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Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate Tolerant MON 87419 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

April 23, 2015

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Submitted by:

Mark Groth, B.A.
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
Phone: (314) 694-3369
Fax: (314) 694-3080
E-mail: mark.e.groth@monsanto.com

Prepared by:
S. Carleton, Ph.D., P. Chinnadurai, M.S., E. Clawson, Ph.D., M. Elrick, Ph.D., G. Frierdich, B.S., C. Garnaat, M.S., M. Graneto, Ph.D., M. Groth, B.A., M. Horak, Ph.D., T. Klusmeyer, Ph.D., W. Li, Ph.D., K. Liu, Ph.D., T. Orr, M.S., L. Shi, Ph.D.

Contributors and/or Principal Investigators:

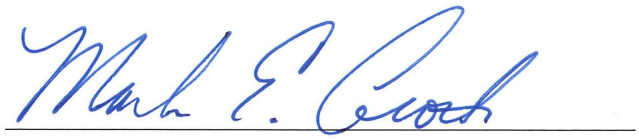
A. Ahmad, Ph.D., B. Baltazar, Ph.D., M. Basu, Ph.D., E. Bell, Ph.D., G. Brown, B.S., L. Burzio, Ph.D., D. Carson, Ph.D., B. Chen, Ph.D., S. Donelson, Ph.D., T. Edrington, Ph.D., K. Glenn, Ph.D., X. Gu, Ph.D., K. Howard, Ph.D., J. Hua, M.D., H. Kang, M.S., K. Klug, M.S., K. Lawry M.S., B. Li, Ph.D., L. Liu, Ph.D., M. McCaan, M.S., M. McPherson, Ph.D., H. Moon, Ph.D., E. Park, Ph.D., J. Saurabh, Ph.D., G. Rogan, M.S., A. Silvanovich, Ph.D., Q. Tian, M.D., Ph.D., C. Wang, Ph.D.

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 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.



Mark E. Groth, B.A.
Regulatory Affairs Manager

Address:
Monsanto Company
800 North Lindbergh Blvd., Mail Stop C3S
St. Louis, MO 63167

Tel: (314)-694-3369
Fax: (314)-694-3080

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 87419, any progeny derived from crosses between MON 87419 and conventional maize, and any progeny derived from crosses of MON 87419 with biotechnology-derived maize that are either outside of the scope of, or have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed MON 87419 maize that is tolerant to dicamba (3,6-dichloro-2-methoxybenzoic acid) and glufosinate (2-amino-4-(hydroxymethylphosphinyl) butanoic acid) herbicides. MON 87419 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and the phosphinothricin N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate herbicide.

MON 87419 will offer maize growers multiple choices for effective weed management including tough to control and herbicide resistant broadleaf weeds. The combination of these two unique herbicide mechanisms-of-action provides an effective weed management system for maize production. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides nonselective control of approximately 120 broadleaf and grass weeds. Additionally, dicamba and glufosinate provide control of herbicide-resistant weeds, including glyphosate-resistant biotypes of Palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza canadensis*), common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*) and waterhemp (*Amaranthus tuberculatus*).

MON 87419 will likely be combined, through traditional breeding methods, with other deregulated herbicide-tolerant (e.g., glyphosate-tolerant) events. The in-crop use of dicamba and glufosinate herbicides, in addition to glyphosate herbicide, provides improved weed management options in maize to control a broad spectrum of grass and broadleaf weed species and effective control of weeds resistant to several herbicide families. Successful integration of MON 87419 into glyphosate-tolerant maize systems

will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system for two additional herbicide mechanisms-of-action for in-crop application in current maize production systems as recommended by weed science experts to manage future weed resistance development; 3) an option to delay or prevent further resistance to glyphosate and other critically important maize herbicides; 4) crop safety to dicamba, glufosinate and glyphosate; and 5) additional weed management tools to enhance weed management systems necessary to maintain or improve maize yield and quality to meet the growing needs of the food, feed, and industrial markets.

Data and Information Presented Confirms the Lack of Plant Pest Potential and the Food and Feed Safety of MON 87419 Compared to Conventional Maize

The data and information presented in this petition demonstrate MON 87419 is agronomically, phenotypically, and compositionally comparable to conventional control and commercially cultivated maize, with the exception of the introduced traits. Moreover, the data and information presented demonstrate MON 87419 is not expected to pose an increased plant pest risk, including weediness, compared to conventional control and commercially cultivated maize. The food, feed, and environmental safety of MON 87419 was confirmed based on multiple, well-established lines of evidence:

- Maize does not possess any of the attributes commonly associated with weeds and has a history of safe consumption. The conventional control used for the transformation process was included in studies to serve as an appropriate basis of comparison for MON 87419.

- 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 DMO protein and previous assessments of PAT protein expressed in MON 87419, confirm they are unlikely to be a toxin or allergen. The PAT protein in MON 87419 has a robust history of safe use and shares a very high level of amino acid identity to the PAT protein produced in several other commercially available crops that have been reviewed by USDA and previously deregulated (e.g., T25 maize, TC1507 maize, DAS-59122-7 maize, etc). The safety of PAT proteins present in biotechnology-derived crops has been thoroughly assessed, and is the subject of numerous publications. The mode-of-action of PAT protein and how it confers glufosinate tolerance has been extensively studied and is well documented in peer reviewed publications.

- A compositional assessment supports the conclusion that MON 87419 grain and forage is compositionally equivalent to grain and forage of conventional maize.

- An extensive evaluation of MON 87419 phenotypic and agronomic characteristics and environmental interactions demonstrates MON 87419 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 87419 is not expected to have an adverse effect on other organisms compared to conventional maize under normal agricultural practices.

- Evaluation of the agronomic and phenotypic characteristics of MON 87419, using current cultivation and management practices, leads to the conclusion that deregulation of MON 87419 is not expected to have an adverse effect on maize agronomic practices or land use.

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.

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 87419, 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 NL6169 is an Appropriate Comparator to MON 87419

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 a conventional line (HCL645) to create F_1 starting control materials. LH244 was used as the control in molecular characterization studies. NL6169 (HCL645 × LH244) was used as the 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 87419

MON 87419 was produced by *Agrobacterium*-mediated transformation of maize tissue using the 2T-DNA transformation plasmid vector PV-ZMHT507801. This plasmid vector contains two separate T-DNAs (transfer DNA), that are each delineated by Right and Left Border regions. The first T-DNA, designated as T-DNA I, contains the *dmo* expression cassette and the *pat* expression cassette. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* expression cassette for selection. During transformation, both T-DNAs were inserted into the maize genome. Subsequently, traditional breeding, segregation, selection and screening were used to isolate plants that contained the *dmo* expression cassette and the *pat* expression cassette (T-DNA I) and did not contain the *cp4 epsps* expression cassette (T-DNA II).

Characterization of the DNA insert in MON 87419 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87419 contains one copy of the intended transfer DNA (T-DNA I) containing the *dmo* expression cassette and the *pat* expression cassette that is stably inherited over multiple generations and segregates according to Mendelian principles. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 87419 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87419 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87419 to determine the presence and identity of sequences derived from PV-ZMHT507801 and demonstrated that MON 87419 contained a single T-DNA I insert with no detectable backbone or T-DNA II sequences.
- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87419 was used to determine the complete sequence of the single DNA insert from PV-ZMHT507801, the adjacent flanking genomic DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the DNA is identical to the corresponding region in the PV-ZMHT507801 T-DNA I. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA I insert in MON 87419 to the sequence of the insertion site in conventional maize. This analysis determined no DNA rearrangement occurred at the insertion site in MON 87419 upon DNA integration, although a 602 bp deletion was observed.
- Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMHT507801 T-DNA I insert in MON 87419 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I in MON 87419.
- Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the nature of the T-DNA I as a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 87419 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 87419.

Data Confirms DMO and PAT Protein Safety

A multistep approach was used to characterize and assess the safety of the DMO and PAT proteins expressed in MON 87419 resulting from the genetic modification. PAT protein expressed in MON 87419 and the wild type PAT protein encoded by *S. viridochromogenes* share 100% amino acid identity except for the N-terminal methionine of MON 87419-produced PAT, which is cleaved during co-translational processing. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins. The PAT protein is produced in numerous commercial soybean, canola, and maize products, including T25, TC1507, and DAS-59122-7 maize, and safety of PAT proteins present in biotechnology-derived crops has been extensively assessed. Thus, the PAT protein produced in MON 87419 represents a highly familiar protein with a robust history of safe use, and additional safety assessment is not required. The safety of DMO has been established through consultations with the FDA in both MON 87708 soybean and MON 88701 cotton (FDA BNF 000125 and BNF 000135, respectively). The expression level of the DMO and PAT proteins in selected tissues of MON 87419 was determined and predicted dietary exposure to humans and animals was evaluated. In addition, the donor organisms for the DMO and PAT protein coding sequences, *S. maltophilia* and *S. viridochromogenes*, respectively, are ubiquitous in the environment and are not commonly known for allergenicity and human or animal pathogenicity. Bioinformatics analysis determined that the DMO and PAT proteins lack structural similarity to known allergens, gliadins, glutenins, or protein toxins and acute toxicology study demonstated that the DMO and PAT proteins have no acute oral toxicity in mice at the levels tested. The DMO and PAT proteins are rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin). Hence, the consumption of the DMO and PAT proteins from MON 87419 or its progeny poses no meaningful risk to human and animal health.

MON 87419 is Compositionally Equivalent to Conventional Maize

Compositional analysis was conducted on grain and forage of MON 87419 treated with dicamba and glufosinate and a conventional control grown at five sites in the U.S. during 2013. Of the 61 components statistically assessed, 60 of the 61 components analyzed showed no significant differences between MON 87419 and the conventional control. One component (manganese in grain) showed a significant difference between MON 87419 and the conventional control. For this one component, the mean difference in the component values between MON 87419 and the conventional control was less than the range value of the conventional control. The MON 87419 mean manganese value was also within the range of values observed in the literature and the ILSI-Crop Composition Database (ILSI-CCDB).

These results support the overall conclusion that MON 87419 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 to the conventional control in levels of these components. These data indicated that the statistically significant difference for the one component was not compositionally meaningful from a food and feed safety perspective.

MON 87419 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 87419 included the genetically similar conventional control as a comparator. This evaluation used a weight of evidence approach and considered statistical differences between MON 87419 and the conventional control with respect to reproducibility, magnitude, and directionality. The observations were taken on plants not treated with dicamba and glufosinate in order to evaluate only the impact of the introduced trait in MON 87419. To further support the trait assessment, similar supplemental observations were also conducted on MON 87419 treated with dicamba and glufosinate herbicides. 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 between MON 87419 and the conventional control. Characteristics assessed included: seed dormancy and germination, and pollen morphology in the laboratory or greenhouse, and plant phenotypic observations and environmental interaction evaluations conducted in the field. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 87419 is comparable to the conventional control. Thus, MON 87419 is not expected to have increased weediness or plant pest potential compared to conventional maize.

Seed germination and dormancy characterization indicated that MON 87419 seed had germination and dormancy 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 87419 compared to the conventional control. For pollen characteristic assessments, there were no statistically significant differences (α=0.05) detected between MON 87419 and the conventional control for percent viable pollen or pollen grain diameter, and no visual differences in general pollen morphology.

Furthermore, field evaluations of phenotypic, agronomic, and environmental characteristics, including phenotypic and agronomic characteristics both treated and not treated with dicamba and glufosinate, support the conclusion that MON 87419 is not

likely to have increased weediness or plant pest potential compared to conventional maize. Evaluations of MON 87419 treated and not treated with dicamba and glufosinate were each conducted at eight replicated field sites across the U.S. corn belt. These assessments included 13 plant growth and development characteristics. For MON 87419 not treated with dicamba and glufosinate, the assessments also included observations for plant responses to abiotic stressors and plant-disease and plant-arthropod interactions. The observed phenotypic characteristics were comparable between MON 87419 and the conventional control. Across sites, data show no statistically significant differences between MON 87419, whether treated or not treated with dicamba and glufosinate, compared to the conventional control for any of the assessed characteristics, including early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield. Thus, the phenotypic characteristics of MON 87419 were not altered in terms of pest/weed potential compared to the conventional control.

In an assessment of abiotic stress response and disease damage, no differences were observed between MON 87419 and the conventional control for any of the 93 comparisons for the assessed abiotic stressors or for any of the 107 comparisons for the assessed diseases among all observations across the sites. In an assessment of arthropod related damage, no differences were detected between MON 87419 and the conventional control for any of the 91 comparisons for the assessed arthropods. Additionally, in the assessments of damage by corn earworm and European corn borer, no statistically significant differences were detected between MON 87419 and the conventional control for eight out of nine comparisons at the three sites where these observations were made. For the one difference observed, MON 87419 had less damage from corn earworm infestation compared to the conventional control. However, the mean damage rating for MON 87419 was within the range of the commercial reference hybrids at the site and no differences were detected at other sites. Thus, this difference was not indicative of a consistent response associated with the trait and is not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize. The lack of biologically meaningful differences in plant response to abiotic stress, disease damage, and arthropod-related damage supports the conclusion that the introduced traits in MON 87419 are not expected to pose an increased plant pest/weed potential compared to the conventional control.

In an assessment of arthropod abundance data from sticky traps, no statistically significant differences were detected between MON 87419 and the conventional control for 21 out of 23 comparisons. Significant differences were detected between MON 87419 and the conventional control for corn rootworm beetles (less abundant on MON 87419) and spiders (more abundant on MON 87419). The mean abundance values for MON 87419 for these arthropods were slightly outside of the respective ranges of the reference hybrids. However, these differences were not consistently detected across collection methods (i.e., in visual counts) and/or sites. In an assessment of arthropod abundance data from visual counts, no statistically significant differences were detected between MON 87419 and the conventional control for 10 out of 11 comparisons. A significant difference was detected between MON 87419 and the conventional control for

minute pirate bugs (less abundant on MON 87419). However, the mean abundance value for MON 87419 was within the range of the reference hybrids. Additionally, this difference was not consistently detected across collection methods (i.e., in sticky traps) or sites. Thus, these three differences in arthropod abundance were not indicative of consistent responses associated with the trait and are not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize.

