3.8 (1.65)	2.0 – 2.8	12.3 (3.12)	12.5 (4.21)	10.5 – 12.0	—	—	—
	PAHM	0.3 (0.33)*	3.3 (1.03)	1.5 – 2.3	51.0 (12.74)	32.0 (6.47)	27.0 – 47.5	—	—	—
5	IABG	2.0 (0.71)	2.5 (0.50)	1.5 – 2.3	5.8 (1.25)	9.0 (3.51)	7.5 – 10.5	—	—	—
	NCBD	0.5 (0.29)†	0.3 (0.25)	0.0 – 0.5	14.5 (2.33)	13.5 (3.23)	10.0 – 15.5	—	—	—
	NEYO	0.8 (0.48)	1.0 (0.41)	1.5 – 2.0	12.8 (4.70)	7.5 (1.26)	7.5 – 14.0	—	—	—
	PAHM	2.0 (0.58)	3.5 (0.50)	3.5 – 5.0	37.0 (10.97)	29.3 (5.28)	32.5 – 41.0	—	—	—

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Minute pirate bugs (Anthocoridae)			Syrphid flies (Syrphidae)			Aphids (Aphididae)		
		Beneficial Arthropod			Beneficial Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]
Coll.[1]	Site[2]	MON 87403	Control		MON 87403	Control		MON 87403	Control	
1	IABG	0.8 (0.48)†	1.0 (0.41)	0.3 – 1.0	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.3 – 1.5
	NCBD	—	—	—	1.3 (0.75)	1.5 (0.50)	1.3 – 4.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	—	—	—	0.8 (0.48)†	0.8 (0.48)	0.3 – 1.0	—	—	—
	PAHM	0.7 (0.67)†	0.5 (0.29)	0.0 – 2.3	4.0 (1.15)	6.8 (1.84)	3.5 – 6.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.0
2	IABG	1.0 (0.58)	2.0 (1.08)	1.3 – 3.5	—	—	—	—	—	—
	NCBD	—	—	—	6.0 (1.00)	4.3 (1.31)	3.5 – 6.5	—	—	—
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	—	—	—	—	—	—
	PAHM	1.0 (0.58)	1.3 (0.48)	1.0 – 1.5	5.3 (2.33)	6.0 (2.74)	4.0 – 10.3	—	—	—
3	IABG	0.8 (0.25)	1.0 (0.41)	0.8 – 1.3	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.5 (0.29)†	0.3 (0.25)	0.3 – 1.0
	NCBD	—	—	—	11.5 (2.40)	7.5 (2.40)	9.3 – 16.3	—	—	—
	NEYO	—	—	—	0.5 (0.29)	0.5 (0.50)	0.8 – 2.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.5
	PAHM	2.0 (0.58)	2.0 (0.71)	1.3 – 4.0	2.3 (1.86)	1.8 (0.48)	1.8 – 4.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
4	IABG	0.5 (0.29)	0.8 (0.25)	0.8 – 3.3	—	—	—	1.0 (0.71)	1.3 (0.95)	1.0 – 2.8
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	22.3 (5.95)	14.8 (2.78)	13.3 – 22.8	—	—	—
	NEYO	—	—	—	1.8 (0.75)	0.8 (0.75)	1.5 – 2.8	1.3 (0.25)	1.0 (0.71)	1.0 – 2.3
	PAHM	0.3 (0.33)†	0.3 (0.25)	0.0 – 1.0	9.3 (2.96)	8.3 (1.38)	8.0 – 14.5	—	—	—
5	IABG	1.5 (0.65)	0.8 (0.48)	0.8 – 4.0	1.5 (0.96)†	0.5 (0.50)	0.5 – 1.5	1.3 (0.48)	3.5 (1.26)	2.0 – 2.5
	NCBD	0.8 (0.25)†	0.3 (0.25)	0.3 – 0.8	72.5 (17.50)	49.0 (6.77)	62.5 – 102.8	0.5 (0.50)†	0.5 (0.29)	0.5 – 1.5
	NEYO	—	—	—	0.8 (0.48)	0.8 (0.25)	1.3 – 2.0	22.5 (3.28)*	13.8 (3.07)	14.5 – 32.0
	PAHM	0.3 (0.33)†	0.8 (0.48)	0.0 – 1.3	6.7 (1.33)	4.0 (1.41)	5.0 – 12.0	—	—	—

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Billbugs (Curculionidae)			Corn flea beetles (*Chaetocnema* sp.)			Corn rootworm beetles (*Diabrotica* sp.)		
		Pest Arthropod			Pest Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]
Coll.[1]	Site[2]	MON 87403	Control		MON 87403	Control		MON 87403	Control	
1	IABG	—	—	—	—	—	—	2.3 (0.63)	1.5 (0.65)	0.8 – 1.8
	NCBD	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	—	—	—
	NEYO	—	—	—	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.8	1.0 (0.41)	0.8 (0.48)	1.3 – 2.8
	PAHM	—	—	—	6.0 (1.73)	6.0 (2.16)	5.3 – 13.8	0.7 (0.33)	1.3 (1.25)	0.7 – 3.5
2	IABG	—	—	—	1.5 (0.29)	2.8 (1.03)	2.5 – 5.0	2.8 (0.75)	1.8 (0.75)	0.5 – 2.8
	NCBD	0.5 (0.50)†	0.5 (0.29)	0.5 – 1.3	—	—	—	0.3 (0.25)†	0.0 (0.00)	0.0 - 0.8
	NEYO	—	—	—	0.5 (0.29)†	0.8 (0.48)	0.5 – 1.0	9.3 (0.63)	9.8 (3.07)	7.8 – 13.8
	PAHM	—	—	—	7.7 (1.45)	4.3 (1.25)	5.0 – 19.3	1.0 (0.58)†	0.5 (0.29)	0.3 – 1.0
3	IABG	—	—	—	0.0 (0.00)*	1.5 (0.65)	1.8 – 2.0	0.8 (0.25)	0.5 (0.50)	1.0 – 2.0
	NCBD	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NEYO	—	—	—	—	—	—	23.0 (3.00)*	34.3 (3.75)	15.0 – 27.8
	PAHM	—	—	—	17.7 (1.76)	16.5 (2.72)	10.5 – 24.5	1.0 (0.00)†	1.0 (0.41)	0.0 – 2.0
4	IABG	—	—	—	1.0 (0.71)	1.0 (0.71)	0.8 – 3.8	1.3 (0.63)	0.3 (0.25)	0.3 – 2.0
	NCBD	—	—	—	7.0 (2.27)	1.8 (0.48)	6.8 – 15.8	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	—	—	—	—	—	—	5.3 (1.03)	9.0 (2.48)	2.3 – 7.5
	PAHM	—	—	—	16.3 (2.19)	7.3 (1.93)	6.0 – 34.3	0.3 (0.33)†	1.0 (0.58)	0.0 – 1.0
5	IABG	—	—	—	1.3 (0.25)*	0.3 (0.25)	2.0 – 3.8	0.5 (0.29)†	0.3 (0.25)	0.3 – 1.3
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	18.3 (2.84)	11.3 (3.12)	21.3 – 29.3	—	—	—
	NEYO	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.3 – 0.5
	PAHM	—	—	—	6.3 (2.73)	4.3 (0.63)	4.0 – 11.0	—	—	—

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Delphacid planthoppers (Delphacidae)			Grasshoppers (Orthoptera)			Leafhoppers (Cicadellidae)		
		Pest Arthropod			Pest Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]
Coll.[1]	Site[2]	MON 87403	Control		MON 87403	Control		MON 87403	Control	
1	IABG	—	—	—	—	—	—	8.3 (0.63)	7.5 (1.89)	8.5 – 14.0
	NCBD	24.0 (3.49)	28.8 (6.70)	27.3 – 41.0	—	—	—	43.5 (5.81)	50.0 (10.06)	43.0 – 70.5
	NEYO	—	—	—	—	—	—	1.8 (0.48)	0.8 (0.75)	0.8 – 1.5
	PAHM	—	—	—	—	—	—	16.3 (1.20)	12.8 (3.33)	14.0 – 33.5
2	IABG	—	—	—	—	—	—	8.8 (2.10)	10.3 (1.11)	7.5 – 11.3
	NCBD	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	30.3 (4.82)	24.3 (2.46)	21.8 – 53.3
	NEYO	—	—	—	—	—	—	1.0 (0.41)†	1.0 (0.41)	0.3 – 1.0
	PAHM	—	—	—	0.3 (0.33)†	0.3 (0.25)	0.0 – 0.5	10.3 (3.71)	11.8 (2.02)	9.0 – 26.8
3	IABG	—	—	—	—	—	—	2.3 (0.63)	4.5 (0.50)	2.3 – 3.8
	NCBD	2.8 (1.38)	1.3 (0.63)	0.5 – 2.3	—	—	—	46.5 (6.28)	37.3 (2.72)	35.8 – 59.3
	NEYO	—	—	—	—	—	—	1.0 (0.71)†	1.3 (0.63)	0.0 – 1.5
	PAHM	—	—	—	0.3 (0.33)†	1.0 (0.41)	0.0 – 0.8	17.0 (4.58)	15.8 (4.39)	7.0 – 26.0
4	IABG	—	—	—	—	—	—	3.8 (0.75)	6.5 (1.04)	2.8 – 6.8
	NCBD	1.3 (0.25)†	0.8 (0.48)	0.5 – 1.3	—	—	—	24.0 (2.12)	24.0 (2.58)	21.3 – 37.0
	NEYO	—	—	—	—	—	—	1.3 (0.95)†	1.0 (0.41)	0.5 – 1.5
	PAHM	—	—	—	0.7 (0.67)†	0.0 (0.00)	0.0 – 1.0	12.3 (1.45)	13.5 (4.33)	6.5 – 26.5
5	IABG	—	—	—	—	—	—	4.5 (1.19)	2.5 (0.65)	2.0 – 6.0
	NCBD	—	—	—	—	—	—	16.3 (2.66)	21.0 (3.11)	15.0 – 22.3
	NEYO	—	—	—	—	—	—	0.8 (0.48)	0.5 (0.29)	0.0 – 2.3
	PAHM	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5	10.7 (2.85)	9.3 (2.56)	6.5 – 24.0