In summary, the phenotypic, agronomic, and environmental interaction data were evaluated to characterize MON 87419, and to assess whether the traits introduced in MON 87419 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 87419 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 87419 not treated with dicamba and glufosinate does not possess increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods. Furthermore, the results indicated that MON 87419, whether treated or not treated with dicamba and glufosinate, does not possess enhanced weediness characteristics or characteristics that would confer a plant pest risk compared to conventional maize.

MON 87419 Will Not Negatively Affect NTOs Including Those Beneficial to Agriculture

An evaluation of the impacts of MON 87419 on non-target organisms (NTOs) is a component of the plant pest risk assessment. Because MON 87419 does not possess pesticidal activity, all organisms that interact with MON 87419 are considered to be NTOs. Data from 2013 U.S. phenotypic and agronomic studies and observational data on environmental interactions such as plant-disease interactions, arthropod damage and arthropod abundance, were collected for MON 87419 and conventional controls. Results from these studies support conclusions of no adverse impacts to non-target arthropod populations and no changes to plant-disease interactions.

Both the DMO and PAT proteins have been assessed in multiple products by USDA-APHIS and U.S. FDA in past years. DMO protein is produced in both MON 87708 soybean and MON 88701 cotton that were granted nonregulated status by USDA-APHIS. Additionally, starting in 1996 with Bayer's T25 maize, a number of glufosinate tolerant crops (canola, cotton, maize, soybean, sugar beet) containing PAT proteins have been granted nonregulated status by USDA-APHIS. After either extensive testing and/or wide scale commercial cultivation, in no instance have adverse impacts to NTOs been associated with exposure to DMO or PAT proteins from these biotechnology derived crops.

The biochemical information and experimental data for evaluation of MON 87419 included molecular characterization, DMO and PAT 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. Taken together, these data support the conclusion that MON 87419 has no reasonable mechanism to harm NTOs, nor does it pose an additional risk to organisms beneficial to agriculture or threatened and endangered species compared to conventional maize.

Deregulation of MON 87419 is Not Expected to Have Adverse Effects on Maize Agronomic Practices or Land Use

An assessment of current maize agronomic practices was conducted to determine whether the cultivation of MON 87419 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 87419 has been developed to offer maize growers multiple choices for effective weed management including tough to control and herbicide resistant broadleaf weeds. The combination of dicamba and glufosinate as two unique herbicide mechanisms-of-action provides an effective weed management system for maize production in the U.S. As dicamba and glufosinate are already labelled for use in maize (Clarity®: EPA Reg No. 7969-137, Liberty®: EPA Reg No. 264-660), the introduction of MON 87419 is not expected to have adverse 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 87419.

MON 87419 has been shown to be comparable to conventional maize in its compositional, phenotypic, and agronomic characteristics and its environmental interactions. When introgressed into existing biotechnology-derived maize hybrids that contain insect protection and herbicide tolerance traits, MON 87419 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.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87419 is not expected to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87419 and any progeny derived from crosses between MON 87419 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
α-Cyano	α-cyano-4-hydroxycinnamic acid
a.e.	acid equivalent
a.i.	active ingredient
AA	Amino Acid
ADF	Acid detergent fiber
ALS	Acetolactate synthase
ANOVA	Analysis of Variance
AOSA	Association of Official Seed Analysts
APHIS	Animal and Plant Health Inspection Service (USDA)
bar	coding sequence of the phosphinothricin N-acetyltransferase gene from *Streptomyces hygroscopicus*
BEH	Bridged Ethyl Hybrid
bp	base pairs
BC	Back-Cross
BIO	Biotechnology Industry Organization
BRS	Biotechnology Regulatory Service (USDA-APHIS)
BSA	Bovise Serum Albumin
Bt	*Bacillus thuringiensis*
bw	body weight
CAB	chlorophyll a/b binding protein
CES	Cooperative Extension Service
CEW	Corn Earworm *(Helicoverpa zea)*
CFR	Code of Federal Regulations (U.S.)
CHT	ceramic hydroxyapatite
CoA	coenzyme A
COA	Certificate of Analysis
CPSC	Consumer Product Safety Commission
CTAB	hexadecyltrimethylammonium bromide
CTP	chloroplast transit peptide
CTP2	chloroplast transit peptide, isolated from *Arabidopsis thaliana* EPSPS
CTP4	chloroplast transit peptide, isolated from *Petunia hybrida* EPSPS
2,4-D	2,4-dichlorophenoxyacetic acid
Da	Dalton
2,4-DB	4-(2,4-dichlorophenoxy)butanoic acid
DCB	Dicamba
DCSA	3,6 dichlorosalicylic acid, also known as 3,6-dichloro-2-hydroxybenzoic acid
DEEM-FCID	Dietary Exposure Evaluation Model - Food Commodity Intake

[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.

	Database
DGA	Diglycolamine
dmo	coding sequence of the dicamba mono-oxygenase gene from *Stenotrophomonas maltophilia* encoding DMO
DMO	dicamba mono-oxygenase protein
DNA	deoxyribonucleic acid
DTT	dichloro diphenyl trichloroethane
dw	dry weight
DWCF	dry weight conversion factor
E. coli	*Escherichia coli* bacteria
ECB	European corn borer
ECL	enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency (U.S.)
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
ETS	Excellence Through Stewardship
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (U.S.)
FIFRA	U.S. Federal Insecticide, Fungicide, and Rodenticide Act
FLt	Full-Length transcript
FOIA	Freedom of Information Act (U.S.)
fw	fresh weight
GLP	Good Laboratory Practice
GM	genetically-modified
GRIN	Germplasm Resources Information Network
ha	hectare
HPLC	high pressure liquid chromatography
HRP	horseradish peroxidase
IAA	Indole acetic acid
IgG	immunoglobulin G
ILSI-CCDB	International Life Sciences Institute Crop Composition Database
ILSI-CERA	International Life Sciences Institute - Center for Environmental Risk Assessment
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
JSA	Junction Sequence Analysis
JSC	Junction Sequence Class
kb	kilobase
kDa	Kilodalton
LB	loading buffer
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m/z	mass-to-charge ratio

MALDI-TOF	Matrix Assisted Laser Desorption Ionization - Time of Flight
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry
MOE	Margin of Exposure
MMT	million metric tons
MRL	Maxiumum residue level
MS	mass spectrometry
MTSA	Monsanto Technology Stewardship Agreement
MW	molecular weight
n	number of samples
NDF	Neutral Detergent Fiber
NADH	nicotinamide adenine dinucleotide
NGS	Next Generation Sequencing
NHANES	National Health and Nutrition Examination Survey
NOAEL	No Observable Adverse Effect Level
NTO	Nontarget Organism
NTP	National Toxicology Program
OD	optical density
ODS	Octadecyl-silica
OECD	Organisation for Economic Co-operation and Development
pat	coding sequence of the phosphinothricin N-acetyltransferase gene from *Streptomyces viridochromogenes*
PAT	phosphinothricin N-acetyltransferase protein
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline containing Tween-20
PCR	polymerase chain reaction
PDA	photo diode array
PI	prediction interval
PIP	plant incorporated protectant
PPA	U.S. Plant Protection Act
PPO	Protoporphyrinogen oxidatse
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene Difluoride
Q-TOF	Quadrupole-Time-of-Flight
RED	Reregistration Eligibility Decision
ROP	repressor of primer
RP	recurrent parent
RT	Room Temperature
S. hygroscopicus	*Streptomyces hygroscopicus*
S. maltophilia	*Stenotrophomonas maltophilia*
S. viridochromogenes	*Streptomyces viridochromogenes*
SA	salicylic acid
SD	standard deviation
SDS	sodium dodecyl sulfate

SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOP	Standard Operating Procedure
TB	Tris-borate buffer
TBS	tris-buffered saline
TDF	Total dietary fiber
T-DNA	Transfer DNA
TCEP	Tris (2-carboxyethyl) phosphine
TFA	Trifluoroacetic Acid
TFE	2,2,2, trifluoroethanol
TI	Trait Integration
TOF	Time of Flight
Tris	Tris(hydroxymethyl)aminomethane
TSSP	Tissue-specific site pool
TUG	Monsanto's Technology Use Guide
Tween-20	Polyoxyethylenesorbitan monolaurate
U	Units (of enzymatic activity)
U.S.	United States
U.S. EPA	United States Environmental Protection Agency
UK	United Kingdom
UPLC	Ultra Performance Liquid Chromatography
USDA	United States Department of Agriculture
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
USHHS-ATSDR	United States Department of Health and Human Services, Agency for Toxic Substances and Disease Registry
USHHS-NTP	United States Department of Health and Human Services, National Toxicology Program
UV	Ultraviolet
UV/VIS	Ultraviolet/Visible Spectrum
V	Volt
VOI	Verification of Identity
v/v	volume to volume ratio
w/v	weight to volume ratio
WHO	World Health Organization
WSSA	Weed Science Society of America

I. RATIONALE FOR THE DEVELOPMENT OF MON 87419

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 87419, any progeny derived from crosses between MON 87419 and conventional maize, and any progeny derived from crosses of MON 87419 with biotechnology-derived maize that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Dicamba and Glufosinate Tolerant Maize MON 87419

Annual and perennial weeds are considered to be the greatest pest problem in corn production (Aref and Pike 1998). Weeds compete with maize for water, nutrients, and light resulting in substantial yield losses when left uncontrolled. Weed species in maize vary from region to region and from state to state. Economic thresholds for controlling weeds in maize require some form of weed management practice on all maize acreage. Weed management practices include mechanical tillage, crop rotations, cultural practices, and herbicide application. Numerous selective herbicides are available for preplant, preemergence, and postemergence control of annual and perennial weeds in maize. Approximately 98% of the maize acreage in the U.S. receives a herbicide application (USDA-NASS 2010c).

Monsanto Company has developed dicamba and glufosinate-tolerant maize, MON 87419, which will allow in-crop applications of dicamba (3,6-dichloro-2-methoxybenzoic acid) herbicide for the control of broadleaf weeds and glufosinate herbicide for broad spectrum weed control. The combination of the two herbicides' distinct mechanisms-of-action provides an effective weed management system. Dicamba provides effective control of over 95 annual and biennial weed species, and suppression of over 100 perennial broadleaf and woody plant species (BASF Corporation 2008) (EPA Reg. No. 7969-137) and glufosinate is a broad-spectrum contact herbicide that provides nonselective control of about 120 broadleaf and grass weeds (Bayer Crop Science 2013) (EPA Reg. No. 264-829). Additionally, dicamba and glufosinate each provide control of many herbicide-resistant weeds, including glyphosate-resistant biotypes of Palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza canadensis*), common ragweed (*Ambrosia artemisiifolia*),

giant ragweed (*Ambrosia trifida*) and waterhemp (*Amaranthus tuberculatus*) that are present in maize production areas.

MON 87419 will likely be combined with Roundup Ready® Corn 2 utilizing traditional breeding techniques. Successful integration of MON 87419 into glyphosate tolerant maize systems will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system for two additional in-crop herbicide modes-of-action in current maize production practices as recommended by weed science experts to manage future weed resistance development; 3) an option to delay or prevent further development of weed populations resistant to glyphosate and other critically important maize herbicides; 4) crop safety to dicamba, glufosinate and glyphosate; and 5) additional weed management tools to enhance weed management systems necessary to maintain yield and quality to meet the growing needs of food and feed.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology (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 U.S. Environmental Protection Agency (EPA). Deregulation of MON 87419 by USDA constitutes only one component of the overall regulatory oversight and review of this product. USDA considers the plant pest potential of regulated genetically modified organisms. As a practical matter, MON 87419 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions. Additionally, EPA must complete its review and assessments prior to approving the revised use and allowable residues of dicamba on MON 87419. Glufosinate is currently registered for use on glufosinate-tolerant maize.

I.C.1. Submission to FDA

MON 87419 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 compliance with this policy, Monsanto will initiate a consultation with the FDA. Monsanto will be submitting a food/feed safety and nutritional assessment summary document to FDA in the near future.

I.C.2. Submission to EPA

Dicamba and glufosinate herbicides are currently labeled for use as preplant and postemergence applications in maize. MON 87419 with the dicamba-tolerance trait will provide improved crop tolerance and provide more effective preemergence and postemergence control of problem weed species compared to currently labeled applications of dicamba in conventional maize hybrids. Glufosinate is currently labeled

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for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on glufosinate-tolerant hybrids only. Glufosinate use in MON 87419 will not change from current labeled uses of glufosinate.

Monsanto will petition EPA to increase the maximum use rate of dicamba in maize from 0.5 lbs. to 1.0 lbs. a.e. per acre for preemergence applications and up to two applications of 0.5 lbs. a.e. of dicamba per acre for postemergence applications through the V8 growth stage or maize height of 30 inches, whichever comes first. The combined maximum annual application rate of dicamba on MON 87419 would be 2.0 lbs. a.e. dicamba per acre per year.

I.C.3. Submissions to Foreign Government Agencies

Consistent with our commitments to the Biotechnology Industry Organization (BIO) and Excellence Through Stewardship® (ETS)[2], Monsanto will meet applicable regulatory requirements for MON 87419 in the country of intended production and for key import countries identified in the trade assessment process that have functioning regulatory systems to assure global compliance and support the flow of international trade. Monsanto will continue 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 re-evaluate its stewardship plans and make appropriate modifications to minimize the potential for trade disruption.

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[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 Germplasm Resources Information Network (GRIN) database (USDA-ARS 2013).

To support the evaluation of the plant pest potential of MON 87419 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.D.

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 the 2013/2014 growing season (October to September), maize was planted globally on ~180 million hectares (ha) with a total grain production of an estimated 989 million metric tons (MMT) (USDA-FAS 2015). The top five production regions were: USA (351 MMT), China (218 MMT), Brazil (80 MMT), EU-28 (64 MMT), and Argentina (26 MMT) (USDA-FAS 2015). In the U.S., maize is grown in almost every state and in 2014, its production value of over $52 billion was the highest of any crop (USDA-NASS 2015).

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. In addition, 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 selection of desirable characteristics in a plant through the generation of unique combinations of genes already present in the plant. However, there is a limit to genetic diversity that is available for use and selection 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 desired by growers and downstream crop users.

II.B. Characteristics of the Recipient Plant

The transformation for MON 87419 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 × LH199) × LH197) 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 MON 87419 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

In studies utilizing hybrid maize, NL6169 (LH244 × HCL645) was used as near isogenic, conventional controls for this submission (hereafter referred to as conventional control). The test is a hybrid of LH244 (MON 87419 expressing DMO and PAT) × HCL645 unless otherwise noted (Figure IV-3). In addition, other conventional 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. NL6169 was used as the conventional control in molecular characterization studies, 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 87419 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 and initiation of commercial breeding efforts were conducted with the R_3 generation (Figure IV-3). Protein characterization and expression analysis, composition analysis, and phenotypic, agronomic and environmental interactions assessment were conducted with various MON 87419 breeding generations as noted in the breeding tree (Figure IV-3).

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 87419 was developed by *Agrobacterium tumefaciens*-mediated transformation of maize immature embryos from line LH244 utilizing plasmid PV-ZMHT507801. This section describes the plasmid vector, the donor genes, and the regulatory elements used in the development of MON 87419. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. 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- ZMHT507801

Plasmid vector PV-ZMHT507801 was used for the transformation of maize to produce MON 87419 and its plasmid map is shown in Figure III-1. The elements included in this plasmid vector are described in Table III-1. Summary of Genetic Elements in PV-ZMHT507801. Plasmid vector PV-ZMHT507801 is approximately 14.6 kb in length and contains two separate T-DNAs, each delineated by Left and Right Border regions. The first T-DNA, designated as T-DNA I, contains the *dmo* expression cassette and the *pat* expression cassette. The *dmo* expression cassette is regulated by the peanut chlorotic streak caulimovirus (*PClSV*) promoter, the *5′* untranslated leader sequence of the *Cab* gene from *Triticum aestivum*, the *Ract1* intron from *Oryza sativa*, the *CTP4* targeting sequence from *Petunia hybrida* and the *3′* untranslated region of heat shock protein 17 (*Hsp17*) from *Triticum aestivum.* The *pat* expression cassette is regulated by the *Ubq* promoter from *Andropogon gerardii*, the *Ubq* *5′* untranslated leader sequence from *Andropogon gerardii*, the *Ubq* intron from *Andropogon gerardii* and the *3′* untranslated region of the *Ara5* gene from *Oryza sativa*. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* expression cassette. The *cp4 epsps* expression cassette is regulated by the *Ract1* promoter from *Oryza sativa*, the *Ract1* *5′* untranslated leader 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*. During transformation, both T-DNAs 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 *dmo* and *pat* expression cassettes (T-DNA I) and do not contain the *cp4 epsps* expression cassette (T-DNA II).

The backbone region of PV-ZMHT507801, located outside of the T-DNAs, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori V*, *ori pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer (ROP) protein for maintenance of plasmid vector copy number in *Escherichia coli* (*E. coli*). A description of the genetic elements and their prefixes (e.g., B, P, L, I, TS, CS, T, and OR) in PV-ZMHT507801 is provided in Table III-1.

III.B. Description of the Transformation System

MON 87419 was developed through *Agrobacterium tumefaciens* mediated transformation of immature maize embryos based on the method described by Sidorov and Duncan (Sidorov and Duncan 2009) utilizing PV-ZMHT507801. 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 87419.

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 were self-pollinated to produce R_1 seed and the unlinked insertions of T-DNA I and T-DNA II were segregated. Subsequently, R_1 plants that were positive for the *dmo* and *pat* expression cassettes (T-DNA I) and did not contain the *cp4 epsps* expression cassette (T-DNA II) were identified by a polymerase chain reaction (PCR) based analysis. The R_1 plants homozygous for T-DNA I 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, thousands of different transformation events (regenerants) were generated in the laboratory using PV-ZMHT507801. After many months of careful selection and evaluation of these thousands of events in the laboratory, greenhouse and field, MON 87419 was selected as the lead event based on superior agronomic, phenotypic, and molecular characteristics (Prado et al. 2014). Studies on MON 87419 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 87419 are depicted in Figure III-2. The result of this process was the production of MON 87419 maize with the *dmo* and *pat* expression cassettes and without the *cp4 epsps* expression cassette.

III.C. The *dmo* Coding Sequence and the MON 87419 DMO Protein

The *dmo* expression cassette encodes for 412 amino acids (340 amino acids encoded by the *dmo* gene and 72 amino acids encoded by the *CTP4* gene). MON 87419 expresses two forms of DMO protein due to alternative processing of CTP. One form, referred to as MON 87419 DMO+12 consists of 352 amino acids, which includes 340 amino acids encoded by the *dmo* gene and 12 amino acids encoded by the *CTP4* gene. The other form of the protein, referred to as MON 87419 DMO+7 consists of 347 amino acids, which includes 340 amino acids encoded by the *dmo* gene and seven amino acids encoded by the *CTP4* gene. MON 87419 DMO+7 does not contain the first five amino acids of MON 87419 DMO+12. Both forms of DMO protein expressed in MON 87419 are indistinguishable by Coomassie stain of SDS-PAGE and western blot analysis because the difference in molecular weight between these two forms is small. Therefore, a ~39.5 kDa MON 87419 DMO protein is observed by Coomassie stain of SDS-PAGE and western blot analysis. The *dmo* open reading frame in the expression cassette includes a codon optimized sequence from *S. maltophilia* that encodes the DMO protein (Herman et al. 2005; Wang et al. 1997). The expression of MON 87419 DMO protein confers tolerance to dicamba herbicide.

III.D. The *pat* Coding Sequence and the PAT Protein

The *pat* expression cassette encodes for 183 amino acids. MON 87419 expresses a ~25 kDa PAT protein consisting of a single polypeptide of 182 amino acids, except for the lead methionine which is cleaved during a co-translational process in MON 87419 (Wehrmann et al. 1996; Wohlleben et al. 1988) (Figure III-4). The *pat* open reading frame in the expression cassette includes sequence from *S. viridochromogenes* that encodes the PAT protein (Wehrmann et al. 1996; Wohlleben et al. 1988). The expression of PAT protein confers glufosinate tolerance.

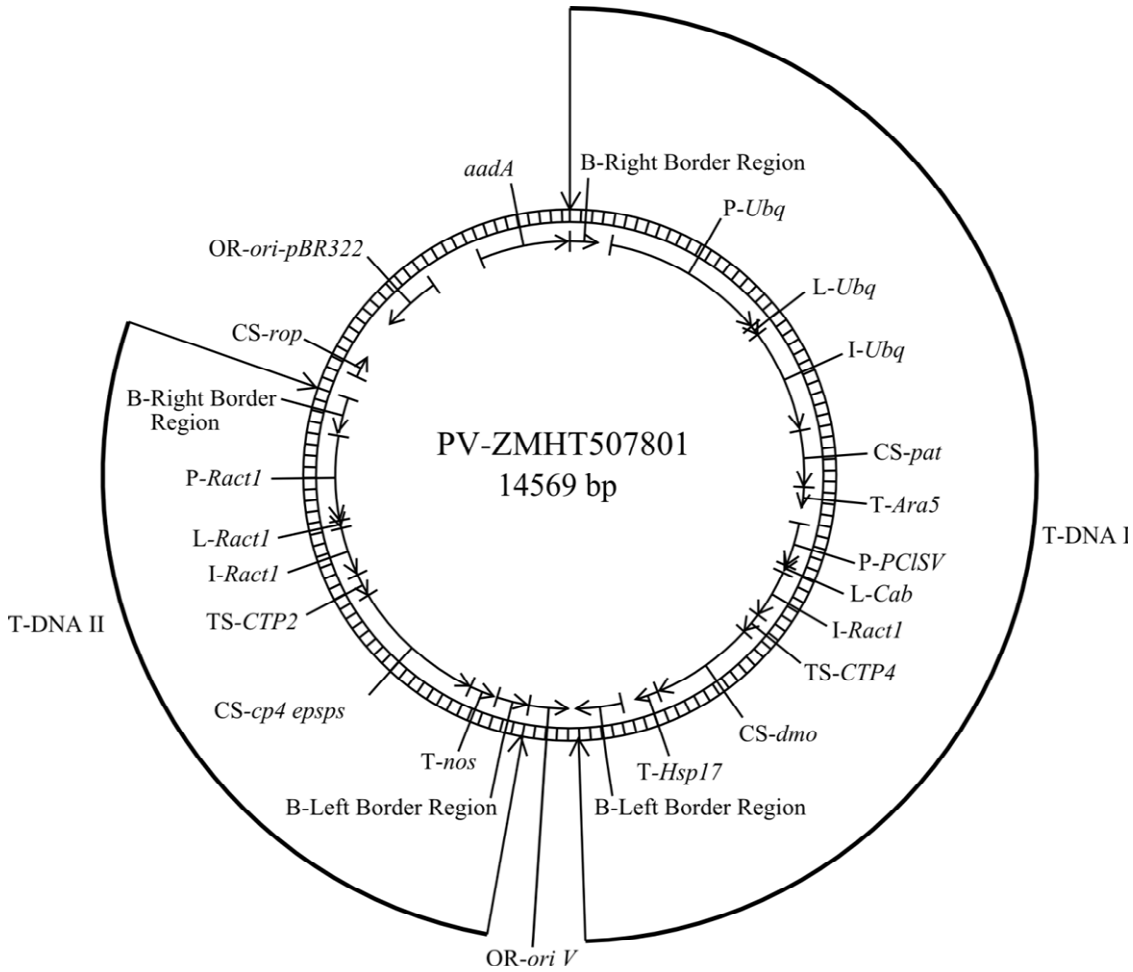

Figure III-1. Circular Map of PV-ZMHT507801
A circular map of PV-ZMHT507801 used to develop MON 87419 is shown. PV-ZMHT507801contains two T-DNAs, designated as T-DNA I and T-DNA II. Genetic elements are shown on the exterior of the map

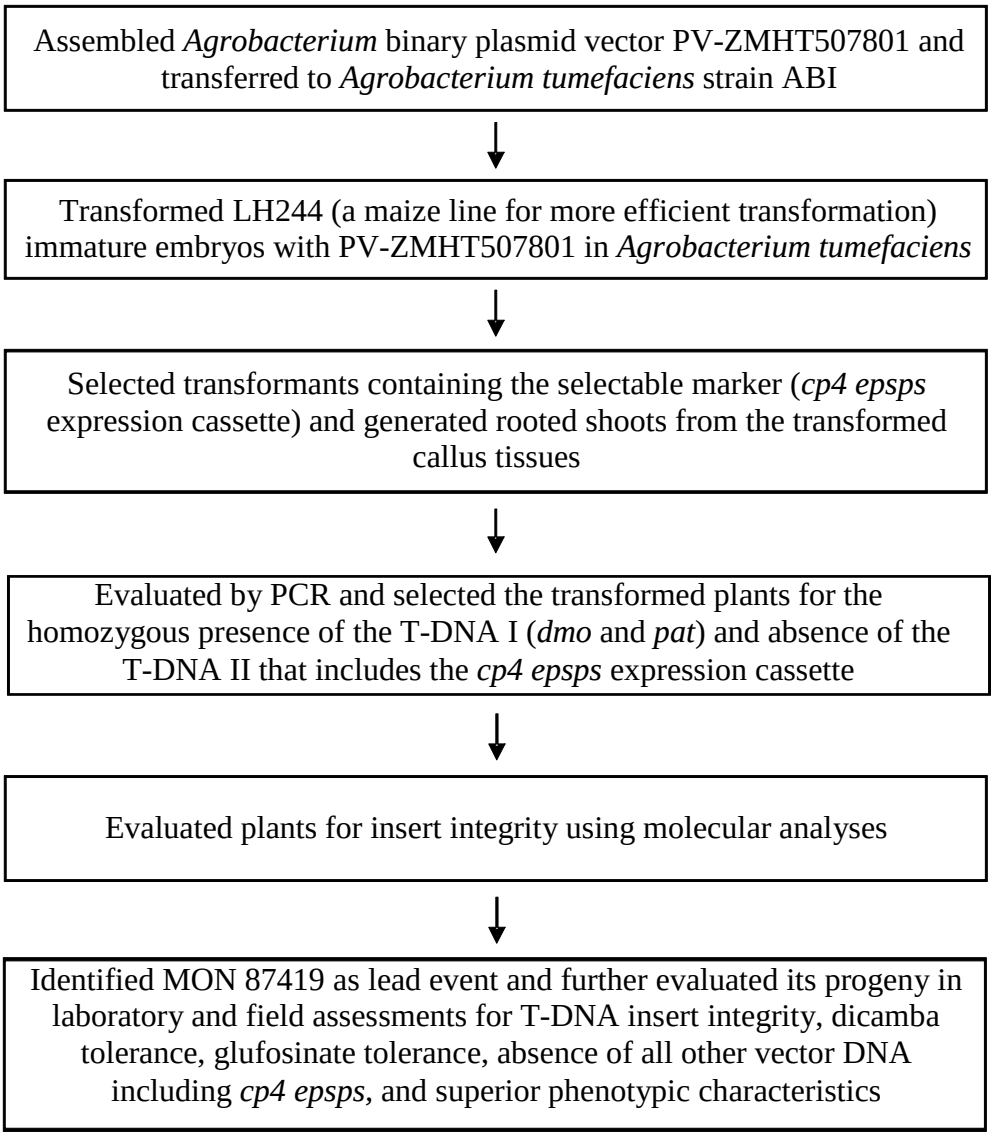

Figure III-2. Schematic of the Development of MON 87419

```
  1  MAQINNMAQG IQTLNPNSNF HKPQVPKSSS FLVFGSKKLK NSANSMLVLK
 51  KDSIFMQKFC SFRISASVAT ACMLTFVRNA WYVAALPEEL SEKPLGRTIL
101  DTPLALYRQP DGVVAALLDI CPHRFAPLSD GILVNGHLQC PYHGLEFDGG
151  GQCVHNPHGN GARPASLNVR SFPVVERDAL IWIWPGDPAL ADPGAIPDFG
201  CRVDPAYRTV GGYGHVDCNY KLLVDNLMDL GHAQYVHRAN AQTDAFDRLE
251  REVIVGDGEI QALMKIPGGT PSVLMAKFLR GANTPVDAWN DIRWNKVSAM
301  LNFIAVAPEG TPKEQSIHSR GTHILTPETE ASCHYFFGSS RNFGIDDPEM
351  DGVLRSWQAQ ALVKEDKVVV EAIERRRAYV EANGIRPAML SCDEAAVRVS
401  REIEKLEQLE AA
```