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Sap beetles (Nitidulidae)			Seedcorn beetles (*Stenolophus* sp.)			Tachinid flies (Tachinidae)		
		Pest Arthropod			Pest Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	0.0 (0.00)†	0.8 (0.48)	0.8 – 1.5	—	—	—	—	—	—
	NCBD	—	—	—	—	—	—	—	—	—
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	—	—	—	—	—	—
	PAHM	—	—	—	—	—	—	—	—	—
2	IABG	2.0 (0.71)†	0.5 (0.50)	0.0 – 1.0	—	—	—	—	—	—
	NCBD	—	—	—	—	—	—	—	—	—
	NEYO	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.8	—	—	—	0.5 (0.50)†	0.0 (0.00)	0.0 – 0.3
	PAHM	—	—	—	—	—	—	—	—	—
3	IABG	1.0 (0.58)†	0.5 (0.29)	0.0 – 1.3	—	—	—	—	—	—
	NCBD	—	—	—	0.3 (0.25)†	0.5 (0.50)	0.0 – 0.3	—	—	—
	NEYO	1.0 (0.71)	0.5 (0.29)	0.5 – 1.5	—	—	—	—	—	—
	PAHM	—	—	—	—	—	—	—	—	—
4	IABG	2.0 (1.68)	1.5 (0.87)	0.8 – 2.3	—	—	—	—	—	—
	NCBD	—	—	—	—	—	—	—	—	—
	NEYO	0.8 (0.48)	1.0 (0.00)	1.0 – 3.0	—	—	—	—	—	—
	PAHM	—	—	—	—	—	—	—	—	—
5	IABG	0.5 (0.50)	0.3 (0.25)	1.0 – 1.5	—	—	—	—	—	—
	NCBD	—	—	—	—	—	—	—	—	—
	NEYO	1.3 (0.75)	2.5 (1.04)	0.3 – 2.0	—	—	—	—	—	—
	PAHM	—	—	—	—	—	—	—	—	—

Table H-9 (continued). Individual Site Analysis: Arthropod Abundance in Sticky Trap Samples Collected from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Thrips (Thripidae)			Tarnished plant bugs (*Lygus* sp.)			Click beetle[5] (Elateridae)		
		Pest Arthropod			Pest Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]
Coll.[1]	Site[2]	MON 87403	Control		MON 87403	Control		MON 87403	Control	
1	IABG	31.5 (11.59)	41.5 (7.79)	26.8 – 50.8	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.5	—	—	—
	NCBD	50.5 (10.90)	40.5 (5.01)	37.5 – 76.3	—	—	—	2.0 (0.71)	3.8 (1.11)	2.0 – 5.0
	NEYO	4.8 (2.25)	1.0 (0.58)	4.0 – 8.0	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.0	—	—	—
	PAHM	12.0 (5.77)	8.8 (5.01)	9.5 – 13.5	1.7 (0.88)	0.0 (0.00)	0.8 – 2.5	—	—	—
2	IABG	86.3 (23.97)	79.3 (27.29)	40.3 – 95.5	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3	—	—	—
	NCBD	32.5 (5.85)*	18.5 (4.03)	20.8 – 34.5	—	—	—	3.0 (0.41)	2.8 (1.31)	3.0 – 7.5
	NEYO	183.0 (54.68)*	71.5 (13.76)	66.8 – 159.0	—	—	—	—	—	—
	PAHM	1.3 (0.88)	2.5 (1.04)	1.8 – 6.7	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.5	—	—	—
3	IABG	38.5 (4.50)	39.0 (18.18)	24.0 – 130.0	—	—	—	—	—	—
	NCBD	39.3 (6.30)	29.8 (6.17)	46.0 – 59.3	—	—	—	2.0 (0.71)	0.8 (0.25)	2.0 – 3.0
	NEYO	319.3 (102.23)	207.3 (58.63)	171.8 – 511.0	—	—	—	—	—	—
	PAHM	9.7 (4.26)*	2.8 (2.14)	2.3 – 4.8	—	—	—	—	—	—
4	IABG	95.5 (6.96)	139.3 (82.00)	44.8 – 167.8	—	—	—	—	—	—
	NCBD	33.8 (5.27)	26.5 (2.90)	27.8 – 85.8	—	—	—	0.8 (0.25)	0.8 (0.48)	0.8 – 3.3
	NEYO	134.3 (36.50)*	60.3 (14.02)	56.3 – 125.0	—	—	—	—	—	—
	PAHM	11.0 (5.29)	7.5 (3.30)	6.0 – 12.3	—	—	—	0.0 (0.00)†	0.8 (0.48)	0.0 – 0.5
5	IABG	30.0 (14.57)	15.3 (2.87)	17.8 – 41.5	—	—	—	—	—	—
	NCBD	33.3 (5.50)	44.3 (15.58)	18.5 – 42.8	—	—	—	1.3 (0.25)	0.8 (0.25)	1.5 – 2.3
	NEYO	138.0 (10.12)*	62.3 (15.52)	102.3 – 173.8	—	—	—	—	—	—
	PAHM	1.7 (0.88)	1.3 (0.48)	0.7 – 3.5	0.7 (0.67)†	0.0 (0.00)	0.0 – 0.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5