Figure III-3. Deduced Amino Acid Sequence of the CTP4 Targeting Sequence and DMO Protein

The amino acid sequence of the MON 87419 DMO precursor protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT507801 (See Table III-1 for more detail). The first 72 amino acids of the precursor protein (underlined) are the chloroplast transit peptide (CTP) from *Petunia hybrida* EPSPS (*CTP4*). CTP targets MON 87419 DMO protein to the chloroplast. CTP4 is partially cleaved in the chloroplast producing the mature 352 amino acid MON 87419 DMO protein that begins with the serine at position 60. The double underline shows the twelve amino acids from *CTP4* that are at the N-terminus of the mature MON 87419 DMO protein, referred to as MON 87419 DMO+12. MON 87419 DMO+7 does not contain the first five amino acids of MON 87419 DMO+12.

```
  1  MSPERRPVEI RPATAADMAA VCDIVNHYIE TSTVNFRTEP QTPQEWIDDL
 51  ERLQDRYPWL VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRHQRL
101  GLGSTLYTHL LKSMEAQGFK SVVAVIGLPN DPSVRLHEAL GYTARGTLRA
151  AGYKHGGWHD VGFWQRDFEL PAPPRPVRPV TQI
```

Figure III-4. Deduced Amino Acid Sequence of the PAT Protein

The amino acid sequence of the MON 87419 produced PAT protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT507801 (See Table III-1 for more detail). The lead methionine of the PAT protein produced in MON 87419 is cleaved during a co-translational process in MON 87419.

III.E. Regulatory Sequences

The *dmo* coding sequence in MON 87419 is under the regulation of the *PClSV* promoter, the chlorophyll a/b binding protein (CAB) leader, the *Ract1* intron, The *CTP4* transit peptide, and the heat shock protein 17 (*Hsp17*) 3′ untranslated region. The *PClSV* promoter is the promoter for the Full-Length transcript (FLt) of peanut chlorotic streak caulimovirus (Maiti and Shepherd 1998) that directs transcription in plant cells. 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 and flanking UTR sequences are from the *act1* gene from *Oryza sativa* (McElroy et al. 1990). The chloroplast transit peptide CTP directs transport of the DMO protein to the chloroplast in MON 87419 and is derived from the chloroplast targeting sequence of the *Petunia hybrida ShkG* gene (Gasser et al. 1988; Herrmann 1995). 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.

The *pat* coding sequence in MON 87419 is under the regulation of the *Ubq* promoter, the *Ubq* leader, the *Ubq* intron and the *Ara5* 3′ untranslated region. The *Ubq* promoter is the promoter for an ubiquitin gene (*Ubq*) from *Andropogon gerardii* (Joung and Kamo 2006) that directs transcription in plant cells. The *Ubq* leader is the 5′ untranslated region from an ubiquitin gene (*Ubq)* from *Andropogon gerardii* (Joung and Kamo 2006) and is involved in regulating gene expression. The *Ubq* intron is the intron from an ubiquitin gene (*Ubq*) from *Andropogon gerardii* (Joung and Kamo 2006). The *Ara5* 3′ untranslated region is the 3′ untranslated region from the alpha-amylase/trypsin inhibitor gene (*Ara5*) gene of *Oryza sativa* encoding the *RA5B* precursor gene and directs polyadenylation of the mRNA (Hunt 1994).

The *cp4 epsps* coding sequence in MON 87419 is under the regulation of the *Ract1* promoter, the *Ract1* leader, the *Ract1* intron, the *CTP2* targeting sequence, and the *nos* 3′ untranslated region. The *Ract1* promoter is the promoter for the *act1* gene from *Oryza sativa* (McElroy et al. 1990) that directs transcription in plant cells. The *Ract1* leader is the leader sequence of the *act1* gene from *Oryza sativa* (McElroy et al. 1990) that is involved in regulating gene expression. The *Ract1* intron is the intron and flanking untranslated sequence of the *act1* gene from *Oryza sativa* (McElroy et al. 1990) that is involved in regulating gene expression. The chloroplast transit peptide CTP2 is the 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). The *nos* 3′ untranslated region is the 3′ untranslated region of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan et al. 1983; Fraley et al. 1983).

III.F. T-DNA Borders

PV-ZMHT507801 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 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 87419.

III.G. 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-ZMHT507801 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-pBR322* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe 1979). 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 selectable marker *aadA* is a bacterial promoter, coding sequence and 3′ untranslated region for an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance (Fling et al. 1985) in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the maize genome. The absence of the backbone and other unintended plasmid sequence in MON 87419 was confirmed by sequencing and bioinformatic analyses (see Section IV.A).

Table III-1. Summary of Genetic Elements in PV-ZMHT507801

Genetic Element	Location in Plasmid Vector	Function (Reference)
T-DNA I		
B[1]-Right Border Region	1-285	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	286-410	Sequence used in DNA cloning
P[2]-*Ubq*	411-2054	Promoter for a ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that initiates and directs transcription (Joung and Kamo 2006)
L[3]-*Ubq*	2055-2153	5' UTR leader sequence for the ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that is involved in regulating gene expression (Joung and Kamo 2006)
I[4]-*Ubq*	2154-3195	Intron sequence of the ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that is involved in regulating gene expression (Joung and Kamo 2006)
Intervening Sequence	3196-3200	Sequence used in DNA cloning
CS[5]-*pat*	3201-3752	Coding sequence for the phosphinothricin N-acetyltransferase (PAT) protein of *Streptomyces viridochromogenes* that confers tolerance to glufosinate (Wehrmann et al. 1996; Wohlleben et al. 1988)
Intervening Sequence	3753-3760	Sequence used in DNA cloning
T[6]-*Ara5*	3761-3973	3'UTR sequence of the *RA5B* precursor gene from *Oryza sativa* (rice), encoding an alpha-amylase/trypsin inhibitor (*Ara5*) that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	3974-4120	Sequence used in DNA cloning
P-*PClSV*	4121-4553	Promoter for the Full-Length transcript (FLt) of peanut chlorotic streak caulimovirus (PClSV) that directs transcription in plant cells (Maiti and Shepherd, 1998)
Intervening Sequence	4554-4558	Sequence used in DNA cloning

Table III-I (continued). Summary of Genetic Elements in PV-ZMHT507801

Genetic Element	Location in Plasmid Vector	Function (Reference)
L-*Cab*	4559-4619	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	4620-4635	Sequence used in DNA cloning
I-*Ract1*	4636-5115	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein (McElroy et al. 1990) that is involved in regulating gene expression
Intervening Sequence	5116-5124	Sequence used in DNA cloning
TS[7]-*CTP4*	5125-5340	Targeting and 5' UTR leader sequence of the *ShkG* gene from *Petunia hybrida* encoding the EPSPS transit peptide region that directs the protein to the chloroplast (Gasser et al. 1988; Herrmann 1995)
CS-*dmo*	5341-6363	Codon optimized coding sequence for the dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba resistance (Herman et al. 2005; Wang et al. 1997)
Intervening Sequence	6364-6393	Sequence used in DNA cloning
T-*Hsp17*	6394-6603	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	6604-6765	Sequence used in DNA cloning
B-Left Border Region	6766-7207	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)
		Backbone
Intervening Sequence	7208-7293	Sequence used in DNA cloning
OR[8]-*ori V*	7294-7690	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al. 1981)
Intervening Sequence	7691-7696	Sequence used in DNA cloning

Table III-I (continued). Summary of Genetic Elements in PV-ZMHT507801

Genetic Element	Location in Plasmid Vector	Function (Reference)
T-DNA II		
B-Left Border Region	7697-8015	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)
Intervening Sequence	8016-8045	Sequence used in DNA cloning
T-*nos*	8046-8298	3' UTR sequence of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan et al. 1983; Fraley et al. 1983)
Intervening Sequence	8299-8313	Sequence used in DNA cloning
CS-*cp4 epsps*	8314-9681	Coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides herbicide tolerance (Barry et al. 2001; Padgette et al. 1996)
TS-*CTP2*	9682-9909	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)
Intervening Sequence	9910-9918	Sequence used in DNA cloning
I-*Ract1*	9919-10396	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein (McElroy et al. 1990) that is involved in regulating gene expression
L-*Ract1*	10397-10476	Leader sequence of the *act1* gene from *Oryza sativa* (rice) encoding the rice Actin 1 protein (McElroy et al. 1990) that is involved in regulating gene expression
P-*Ract1*	10477-11317	Promoter of the *act1* gene from *Oryza sativa* (rice) encoding the rice Actin 1 protein (McElroy et al. 1990) that directs transcription in plant cells
Intervening Sequence	11318-11343	Sequence used in DNA cloning
B-Right Border Region	11344-11700	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)

Table III-I (continued). Summary of Genetic Elements in PV-ZMHT507801

Genetic Element	Location in Plasmid Vector	Function (Reference)
Backbone		
Intervening Sequence	11701-11926	Sequence used in DNA cloning
CS-*rop*	11927-12118	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	12119-12545	Sequence used in DNA cloning
OR-*ori-pBR322*	12546-13134	Origin of replication from plasmid pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe 1979)
Intervening Sequence	13135-13664	Sequence used in DNA cloning
aadA	13665-14553	Bacterial promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3''(9)-*O*-nucleotidyltransferase from the transposon Tn*7* (Fling et al. 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	14554-14569	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 87419 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87419 contains one copy of the intended transfer DNA (T-DNA I) containing the *dmo* and *pat* expression cassettes that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple breeding generations. These conclusions are based on several lines of evidence:

1. Molecular characterization of MON 87419 by Next Generation Sequencing and Junction Sequence Analysis (NGS/JSA) demonstrated that MON 87419 contained a single intended DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 87419 to determine the presence and identity of sequences derived from PV-ZMHT507801 (DuBose et al. 2013; Kovalic et al. 2012), demonstrating that MON 87419 contained a single T-DNA I insert with no detectable backbone or T-DNA II sequences.
2. Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87419 was used to determine the complete sequence of the single DNA insert from PV-ZMHT507801, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the DNA is identical to the corresponding region in the PV-ZMHT507801 T-DNA I. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA I insert in MON 87419 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 87419 upon DNA integration.
3. Generational stability analysis by NGS/JSA demonstrated that the single PV-ZMHT507801 T-DNA I insert in MON 87419 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I insert in MON 87419.
4. Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the nature of the T-DNA I insert at a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 87419 demonstrates that a single copy of the intended T-DNA I was stably integrated at a single locus of the maize genome and that no plasmid backbone or T-DNA II sequences are present in MON 87419.

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 B provides an additional overview of these techniques, their use in DNA characterization in crop plants and the materials and methods.