Note: The experimental design was a randomized complete block with four replicates per site.
[*] Indicates a statistically significant difference between MON 87403 and the conventional control ($\alpha = 0.05$) using ANOVA.
[†] Indicates p-values could not be generated where the taxa did not meet inclusion criteria. Among taxa that did not meet inclusion criteria, centipedes, millipedes, and corn earworms data are not presented due to low probability of capture on sticky traps.
(—)A dash indicates data not available.
[1] Arthropods were enumerated at five crop development stages: Collection 1 = late vegetative – VT growth stage; Collection 2 = R1 growth stage; Collection 3 = R2 growth stage; Collection 4 = R3 growth stage; Collection 5 = R4 growth stage.
[2] Site code: IABG = Greene, IA; NCBD = Perquimans, NC; NEYO = York, NE; PAHM = Berks, PA.
[3] MON 87403 and the conventional control values represent means with standard error in parentheses. N = 4 (IABG, NCBD, and NEYO sites); N= 3 (PAHM site).
[4] Reference range is calculated from the minimum and maximum mean values from among reference materials at each site.
[5] Click beetle = wireworm adults; Damsel bug = Nabis bug

Table H-10. Individual Site Analysis: Arthropod Abundance in Visual Counts from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Ant-like flower beetles (Anthicidae)			Click beetles (Elateridae)			Corn flea beetles (*Chaetocnema* sp.)		
		Pollen Feeder			Pest Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	—	—	—	1.0 (0.71)	2.3 (1.31)	2.0 – 4.3	0.5 (0.29)†	0.0 (0.00)	0.3 – 2.0
	NEYO	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	—	—	—	—	—	—	8.7 (3.53)	6.5 (2.40)	3.0 – 8.5
2	IABG	1.0 (0.41)	1.8 (0.75)	1.3 – 5.0	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	—	—	—	1.5 (0.87)	0.5 (0.29)	0.5 – 2.0	0.3 (0.25)†	0.3 (0.25)	0.0 – 1.0
	NEYO	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	—	—	—	—	—	—	9.3 (2.73)	9.3 (3.64)	6.5 – 9.5
3	IABG	2.0 (1.00)	2.3 (1.03)	1.8 – 4.3	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	—	—	—	0.8 (0.25)†	0.3 (0.25)	0.3 – 0.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	—	—	—	—	—	—	6.7 (4.06)	6.3 (1.65)	8.8 – 11.0
4	IABG	3.8 (0.85)	3.5 (0.87)	3.5 – 4.5	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.8	0.5 (0.29)†	0.0 (0.00)	0.0 – 1.0
	NEYO	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	—	—	—	—	—	—	3.3 (0.88)	3.3 (1.11)	4.0 – 8.3
5	IABG	1.0 (0.71)†	0.8 (0.48)	0.5 – 1.3	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	1.0 (0.71)	0.5 (0.29)	0.5 – 3.0
	NEYO	—	—	—	—	—	—	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	—	—	—	—	—	—	3.3 (1.33)	3.8 (2.25)	3.7 – 4.8

Table H-10 (continued). Individual Site Analysis: Arthropod Abundance in Visual Counts from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Japanese beetles (Scarabaiedae)			Lacewing adults (Chrysopidae)			Lacewing larvae (Chrysopidae)		
		Pest Arthropod			Beneficial Arthropod			Beneficial Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.5 (0.29)†	0.5 (0.29)	0.3 – 0.5
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.5	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3
2	IABG	0.8 (0.75)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	0.3 (0.25)	0.0 – 1.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	1.0 (0.41)	0.5 – 1.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.33)†	0.3 (0.25)	0.0 – 0.5	0.3 (0.33)†	0.0 (0.00)	0.0 – 0.7
3	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.8
	NCBD	0.5 (0.50)†	0.0 (0.00)	0.0 – 0.5	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3	0.5 (0.50)†	0.5 (0.29)	0.0 – 0.3
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.5 (0.50)	0.0 – 0.3
4	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.5 (0.29)†	0.3 (0.25)	0.0 – 0.5
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.0	0.3 (0.33)†	0.3 (0.25)	0.0 – 0.3
5	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	0.3 (0.25)	0.3 – 1.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0

Table H-10 (continued). Individual Site Analysis: Arthropod Abundance in Visual Counts from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Ladybird beetles adult (Coccinellidae)			Ladybird beetles Larvae (Coccinellidae)			Minute pirate bugs (Anthocoridae)		
		Beneficial Arthropod			Beneficial Arthropod			Beneficial Arthropod		
		Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]	Mean (S.E.)[3]		Reference range[4]
Coll.[1]	Site[2]	MON 87403	Control		MON 87403	Control		MON 87403	Control	
1	IABG	0.3 (0.25)†	0.0 (0.00)	0.3 – 0.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	0.0 (0.00)	0.3 – 0.5
	NCBD	0.0 (0.00)†	0.5 (0.50)	0.3 – 1.3	0.0 (0.00)†	0.5 (0.29)	0.3 – 1.3	2.3 (1.03)	2.5 (1.32)	1.8 – 4.8
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	1.0 (0.58)	2.5 (1.32)	2.3 – 6.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5	6.7 (1.67)	9.5 (2.40)	8.5 – 11.7
2	IABG	0.3 (0.25)†	0.5 (0.29)	0.0 – 0.8	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.8	6.3 (1.65)	4.5 (0.96)	3.0 – 11.0
	NCBD	0.3 (0.25)†	0.0 (0.00)	0.3 – 0.8	1.3 (0.75)	2.0 (0.58)	0.5 – 2.3	1.0 (0.41)	1.8 (0.63)	0.8 – 3.8
	NEYO	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	2.7 (0.33)	3.5 (1.76)	0.8 – 3.7	0.3 (0.33)†	0.8 (0.48)	0.0 – 0.5	5.7 (2.03)	6.0 (2.20)	5.3 – 8.0
3	IABG	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	1.8 (0.75)	2.5 (0.96)	2.0 – 4.0
	NCBD	0.5 (0.29)†	0.3 (0.25)	0.0 – 1.0	1.3 (0.95)†	0.8 (0.25)	0.5 – 1.0	1.0 (1.00)†	0.3 (0.25)	0.3 – 1.8
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.5 (0.50)	0.0 – 1.3	1.7 (0.33)	1.8 (0.25)	1.3 – 2.0	8.7 (4.67)	5.8 (1.55)	6.3 – 11.5
4	IABG	0.5 (0.29)†	0.3 (0.25)	0.0 – 0.8	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	2.3 (1.44)	2.5 (1.55)	1.5 – 3.3
	NCBD	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.3	0.5 (0.50)†	0.5 (0.50)	0.5 – 1.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.8	0.7 (0.67)†	2.0 (0.71)	0.3 – 1.5	0.0 (0.00)*	1.0 (0.41)	0.5 – 3.7
5	IABG	0.0 (0.00)†	0.3 (0.25)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	1.5 (0.87)	1.0 (0.58)	0.8 – 2.5
	NCBD	0.0 (0.00)†	0.5 (0.50)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5	0.0 (0.00)†	0.5 (0.29)	0.0 – 1.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.3 (0.33)†	0.3 (0.25)	0.0 – 0.5	0.7 (0.33)†	0.8 (0.48)	0.5 – 1.0	1.0 (0.58)	1.8 (0.85)	1.3 – 5.5