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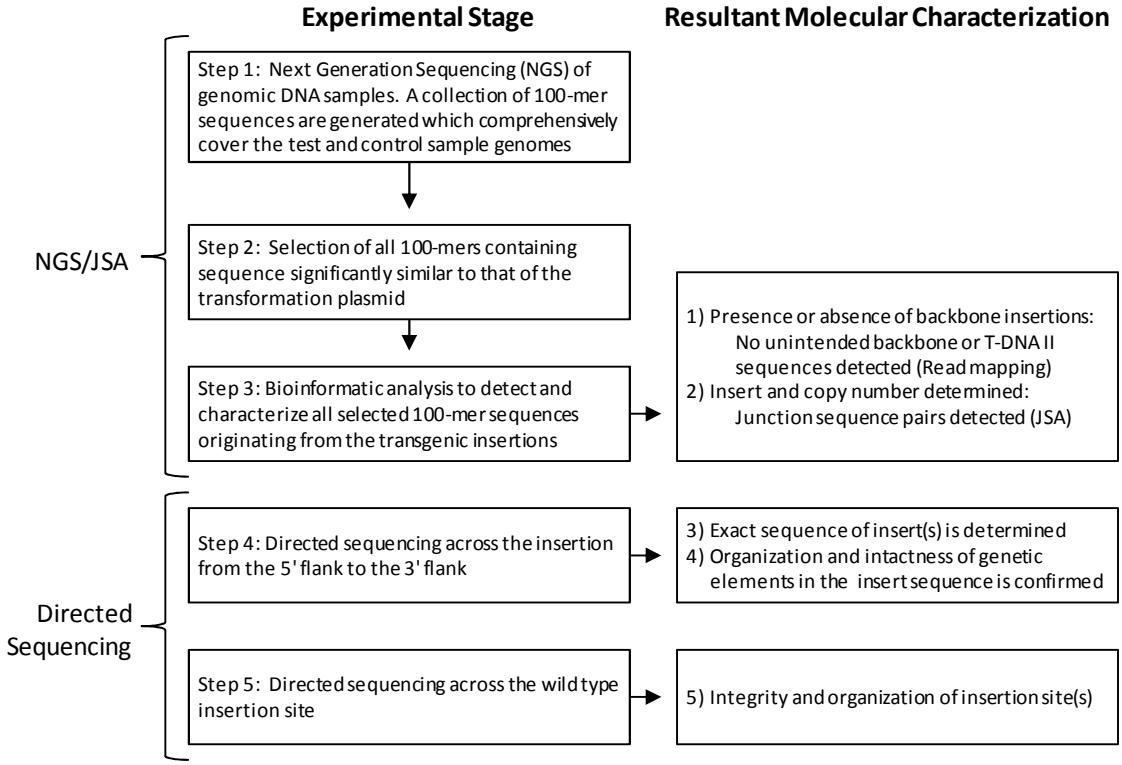

Figure IV-1. Molecular Characterization using Sequencing and Bioinformatics
Genomic DNA from MON 87419 and the conventional control was sequenced using technology that produces a set of short, randomly distributed sequence reads (each approximately 100 bp long) that comprehensively covers both test and control genomes (Step 1). Utilizing these genomic sequences, 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 T-DNA II 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 loci (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 87419 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 been previously 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 achieved in this study was sufficient to detect ≥ 99% of the plasmid sequence when present at 1/10th the mean coverage of the conventional control genome. Analysis of the sampling data thus confirmed 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 insert(s). NGS/JSA was run on all five generations of MON 87419 samples and the conventional controls. NGS/JSA methodology utilizes sequencing and bioinformatics to produce characterizations equivalent to those achieved previously by traditional Southern blotting (See Section IV.F) (Kovalic et al. 2012). Results of NGS/JSA are shown in Sections IV.A and IV.D.

Directed sequencing (locus-specific PCR and DNA sequencing analyses, Figure IV-1, Step 4) complements the NGS/JSA. 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 I in PV-ZMHT507801 and if each genetic element in the insert is intact. Results are described in Section IV.B and Section IV.C; methods are presented in Appendix B.

The stability of the T-DNA I present in MON 87419 across multiple breeding generations was evaluated by NGS/JSA as described above. This information was used to determine the number and identity of the DNA inserts. For a single copy T-DNA I insert, two junction sequence classes are expected. In the case of an event where a single locus is stably inherited over multiple breeding generations, two identical junction sequence classes would be detected in all the breeding generations tested. Results are described in Section IV.D; methods are presented in Appendix B.

Segregation analysis of the T-DNA I was conducted to determine the inheritance and stability of the insert in MON 87419. Segregation analysis corroborates the insert stability demonstrated by NGS/JSA and independently establishes the genetic behavior of the T-DNA I. Results are described in Section IV.E; methods are presented in Appendix B.

NGS/JSA methodology arrives at the same conclusions as previously determined by traditional Southern blotting (See Section IV.F) (Kovalic et al. 2012).

IV.A. Determining the Number and Identity of DNA Inserts in MON 87419

The number of insertion sites of PV-ZMHT507801 DNA in MON 87419 was assessed by performing NGS/JSA on MON 87419 genomic DNA. A plasmid map of PV-ZMHT507801 is shown in Figure III-1. Table IV-1 provides descriptions of the genetic elements present in MON 87419. A schematic representation of the insert and flanking sequences in MON 87419 is shown in Figure IV-2. For full details on materials and methods, see Appendix B.

IV.A.1. Next Generation Sequencing of MON 87419 and Conventional Control Genomic DNA

Genomic DNA from five breeding generations of MON 87419 (Figure IV-3) and the conventional control was isolated from seed and prepared for sequencing using the Illumina TruSeq DNA Sample Preparation Kit (Illumina). For material and method details see Appendix B). 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).

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) in each of the five breeding generations. 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 87419 (R_3, R_4, R_5, R_3F_1, and R_4F_1) and the conventional control. It has been previously 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-ZMHT507801 transformation plasmid, a sample of conventional control genomic DNA spiked with PV-ZMHT507801 DNA was analyzed by NGS and bioinformatics. The level of sensitivity of this method was demonstrated to a level of one genome equivalent, 100% nucleotide identity was observed over 100% of PV-ZMHT507801. This result demonstrates that all nucleotides of the transformation plasmid are observed by the sequencing and bioinformatic assessments performed (Figure IV-4). Also, observed coverage was adequate (Clarke and Carbon 1976) at a level 1/10th genomic equivalent (99.43% coverage at 100% identity) and, hence, a detection level of at most 1/10th genome equivalent was achieved for the plasmid DNA sequence assessment (Figure IV-4).

Table IV-1. Summary of Genetic Elements in MON 87419

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
Flanking DNA	1-1246	Flanking DNA
B[3]-Right Border Region [r1]	1247-1317	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	1318-1442	Sequence used in DNA cloning
P[4]-*Ubq*	1443-3086	Promoter for a ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that initiates and directs transcription (Joung and Kamo 2006)
L[5]-*Ubq*	3087-3185	5' UTR leader sequence for the ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that is involved in regulating gene expression (Joung and Kamo 2006)
I[6]-*Ubq*	3186-4227	Intron sequence of the ubiquitin gene (*Ubq*) from *Andropogon gerardii* (big bluestem grass) that is involved in regulating gene expression (Joung and Kamo 2006)
Intervening Sequence	4228-4232	Sequence used in DNA cloning
CS[7]-*pat*	4233-4784	Coding sequence for the phosphinothricin N-acetyltransferase (PAT) protein of *Streptomyces viridochromogenes* that confers tolerance to glufosinate (Wehrmann et al. 1996; Wohlleben et al. 1988)
Intervening Sequence	4785-4792	Sequence used in DNA cloning
T[8]-*Ara5*	4793-5005	3'UTR sequence of the RA5B precursor gene from *Oryza sativa* (rice), encoding an alpha-amylase/trypsin inhibitor (*Ara5*) that directs polyadenylation of mRNA (Hunt 1994)
Intervening Sequence	5006-5152	Sequence used in DNA cloning
P-*PClSV*	5153-5585	Promoter for the Full-Length transcript (FLt) of peanut chlorotic streak caulimovirus (PClSV) that directs transcription in plant cells (Maiti and Shepherd 1998)
Intervening Sequence	5586-5590	Sequence used in DNA cloning

Table IV-1 (continued). Summary of Genetic Elements in MON 87419

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
L-*Cab*	5591-5651	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	5652-5667	Sequence used in DNA cloning
I-*Ract1*	5668-6147	Intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 protein (McElroy et al. 1990) that is involved in regulating gene expression
Intervening Sequence	6148-6156	Sequence used in DNA cloning
TS[9]-*CTP4*	6157-6372	Targeting and 5' UTR leader sequence of the *ShkG* gene from *Petunia hybrida* encoding the EPSPS transit peptide region that directs the protein to the chloroplast (Gasser et al. 1988; Herrmann 1995)
CS-*dmo*	6373-7395	Codon optimized coding sequence for the dicamba mono-oxygenase (DMO) protein of *Stenotrophomonas maltophilia* that confers dicamba resistance (Herman et al. 2005; Wang et al. 1997)
Intervening Sequence	7396-7425	Sequence used in DNA cloning
T-*Hsp17*	7426-7635	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	7636-7797	Sequence used in DNA cloning
B-Left Border Region [r1]	7798-8008	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)
Flanking DNA	8009-9259	Flanking DNA

[1] Although flanking sequences and intervening sequence are not functional genetic elements, they comprise a portion of the sequence.
[2] Numbering refers to the sequence of the insert in MON 87419 and adjacent DNA.
[3] B, Border
[4] P, Promoter
[5] L, Leader
[6] I, Intron
[7] CS, Coding Sequence
[8] T, Transcription Termination Sequence
[9] TS, Targeting Sequence
[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87419 was truncated compared to the sequences in PV-ZMHT507801.

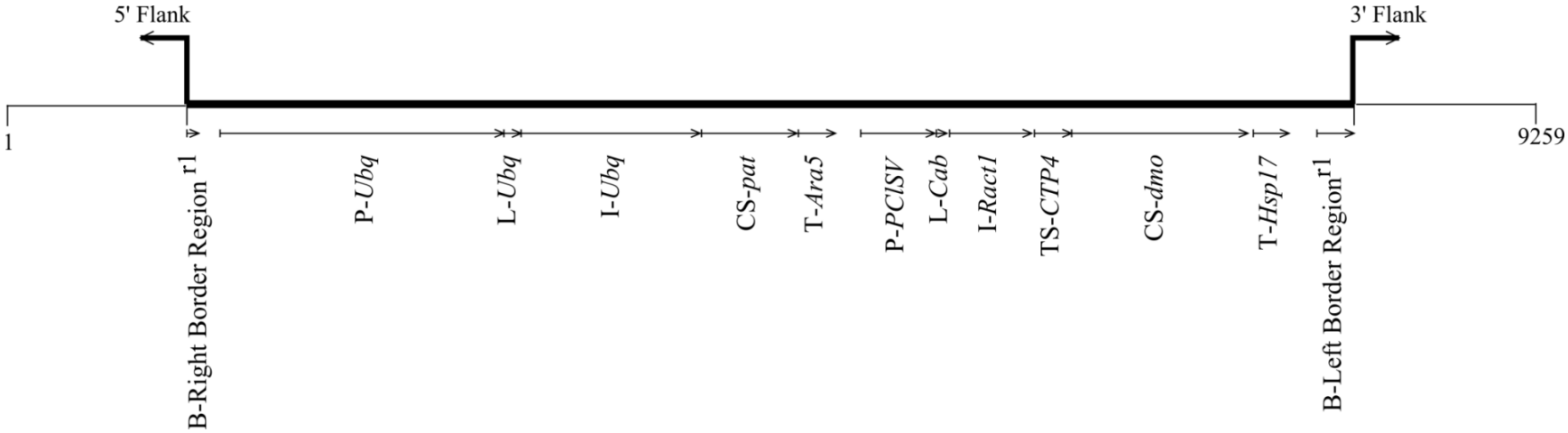

Figure IV-2. Schematic Representation of the Insert and Flanking Sequences in MON 87419
DNA derived from T-DNA I of PV-ZMHT507801 integrated in MON 87419. Right-angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking sequence. Identified on the map are genetic elements within the insert. This schematic diagram is drawn to scale. The exact coordinates of every element are shown in Table IV-1.
[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87419 was truncated compared to the sequences in PV-ZMHT507801.

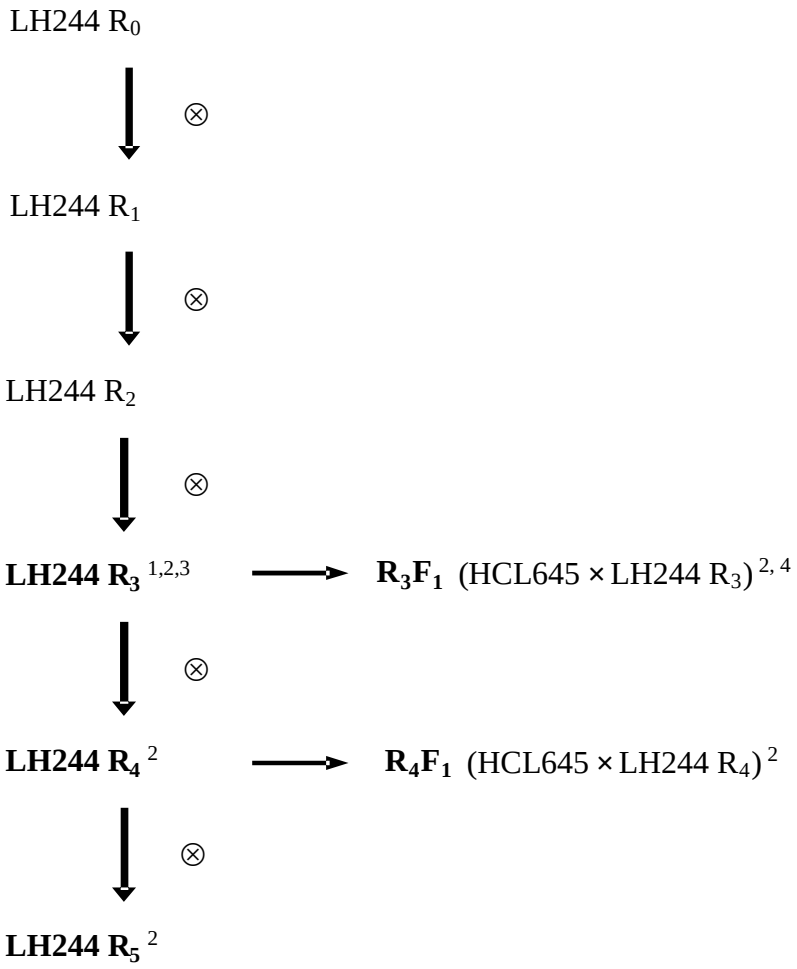

Figure IV-3. Breeding History of MON 87419
R_0 corresponds to the transformed plant, F# is the filial generation, ⊗ designates self pollination.
[1] Generation used for molecular characterization
[2] Generations used to confirm insert stability
[3] Generation used for commercial development of MON 87419
[4] Generation used for agronomic/phenotypic and compositional analysis studies

IV.A.2. Characterization of Insert Number in MON 87419 using Bioinformatic Analysis

The number of insertion sites of DNA from PV-ZMHT507801 in MON 87419 was assessed by performing NGS/JSA on MON 87419 genomic DNA using the R_3 generation (Figure IV-3).