Table H-10 (continued). Individual Site Analysis: Arthropod Abundance in Visual Counts from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Corn rootworm beetles (Chrysomelidae)			Sap beetles (Nitidulidae)			Shining flower beetles (Phlacridae)		
		Pest Arthropod			Pest Arthropod			Pollen Feeder		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	—	—	—	0.5 (0.29)†	0.3 (0.25)	0.3 – 0.8	—	—	—
	NCBD	1.0 (0.58)	1.3 (0.75)	1.0 – 2.0	4.3 (3.92)	4.5 (2.87)	2.5 – 5.3	5.0 (0.91)	6.0 (0.71)	3.8 – 6.8
	NEYO	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.5	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	—	—	—
	PAHM	0.3 (0.33)*	2.0 (0.71)	1.0 – 2.0	4.3 (0.67)	8.0 (1.08)	1.8 – 8.5	1.3 (0.88)	3.5 (1.55)	1.7 – 3.0
2	IABG	3.0 (1.29)	2.5 (0.65)	1.5 – 6.0	4.5 (0.96)	9.5 (3.97)	8.8 – 19.0	—	—	—
	NCBD	0.3 (0.25)†	0.5 (0.50)	0.0 – 1.0	6.3 (5.27)	7.3 (3.59)	4.3 – 9.8	7.0 (3.19)*	12.3 (1.70)	6.8 – 10.5
	NEYO	1.0 (0.41)	1.5 (0.65)	1.3 – 2.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	—	—	—
	PAHM	0.3 (0.33)	1.3 (0.63)	0.7 – 1.8	2.7 (0.33)	4.3 (0.75)	2.5 – 6.0	2.3 (0.33)	1.8 (0.75)	1.0 – 2.0
3	IABG	0.5 (0.29)†	1.0 (0.41)	0.5 – 1.3	4.8 (1.03)	2.8 (0.85)	3.5 – 7.5	—	—	—
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	1.5 (0.87)	2.5 (1.19)	1.5 – 3.8	1.5 (0.65)	1.0 (0.00)	1.0 – 3.0
	NEYO	3.8 (1.75)	6.8 (1.03)	1.8 – 6.0	1.0 (0.00)*	4.5 (0.96)	0.3 – 1.3	—	—	—
	PAHM	1.0 (0.58)†	0.0 (0.00)	0.0 – 1.0	2.3 (2.33)	1.0 (1.00)	1.0 – 3.3	2.0 (1.53)	1.0 (0.41)	0.3 – 2.7
4	IABG	1.3 (0.95)	0.8 (0.48)	0.8 – 2.3	3.3 (1.38)	7.5 (1.89)	4.3 – 9.0	—	—	—
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.3 (0.25)†	0.3 (0.25)	0.3 – 1.3	0.8 (0.25)†	0.0 (0.00)	0.0 – 2.0
	NEYO	0.8 (0.25)†	1.3 (0.48)	0.3 – 1.0	2.0 (1.08)	0.8 (0.48)	0.3 – 2.3	—	—	—
	PAHM	0.0 (0.00)†	0.8 (0.48)	0.0 – 0.5	1.3 (0.88)	1.8 (0.75)	1.5 – 4.0	0.0 (0.00)†	0.5 (0.29)	0.0 – 1.3
5	IABG	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.8	5.3 (2.72)	5.0 (1.78)	1.8 – 5.8	—	—	—
	NCBD	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	1.3 (0.48)*	4.5 (1.19)	1.0 – 5.5	0.8 (0.48)†	0.3 (0.25)	0.0 – 0.8
	NEYO	—	—	—	0.0 (0.00)†	1.0 (0.71)	0.0 – 1.0	—	—	—
	PAHM	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	1.3 (0.67)	1.5 (0.65)	1.5 – 4.8	1.0 (1.00)†	0.5 (0.29)	0.0 – 0.3

Table H-10 (continued). Individual Site Analysis: Arthropod Abundance in Visual Counts from MON 87403 Compared to the Conventional Control in 2012 U.S. Field Trials

		Spiders (Araneae)			Stink bugs (Pentatomidae)		
		Beneficial Arthropod			Pest Arthropod		
		Mean (S.E.)[3]		Reference	Mean (S.E.)[3]		Reference
Coll.[1]	Site[2]	MON 87403	Control	range[4]	MON 87403	Control	range[4]
1	IABG	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NCBD	4.8 (1.25)	3.0 (0.91)	3.5 – 4.5	0.8 (0.48)†	1.3 (0.48)	0.0 – 1.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.0
	PAHM	0.7 (0.33)	1.5 (0.50)	0.0 – 2.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
2	IABG	1.3 (0.75)†	0.0 (0.00)	0.0 – 1.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NCBD	3.8 (0.75)	2.8 (1.18)	3.5 – 5.0	1.5 (0.50)†	0.5 (0.29)	0.3 – 1.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	1.7 (0.88)	2.3 (1.44)	0.7 – 2.0	0.3 (0.33)†	0.0 (0.00)	0.0 – 0.3
3	IABG	1.5 (0.50)	0.8 (0.25)	0.8 – 1.0	0.3 (0.25)†	0.3 (0.25)	0.3 – 0.5
	NCBD	3.0 (1.22)	3.8 (1.11)	4.3 – 6.0	0.3 (0.25)†	0.3 (0.25)	0.0 – 0.5
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.8	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	PAHM	4.0 (1.53)	3.5 (2.22)	2.5 – 3.7	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
4	IABG	0.5 (0.29)	0.8 (0.25)	0.8 – 2.3	1.0 (0.71)†	0.5 (0.29)	0.0 – 1.0
	NCBD	2.5 (0.65)	3.0 (1.29)	2.8 – 5.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	2.0 (0.58)	1.5 (0.65)	1.0 – 3.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.3
5	IABG	0.3 (0.25)†	0.0 (0.00)	0.3 – 0.3	0.3 (0.25)†	0.0 (0.00)	0.0 – 0.3
	NCBD	3.0 (0.71)	2.3 (0.48)	2.0 – 3.3	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	NEYO	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.0
	PAHM	2.7 (0.33)	2.8 (0.75)	1.5 – 5.0	0.0 (0.00)†	0.0 (0.00)	0.0 – 0.5