IV.A.2.1. Selection of Sequence Reads Containing Sequence of the PV-ZMHT507801

PV-ZMHT507801 was transformed into the parental variety LH244 to produce MON 87419. Consequently, any DNA inserted into MON 87419 will consist of sequences that are similar to the PV-ZMHT507801 DNA sequence. Therefore, to fully characterize the DNA from PV-ZMHT507801 inserted in MON 87419, 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 B), sequence reads similar to the transformation plasmid were selected from MON 87419, and the conventional control sequence datasets were mapped and 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 I, T-DNA II, or backbone, can be identified by aligning reads to the transformation plasmid sequence while the number of inserted DNA molecules can be determined using JSA.

Therefore where a traditional Southern blot analysis separately hybridizes T-DNA or backbone probes, NGS/JSA determines the T-DNA insert number and the absence of backbone, T-DNA II, or unintended sequences by the identification of sequence reads that match the transformation plasmid and the determination of the overall insert number in the genome. This alternative method reaches the same conclusions regarding the number of inserts and presence or absence of backbone or T-DNA II as those determined by traditional Southern blots.

By evaluating the number of unique junction classes, the number of DNA insertion sites can be determined (Figure IV-1, Step 3). If MON 87419 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 87419, 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 87419	2
LH244	0

The location and orientation of the junction sequences relative to the T-DNA insert determined for MON 87419 (as described in Section IV.B) are illustrated in Figure IV-4. As shown in the figure, there are two junction sequence classes identified in MON 87419. Junction Sequence Class A and Class B (JSC A and JSC B) both contain partial T-DNA border sequence joined to genomic flanking sequence, indicating that they represent the sequences at the junctions of the intended T-DNA I insert and genomic flanking sequence.

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

To determine the identity of inserted DNA, all selected sequences described in Section IV.A.2.1 were mapped to the transformation plasmid sequence. Zero reads uniquely mapped to the plasmid backbone or T-DNA II while thousands of sequence reads from the R_3 generation mapped to the plasmid T-DNA I sequence. From this result it was determined that MON 87419 does not contain any sequence from the transformation plasmid backbone or T-DNA II.

Based on the comprehensive NGS/JSA study, it was concluded that MON 87419 contains one T-DNA I inserted into a single locus, as shown in Figure IV-2 and is devoid of backbone or T-DNA II 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, demonstrating MON 87419 contains only T-DNA I elements from the plasmid.

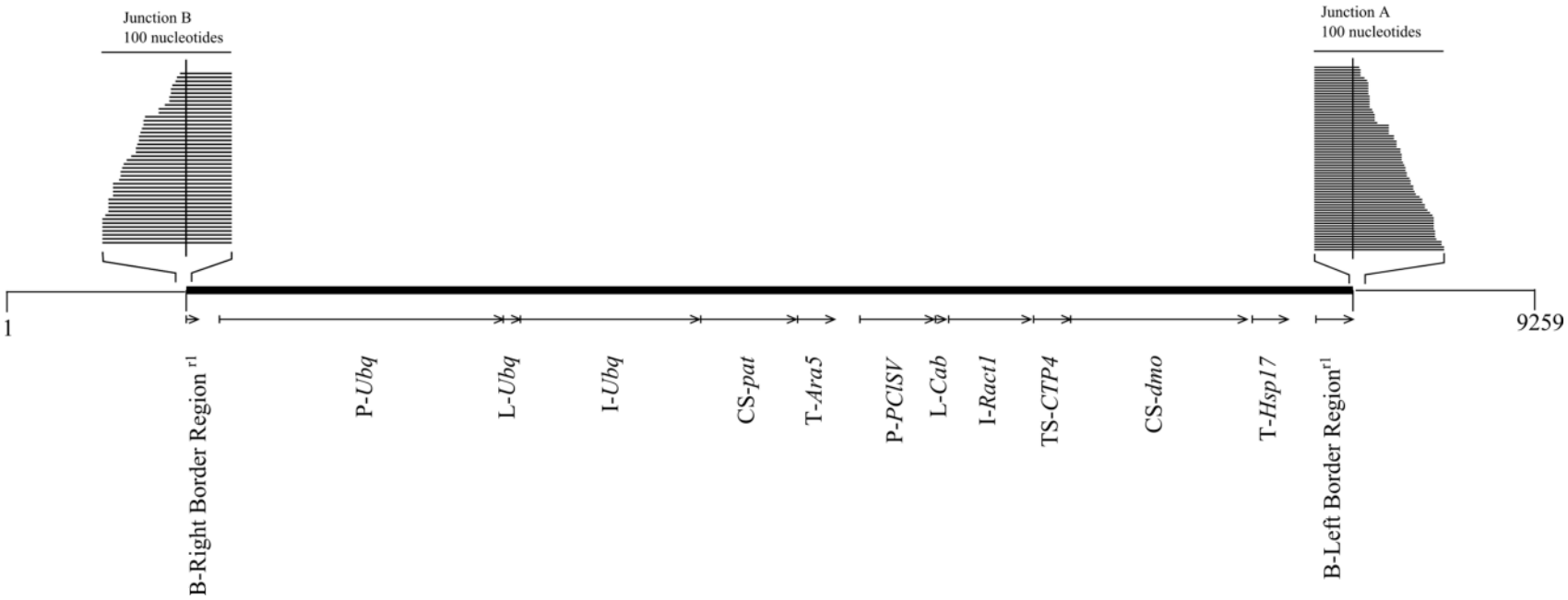

Figure IV-4. Junction Sequences Detected by NGS/JSA

Linear map of MON 87419 illustrating the relationship of the detected junction sequences to the event 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 87419 was truncated compared to the sequences in PV-ZMHT507801.

IV.B. Organization and Sequence of the Insert and Adjacent DNA in MON 87419

The organization of the elements within the DNA insert and the adjacent genomic DNA was assessed using directed DNA sequence analysis (see Figure IV-5, Step 4). PCR primers were designed to amplify six overlapping regions of the MON 87419 genomic DNA that span the entire length of the insert (Figure IV-5). The amplified PCR products were subjected to DNA sequencing analyses. The results of this analysis confirm that the MON 87419 insert is 6,762 bp and that each genetic element within the T-DNA I is intact compared to the transformation plasmid PV-ZMHT507801, 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-ZMHT507801. The sequence and organization of the insert was also shown to be identical to the corresponding T-DNA I of PV-ZMHT507801 as intended. This analysis also shows that only T-DNA I 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-ZMHT507801 backbone or T-DNA II elements are present in MON 87419.

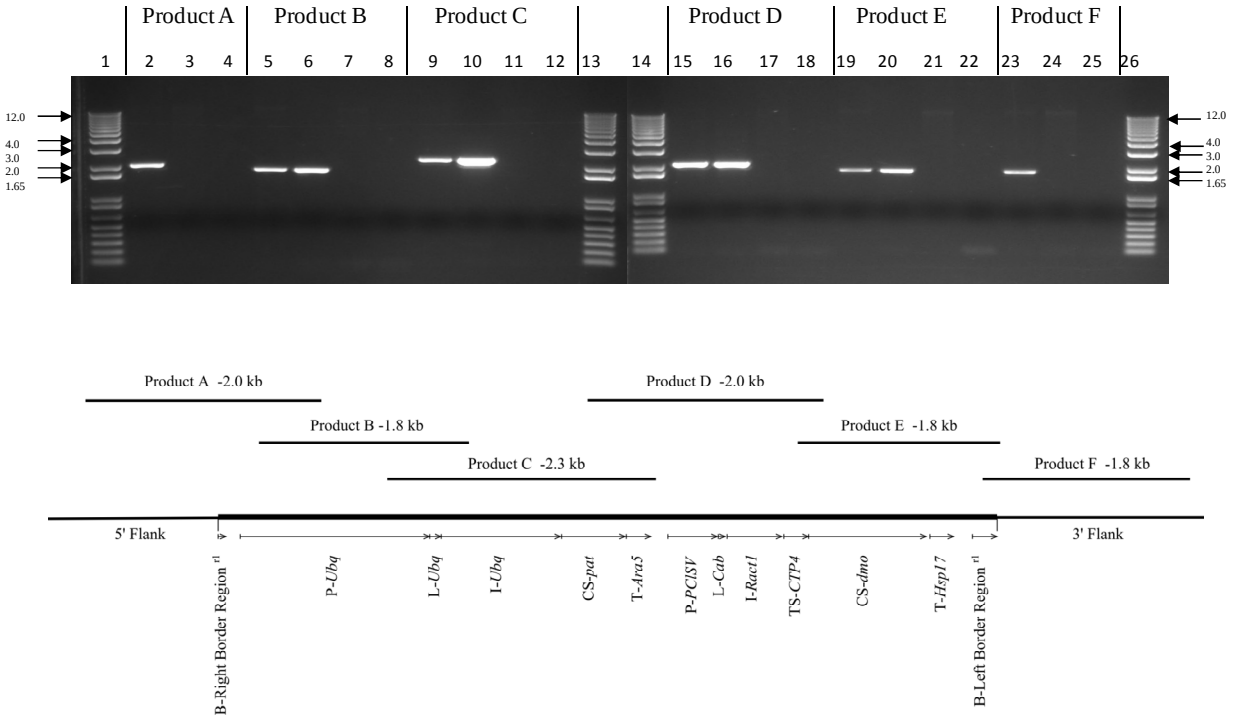

Figure IV-5. Overlapping PCR Analysis Across the Insert in MON 87419

PCR was performed on both conventional control genomic DNA and MON 87419 genomic DNA using six pairs of primers to generate overlapping PCR fragments from MON 87419 for sequencing analysis. To verify the PCR products, a portion of each PCR was loaded on the gel. 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	14	1 Kb Plus DNA Ladder
2	MON 87419	15	MON 87419
3	Conventional Control LH244	16	PV-ZMHT507801
4	No template control	17	Conventional Control LH244
5	MON 87419	18	No template control
6	PV ZMHT507801	19	MON 87419
7	Conventional Control LH244	20	PV-ZMHT507801
8	No template control	21	Conventional Control LH244
9	MON 87419	22	No template control
10	PV ZMHT507801	23	MON 87419
11	Conventional Control LH244	24	Conventional Control LH244
12	No template control	25	No template control
13	1 Kb Plus DNA Ladder	26	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) on the ethidium bromide stained gel.

[r1]Superscript in Left and Right Border Regions indicate that the sequence in MON 87419 was truncated compared to the sequences in PV-ZMHT507801.

IV.C. Sequencing of the MON 87419 Insertion Site

PCR and sequence analysis were performed on genomic DNA extracted from the conventional control to examine the insertion site in conventional maize (see Figure IV-1, Step 5). The PCR was performed with one primer specific to the genomic DNA sequence flanking the 5' end of the MON 87419 insert paired with a second primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-6). 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 87419 indicates that 602 bases of maize genomic DNA were deleted during integration of the T-DNA I. The remainder of the flanks in MON 87419 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).

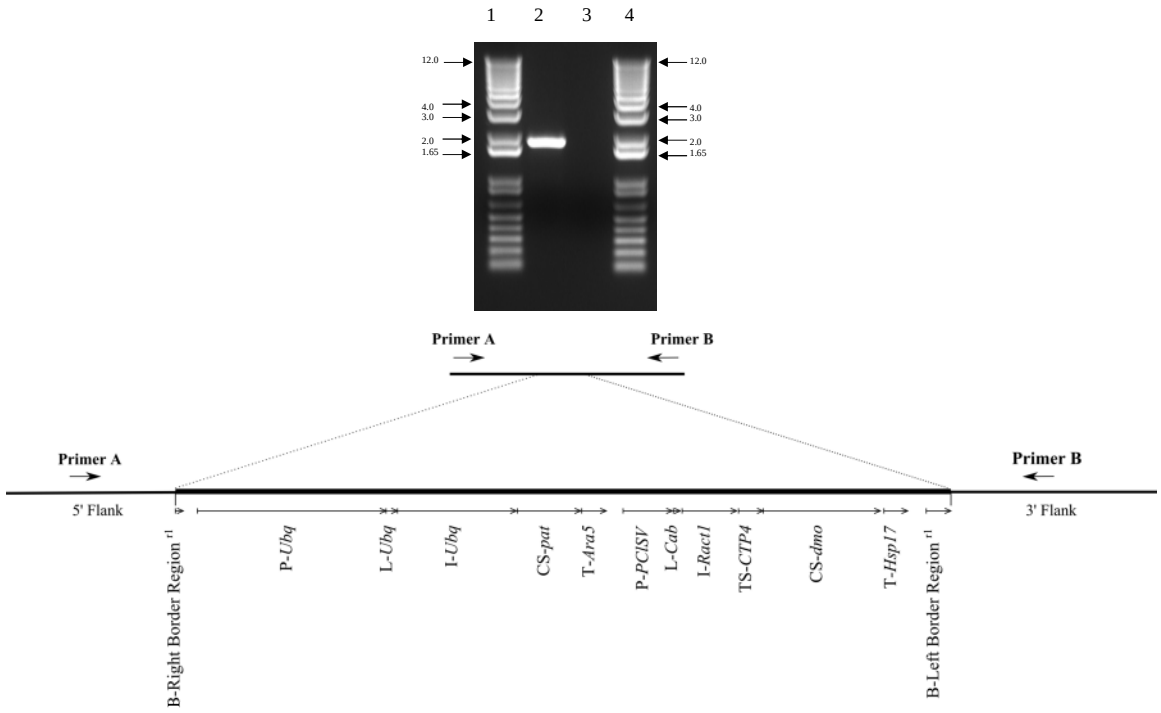

Figure IV-6. PCR Amplification of the MON 87419 Insertion Site

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 87419. The amplicon generated from the conventional control PCR was used for sequencing analysis. This illustration depicts the MON 87419 insertion site in the conventional control (upper panel) and the MON 87419 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
4	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 87419 was truncated compared to the sequences in PV-ZMHT507801.

IV.D. Determination of Insert Stability over Multiple Generations of MON 87419

In order to demonstrate the stability of the T-DNA present in MON 87419 through multiple breeding generations, NGS/JSA was performed using DNA obtained from five breeding generations of MON 87419. The breeding history of MON 87419 is presented in Figure IV-3, and the specific generations tested are indicated in the figure legend. The MON 87419 R_3 generation was used for the molecular characterization analyses discussed in Sections IV.A-IV.C and shown in Figure IV-3. To assess stability, four additional generations were evaluated by NGS/JSA 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, with similar background genetics to the R_3, R_4 and the R_5 generations and represents the original transformation line; and NL6169, a hybrid with similar background genetics to the R_3F_1 hybrid and to the R_4F_1 hybrid. Genomic DNA isolated from each of the selected generations of MON 87419 and conventional control was used for NGS/JSA.

To determine the insert number in the MON 87419 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-ZMHT507801 DNA sequence determined by this analysis.

Table IV-3. Junction Sequence Classes Detected

Sample	Junction Sequence Classes Detected
MON 87419 (R_3)	2
MON 87419 (R_3F_1)	2
MON 87419 (R_4)	2
MON 87419 (R_4F_1)	2
MON 87419 (R_5)	2
LH244	0
HCL645 × LH244	0

Alignment of the JSCs from each of the assessed MON 87419 generations (R_4, R_5, R_3F_1, and R_4F_1) to the full flank/insert sequence and JSCs determined for the MON 87419 R_3 generation, confirms that the pair of JSCs originates from the same region of the MON 87419 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-ZMHT507801 T-DNA I at a single locus in the genome of MON 87419. The consistency of these JSC data across all generations tested demonstrates that this single locus was stably maintained throughout the MON 87419 breeding process; thereby confirming the stability of the insert. Based on this comprehensive sequence data and bioinformatic analysis (NGS/JSA), it is concluded that MON 87419 contains a single and stable T-DNA I insertion.

IV.E. Inheritance of the Genetic Insert in MON 87419

The MON 87419 T-DNA I 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 87419, phenotypic and genotypic segregation data were recorded to assess the inheritance and stability of the MON 87419 T-DNA I 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 87419 breeding path for generating segregation data is described in Figure IV-7. The transformed R_0 plant was self-pollinated to generate R_1 seed. An individual plant homozygous for the MON 87419 T-DNA I was identified in the R_1 segregating population via a Real-Time 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. R_3 plants homozygous for the MON 87419 T-DNA I were crossed via traditional breeding techniques to a Monsanto proprietary recurrent parent that does not contain the *dmo* or *pat* coding sequences to produce hemizygous R_3F_1 seed. The R_3F_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 I by End-Point TaqMan PCR to select for hemizygous MON 87419 plants. BC_1F_1 plants hemizygous for MON 87419 T-DNA I were crossed with the recurrent parent to produce the BC_2F_1 plants. The BC_2F_1 plants were assessed using a glufosinate spray treatment to select for plants containing the MON 87419 T-DNA I. The surviving BC_2F_1 plants were self-pollinated to produce the BC_2F_2 plants.

The inheritance of the MON 87419 T-DNA I was assessed in the BC_1F_1, BC_2F_1, and BC_2F_2 generations. At the BC_1F_1 and BC_2F_1 generations, the MON 87419 T-DNA I was predicted to segregate at a 1:1 ratio (hemizygous positive: homozygous negative) according to Mendelian inheritance principles. At the BC_2F_2 generation, the MON 87419 T-DNA I was predicted to segregate at a 1:2:1 ratio (homozygous positive: 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 MON 87419 T-DNA I coding sequence to the expected ratios.

The Chi square was calculated as:

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

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% ($\alpha = 0.05$).

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

The results of the χ^2 analysis of the segregating progeny of MON 87419 are presented in Table IV-4. The χ^2 value in the BC_1F_1 and BC_2F_1 generations indicated no statistically significant difference between the observed and expected 1:1 segregation ratio (hemizygous positive: homozygous negative) of MON 87419 T-DNA I. The χ^2 value in the BC_2F_2 generation indicated no statistically significant difference between the observed and expected 1:2:1 ratio (homozygous positive: hemizygous positive: homozygous negative) of MON 87419 T-DNA I. These results support the conclusion that the MON 87419 T-DNA I 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 87419 contains a single intact copy of the *dmo* expression cassette and *pat* expression cassette inserted at a single locus in the maize genome.

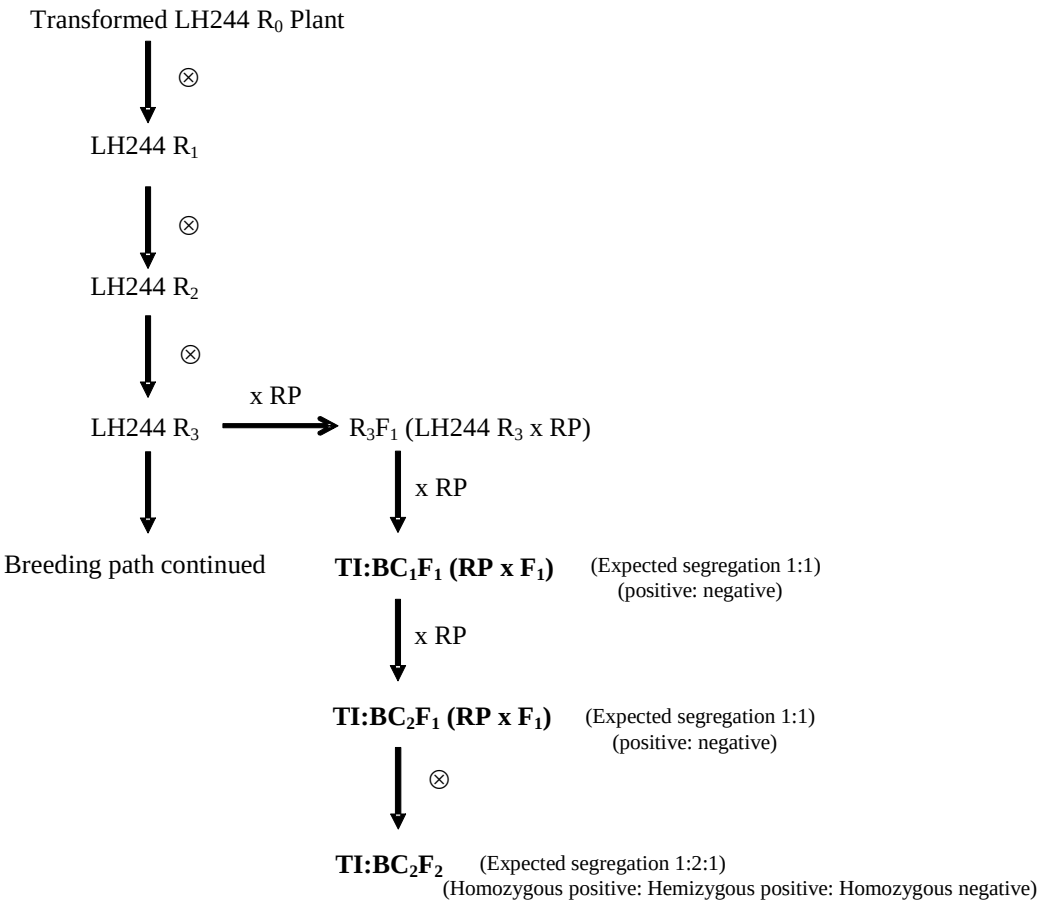

Figure IV-7. Breeding Path for Generating Segregation Data for MON 87419
Chi-square analysis was conducted on segregation data from BC_1F_1, BC_2F_1, and BC_2F_2 generations (bolded text).
TI: Trait Integration: Replacement of genetic background of MON 87419 by recurrent background except inserted gene.
RP: Recurring parent.
BC: Back-Cross.
⊗: Self-Pollinated.

Table IV-4. Segregation of the Expression Cassette During the Development of MON 87419

				1:1 Segregation			
Generation	**Number of Plants**	**Observed Positives**	**Observed Negatives**	**Expected Positives**	**Expected Negatives**	**χ^2**	**Probability**
BC_1F_1	126	64	62	63	63	0.03	0.859
BC_2F_1	381	192	189	190.5	190.5	0.02	0.878

					1:2:1 Segregation				
Generation	**Number of Plants**	**Observed Homozygous Positives**	**Observed Hemizygous Positives**	**Observed Homozygous Negatives**	**Expected Homozygous Positives**	**Expected Hemizygous Positives**	**Expected Homozygous Negatives**	**χ^2**	**Probability**
BC_2F_2	164	48	83	33	41	82	41	2.77	0.251

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

Molecular characterization of MON 87419 by NGS/JSA and directed sequencing demonstrated that a single copy of the intended transfer DNA I (T-DNA I) containing the *dmo* and the *pat* expression cassettes from PV-ZMHT507801 was integrated into the maize genome at a single locus. These analyses also showed no PV-ZMHT507801 backbone elements or T-DNA II sequences were present in the event.

Directed sequence analyses performed on MON 87419 confirmed the organization and intactness of the full T-DNA I 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 *dmo* expression cassette or the *pat* expression cassette. Analysis of the T-DNA I insertion site in maize showed that the 5′ and 3′ genomic DNA flanking the T-DNA I insert in MON 87419 are identical to the conventional control, except for a 602 base pair deletion of genomic DNA at the insertion site in MON 87419.

Generational stability analysis by NGS/JSA demonstrated that the T-DNA I in MON 87419 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 breeding generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA I in MON 87419 at a single chromosomal locus.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE MON 87419 DMO and PAT PROTEINS PRODUCED IN MON 87419

Characterization of the introduced protein(s) in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. As described in Section IV, MON 87419 contains *dmo* and *pat* expression cassettes that, when transcribed and translated, result in the expression of the MON 87419 DMO and PAT proteins, respectively.

PAT protein, encoded by both *pat* and *bar* genes, has been previously characterized and assessed for allergenicity and toxicity (Hérouet et al. 2005; ILSI-CERA 2011) demonstrating no sequence homology with any known allergens or toxins which could have adverse effects to human or animal health. PAT protein expressed in MON 87419 is identical to PAT protein predicted to be expressed based upon the *pat* gene sequence, except for the lead methionine that is removed during a co-translational process. The PAT protein encoded by *pat* gene is expressed in several commercially available glufosinate tolerant maize products including T25, TC1507 and DAS-59122-7 and has an extensive history of safe use (Hérouet et al. 2005; ILSI-CERA 2011). Therefore, based on the protein characteristics and robust history of safe use with PAT, characterization, equivalence, allergenicity and toxicity will not be discussed in detail here.

The DMO proteins produced in MON 87419 maintain a very high level of homology to the DMO proteins previously assessed and deregulated by USDA-APHIS (MON 87708, USDA-APHIS Petition #10-188-01p and MON 88701, USDA-APHIS Petition #12-185-01p). DMO proteins in both MON 87708 and MON 88701 also completed consultation with U.S. FDA (BNF 000125 and BNF 000135), which determined that food and feed derived from these events are not materially different than conventional crops. Alternate N-terminal processing, common in plants, resulted in two forms of DMO produced in MON 87419. Both forms are discussed herein; but, are not anticipated to pose a plant risk potential as the minor differences in the amino acid sequences are not expected to have an effect on structure of the catalytic site, functional activity, immunoreactivity or specificity because the N-terminus is sterically distant from the catalytic site (D'Ordine et al. 2009; Dumitru et al. 2009).

This section summarizes: 1) the identity and function of the DMO and PAT proteins produced in MON 87419; 2) assessment of equivalence between the plant-produced and *E. coli*-produced DMO proteins; 3) the level of the DMO and PAT proteins in plant tissues from MON 87419; 4) assessment of the potential allergenicity of the DMO protein produced in MON 87419; and 5) the food and feed safety assessment of the DMO and PAT proteins produced in MON 87419. The data are consistent with prior safety assessment of these proteins and support a conclusion that the proteins produced in MON 87419 are safe for human or animal consumption based on several lines of evidence summarized below.

V.A. Identity and Function of the DMO and PAT Proteins from MON 87419

V.A.1. Identity and Function of the DMO Protein from MON 87419

MON 87419 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide. DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound 3,6 dichlorosalicylic acid (DCSA) and formaldehyde (Chakraborty et al. 2005). DCSA is a known metabolite of dicamba in cotton, soybean, soil, and livestock, whose safety has been evaluated by the FAO-WHO and EPA (FAO-WHO 2011b; a; U.S. EPA 2009). The other reaction product, formaldehyde, is found naturally in many plants at levels up to several hundred ppm (Adrian-Romero et al. 1999). An assessment of the safety and potential effects of the DMO reaction products is provided in Appendix C.1.

MON 87419 DMO is targeted to chloroplasts by CTP to allow co-localization with the endogenous reductase and ferredoxin enzymes that supply electrons for the DMO demethylation reaction as described by Behrens et al. (2007). In the construction of the plasmid vector used in the development of MON 87419, PV-ZMHT507801, a chloroplast transit peptide coding sequence from *Petunia hybrida* EPSPS (*CTP4*, Table IV-1) was joined to the *dmo* coding sequence; this coding sequence results in the production of a precursor protein consisting of the DMO protein and a N-terminal 72 amino acid CTP, which is utilized to target the precursor protein to the chloroplast (Herrmann 1995; Klee et al. 1987). Typically, transit peptides are precisely removed from the precursor protein following delivery to the targeted plastid (Della-Cioppa et al. 1986) resulting in the full length protein. However, there are examples in the literature of alternatively processed forms of a protein targeted to a plant's chloroplast (Behrens et al. 2007; Clark and Lamppa 1992). Such alternative processing is observed with the DMO precursor protein produced in MON 87419.