Note: The experimental design was a randomized complete block with four replicates per site.
[*] Indicates a statistically significant difference between MON 87403 and the conventional control ($\alpha = 0.05$) using ANOVA.
[†] Indicates p-values could not be generated where the taxa did not meet inclusion criteria
(—) A dash indicates data not available.
[1] Arthropods were enumerated at five crop development stages: Collection 1 = VT-R1 growth stage; Collection 2 = R1 growth stage; Collection 3 = R2 growth stage; Collection 4 = R3 growth stage; Collection 5 = R4-R5 growth stage.
[2] Site code: IABG = Greene, IA; NCBD = Perquimans, NC; NEYO = York, NE; PAHM = Berks, PA.
[3] MON 87403 and the conventional control values represent means with standard error in parentheses. N = 4.
[4] Reference range is calculated from the minimum and maximum mean values from among reference materials at each site.

References for Appendix H

SAS Software Release 9.3 (TS1M0). 2010. Copyright 2002-2010 by SAS Institute, Inc., Cary, North Carolina.

Appendix I: Materials and Methods for Pollen Morphology and Viability Assessment

I.1. Plant Production

MON 87403, the conventional control, and reference hybrids were grown under similar agronomic conditions in a field trial in Story County, Iowa (Table I-1). The trial was arranged in a randomized complete block design with four replications. Each plot consisted of eight rows approximately 6 m in length.

I.2. Flower Collection and Pollen Sample Preparation

Tassel bags were placed on three non-systematically selected plants during pollen shed. The following morning, pollen was collected from three plants per plot and transferred to a uniquely labeled tube. Pollen collected from each plant in a plot represented a subsample. Within approximately 30 minutes of collection, Alexander's stain solution (Alexander, 1980), in a 1:5 dilution with distilled water, was added to each tube (at least 2:1 (v/v) stain to pollen) to fix and stain the pollen, rendering the pollen non-viable. The tubes were closed and the contents shaken until thoroughly mixed. Subsamples were placed on wet ice immediately after pollen collection and maintained under those conditions until receipt at the performing laboratory.

I.3. Data Collection

Slides were prepared by aliquoting suspended pollen/stain solution onto a slide. Pollen characteristics were assessed under an Olympus©[9] BX53 light microscope equipped with an Olympus© DP72 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows XP®[10] and installed with an Olympus© cellSens (version 1.4.1) software.

I.3.1. Pollen Viability

When pollen grains were exposed to the staining solution, viable pollen grains stained red to purple due to the presence of living cytoplasmic content. Non-viable pollen grains stained light blue to green or colorless, and the shape appeared round to collapsed depending on the degree of hydration. For each pollen sample, the number of viable and non-viable pollen grains was counted from a random field of view under the microscope. A minimum of 100 pollen grains were counted for each of the three subsamples per plot. Mean pollen viability for each replicate was calculated from the subsamples as shown in Table VII-8.

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I.3.2. Pollen Diameter

For a single predetermined subsample per plot, pollen grain diameter was measured along two perpendicular axes for 10 representative pollen grains per replication. Mean pollen diameter for each replicate was calculated from the total of 20 diameter measurements as shown in Table VII-8.

I.3.3. General Pollen Morphology

General pollen morphology of MON 87403, the conventional control, and the reference hybrids was observed Figure I-1.

I.4. Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS© (SAS, 2012). The level of statistical significance was predetermined to be 5% (α=0.05). MON 87403 was compared to the conventional control material for percent viable pollen and pollen grain diameter. MON 87403 and conventional control were not statistically compared to the reference hybrids. Minimum and maximum mean values were calculated for each characteristic from the four reference hybrids. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations.

Table I-1. Starting Seed for Pollen Morphology and Viability Assessment

Material Name	Material Type	Phenotype	Monsanto Lot Number
MPA640B	Control	Conventional	11332601
Lewis 7007	Reference	Conventional	11226559
Mycogen 2M746	Reference	Conventional	11226705
NC+ 5220	Reference	Conventional	11226701
Phillips 717	Reference	Conventional	11300073
MON 87403	Test	Increased Ear Biomass Maize	11332602

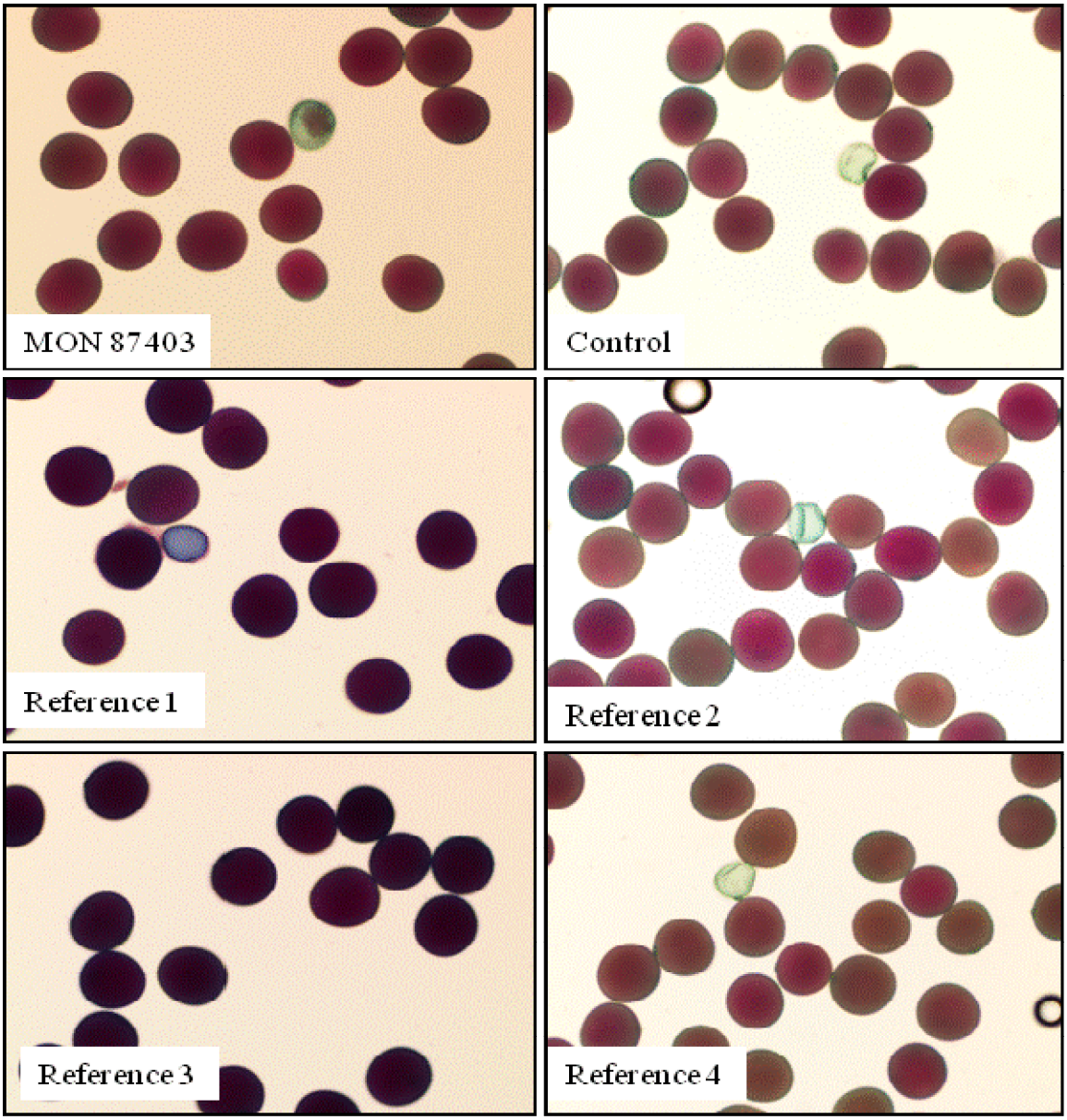

Figure I-1. General Morphology of Pollen from MON 87403, the Conventional Control, and Commercially Available Conventional Reference Materials under 200X Magnification

The maize pollen samples were stained with Alexander's stain diluted 1:5 with distilled water. Viable pollen grains stained red to purple, while non-viable pollen grains stained blue to green and the shape appeared round to collapsed depending on the degree of hydration.

References for Appendix I

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55: 13-18.

SAS. 2012. SAS/STAT software version 9.3. SAS Institute, Inc., Cary, North Carolina.