Two forms of DMO have been identified in MON 87419, herein referred to as MON 87419 DMO+12 and MON 87419 DMO+7. MON 87419 DMO+7 does not contain the first five amino acids of MON 87419 DMO+12. The amino acid differences between these two forms occur at the N-terminus, which are derived from *CTP4*. The difference in molecular weight between these two forms is small and results in only one single band observable by Coomassie stain of SDS-PAGE and western blot analyses, which were utilized to characterize the protein. Because the amino acid residues present in MON 87419 DMO+7 are also present in MON 87419 DMO+12 and both forms of DMO protein are indistinguishable by Coomassie stain of SDS-PAGE and western blot analysis, and functional activity assay, MON 87419 DMO+12 and MON 87419 DMO+7 will be referred to as MON 87419 DMO in this petition, except where stated. Additionally, all characterization and protein safety assessments have been conducted assuming the *E. coli*-produced MON 87419 DMO+12 as the test substance. MON 87419 DMO protein had an apparent molecular weight of ~39.5 kDa. Except for the amino acids derived from the CTP4 (+7 or +12) and an additional leucine at position two, the MON 87419 DMO protein has an identical sequence to the wild-type DMO protein from the DI-6 strain of *S. maltophilia* (Herman et al. 2005). The differences in the amino acid

sequence between the wild-type DMO protein and MON 87419 DMO protein are not anticipated to have an effect on structure of the catalytic site, functional activity, immunoreactivity, or specificity because the N-terminus and position two are sterically distant from the catalytic site (D'Ordine et al. 2009; Dumitru et al. 2009). MON 87419 DMO is also identical in structure of the catalytic site, functional activity, immunoreactivity and specificity to DMO proteins previously assessed and deregulated by USDA-APHIS (MON 87708, USDA-APHIS Petition #10-188-01p and MON 88701, USDA-APHIS Petition #12-185-01p). DMO proteins in both MON 87708 and MON 88701 also completed consultation with U.S. FDA (BNF 000125 and BNF 000135), which determined that food and feed products from MON 87708 and MON 88701 are as safe as food and feed from soybean and cotton currently available on the market.

V.A.2. Identity and Function of the PAT Protein

Phosphinothricin N-acetyltransferase (PAT) proteins have been isolated from two separate species of *Streptomyces*, *S. hygroscopicus* (Thompson et al. 1987) and *S. viridochromogenes* (Wohlleben et al. 1988). The PAT protein isolated from *S. hygroscopicus* is encoded by the *bar* gene, and the PAT protein isolated from *S. viridochromogenes* is encoded by the *pat* gene. These PAT proteins are made up of 183 amino acids with 85% identity at the amino acid level (Wohlleben et al. 1988). Based on previous studies (Wehrmann et al. 1996) that have extensively characterized PAT proteins produced from *bar* and *pat* genes, OECD recognizes both proteins to be equivalent with regard to function and safety (OECD 1999). The PAT protein produced in MON 87419 is from the *pat* gene and is identical to the wild type PAT protein encoded by *S. viridochromogenes*, except that the first methionine of the MON 87419 PAT protein is cleaved during co-translational process, which results in a single polypeptide of 182 amino acids that has an apparent molecular weight of ~25.2 kDa protein. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinnel and Giglione 2008). The PAT protein in MON 87419 also shares very high level of amino acid identity with PAT protein expressed in several commercially available glufosinate tolerant maize products including T25, TC1507 and DAS-59122-7 and therefore has an extensive history of safe use (Hérouet et al. 2005; ILSI-CERA 2011). Thus, the mode-of-action for PAT protein has been extensively assessed, as numerous glufosinate-tolerant products including maize, canola, soy, sugar beet, rice and cotton have been reviewed by the FDA (U.S. FDA 1995b; a; 1996; 1997; 1998b; a; 1999; 2002) and many other regulatory agencies (ILSI-CERA 2011; OECD 1999; 2002c).

PAT, including the PAT protein that is produced in MON 87419, is an enzyme classified as an acetyltransferase which acetylates glufosinate to produce non-herbicidal N-acetyl glufosinate. The PAT proteins are highly specific for glufosinate in the presence of acetyl CoA (Thompson et al. 1987; Wehrmann et al. 1996). Glufosinate is a racemic mixture of the D- and L-forms of phosphinothricin. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthetase (OECD 1999; 2002c). Other L-amino acids are unable to be acetylated by PAT protein and competition assays containing glufosinate, high concentrations of other amino acids and PAT showed no inhibition of glufosinate acetylation (Wehrmann et al. 1996). Furthermore, the presence of L-glutamate, an analogue of glufosinate, also showed no inhibition of

glufosinate acetylation in competition assays (Wehrmann et al. 1996). Thus, the PAT protein has high substrate specificity for L-phosphinothricin, the herbicidal component of glufosinate, and it has been shown in other PAT-expressing maize products (e.g., T25, TC1507 and DAS-59122-7) that PAT does not affect maize metabolism.

V.B. Characterization and Equivalence of MON 87419 DMO Protein from MON 87419

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties of the protein(s) produced from the inserted DNA, and confirmation of the safety of the protein(s). For safety data generated using *E. coli*-produced protein(s) to be applied to plant-produced protein(s), the equivalence of the plant- and *E. coli*-produced proteins must be assessed. The physicochemical and functional characteristics of the MON 87419-produced DMO protein was determined and it was shown to be equivalent to its *E. coli*-produced MON 87419 DMO protein. A summary of the analytical results for the protein is shown below and the details of the materials, methods, and results are described in Appendix C.

The MON 87419-produced DMO protein purified from grain of MON 87419 was characterized and the equivalence of the physicochemical and functional properties between the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins was established using a panel of analytical tests: 1) N-terminal sequence analysis of MON 87419-produced DMO determined the expected N-terminal sequence; 2) MALDI TOF MS analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 87419-produced DMO sequence; 3) western blot analysis with an antibody specific for DMO protein demonstrated that the immunoreactive properties of the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO were equivalent; 4) SDS-PAGE analysis showed that the electrophoretic mobility and apparent molecular weight of the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins were equivalent; 5) MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins were both determined to be non-glycosylated; and 6) functional activity analysis demonstrated that MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins had equivalent enzyme activity (See Appendix C).

Taken together, these data provide a detailed characterization of the MON 87419-produced DMO protein and establish its equivalence to *E. coli*-produced DMO protein. This equivalence justifies the use of the *E. coli*-produced DMO protein in studies to establish the safety of the DMO protein expressed in MON 87419, summarized in Section V.E.

V.C. Expression Levels of DMO and PAT Proteins in MON 87419

The protein expression levels determined in MON 87419 are used to assess exposure to the introduced proteins via food or feed ingestion and potential environmental exposure. The most appropriate tissues to evaluate DMO and PAT protein levels are leaf, root, forage, and grain tissue samples. Levels of the introduced proteins were determined in

forage and grain tissue to evaluate food and feed exposure in humans and animals, where the levels are utilized to also calculate margins of exposure for each protein. Leaf and root tissues are distinct above and below ground plant tissues that are important to estimate environmental exposure.

MON 87419 DMO and PAT protein levels in various tissues of MON 87419 relevant to the characterization and risk assessment were determined by a validated immunoassay. Tissues of MON 87419 were collected from four replicate plots planted in a randomized complete block field design during the 2013 growing season from the following five field sites in the U.S.: Boone County, Iowa (IAPY), Clinton County, Indiana (INKI), Pawnee County, Kansas (KSLA), York County, Nebraska (NEYO), and Lehigh County, Pennsylvania (PAGR). The field sites were representative of maize-producing regions suitable for commercial production. Leaf, root, forage, and grain tissue samples were collected from each replicated plot at all field sites treated with dicamba and glufosinate.

V.C.1. Expression Levels of MON 87419 DMO Protein

MON 87419 DMO protein levels were determined in all four tissue types. The results obtained from immunoassay are summarized in Table V-1 and the details of the materials and methods are described in Appendix D. The mean DMO protein levels were determined across five sites treated with dicamba and glufosinate. Samples with values determined to be less than the LOD or LOQ were not included in mean determinations. The individual DMO protein levels in MON 87419 across all samples analyzed from all sites ranged from 0.14 to 37 µg/g dw. The mean DMO protein level among all tissue types was highest in leaf at 26 µg/g dw and lowest in grain at 0.19 µg/g dw.

Table V-1. Summary of MON 87419 DMO Protein Levels in Tissues from MON 87419 Grown in 2013 U.S. Field Trials (Treated with Dicamba and Glufosinate)

Tissue Type	Development Stage[1]	Mean (SD) Range (µg/g fw)[2]	Mean (SD) Range (µg/g dw)[3]	LOQ/LOD (µg/g fw)[4]
Leaf	V3	3.7 (0.77) 1.9-5.1	26 (6.6) 13-37	0.157/0.027
Root	V3	0.81 (0.16) 0.58-1.1	7.4 (1.4) 5.0-11	0.125/0.038
Forage	R5	1.8 (0.62) 1.0-3.7	6.0 (2.7) 3.1-14	0.157/0.024
Grain	R6	0.17 (0.044) 0.13-0.29	0.19 (0.048) 0.14-0.31	0.125/0.022

[1] The crop development stage each tissue was collected.
[2] DMO protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (number of sites (n)=20 except grain where n=11 due to nine samples having levels <LOQ).
[3] DMO 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 (dw). The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factor obtained from moisture analysis data.
[4] LOQ=limit of quantitation; LOD=limit of detection.

V.C.2. Expression Levels of PAT Protein

PAT protein levels were determined in all four tissue types. The results obtained from immunoassay are summarized in Table V-2 and the details of the materials and methods are described in Appendix D. The mean PAT protein levels were determined across five sites treated with dicamba and glufosinate. The individual PAT protein levels in MON 87419 across all samples analyzed from all sites ranged from 0.56 to 17 µg/g dw. The mean PAT protein level among all tissue types was highest in leaf at 11 µg/g dw and lowest in grain at 0.93 µg/g dw.

Table V-2. Summary of PAT Protein Levels in Tissues from MON 87419 Grown in 2013 U.S. Field Trials (Treated with Dicamba and Glufosinate)

Tissue Type	Development Stage[1]	Mean (SD) Range (µg/g fw)[2]	Mean (SD) Range (µg/g dw)[3]	LOQ/LOD (µg/g fw)[4]
Leaf	V3	1.5 (0.35) 1.1-2.4	11 (2.7) 7.0-17	0.094/0.043
Root	V3	0.84 (0.18) 0.49-1.3	7.7 (1.3) 4.7-11	0.094/0.037
Forage	R5	1.6 (0.50) 0.92-2.3	5.0 (1.6) 2.8-8.5	0.094/0.014
Grain	R6	0.85 (0.25) 0.50-1.4	0.93 (0.27) 0.56-1.6	0.094/0.007

[1]The crop development stage each tissue was collected.
[2]PAT protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20).
[3]PAT 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 (dw). The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factor obtained from moisture analysis data.
[4]LOQ=limit of quantitation; LOD=limit of detection.

V.D. Assessment of Potential Allergenicity of the DMO 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 shows susceptibility to pepsin and pancreatin treatments.

MON 87419 DMO protein has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) MON 87419 DMO protein originates from *S. maltophilia*, an organism that has not been reported to be a source of known allergens.

2) MON 87419 DMO protein represents no more than 0.00016% of the total protein in the grain that could be consumed from MON 87419 maize.

3) Bioinformatics analyses demonstrated that MON 87419 DMO 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* experiments conducted with the MON 87419 DMO protein demonstrated that the protein is rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin) under physiological conditions, respectively.

Taken together, these data support the conclusion that MON 87419 DMO protein does not pose a significant allergenic risk to humans or animals.

V.E. Safety Assessment of DMO and PAT Proteins in MON 87419

V.E.1. Safety Assessment of DMO Protein in MON 87419

A comprehensive set of factors have been considered in the safety assessment of the DMO protein, which include but are not limited to donor organism safety, DMO protein safety, and DMO protein specificity. The results are summarized below, along with the conclusions reached from each assessment.

V.E.1.1. MON 87419 DMO Donor Organism, History of Safe Use, and Specificity

V.E.1.1.1. The MON 87419 DMO Donor Organism is Safe

The *dmo* gene is derived from the bacterium *S. maltophilia* (Herman et al. 2005; Palleroni and Bradbury 1993). *S. maltophilia* is ubiquitous in the environment and is found associated with the rhizosphere of plants (Berg et al. 1999; Echemendia 2010; Ryan et al. 2009). *S. maltophilia* can be found in a variety of foods and feeds (Echemendia 2010;

Qureshi et al. 2005), and is widespread in the home environment (Denton and Kerr 1998; Denton et al. 1998). Exposure to *S. maltophilia* is incidental to its presence in food. *S. maltophilia* can be found in healthy individuals without causing any harm to human health (Denton and Kerr 1998) and infections caused by *S. maltophilia* are extremely uncommon (Cunha 2009). Additionally, *S. maltophilia* has not been reported to be a source of allergens.

V.E.1.1.2. DMO Protein has been Previously Reviewed

MON 87419 DMO is classified as an oxygenase. Oxygenases are enzymes that incorporate one or two oxygen atoms into substrates and are widely distributed in many universal metabolic pathways (Harayama et al. 1992). DMO protein has been previously reviewed as a part of the safety assessment for USDA-APHIS deregulations of dicamba-tolerant soybean, MON 87708 (USDA-APHIS Petition #10-188-01p), and dicamba and glufosinate-tolerant cotton, MON 88701 (USDA-APHIS Petition #12-185-01p). The DMO protein in MON 87419 is highly homologous to the DMO protein in MON 88701 cotton. The differences on amino acid sequence at the N-terminus are derived from their respective CTPs; in MON 87419 five of the 12 amino acids from CTP4 are identical to those in MON 88701, which are from CTP2. The DMO protein in MON 87419 is also highly homologous to MON 87708 soybean fully processed DMO with an addition of 13 amino acids at the N-terminus (12 amino acids from CTP and one methionine remained from the *dmo* gene) and two amino acid variations at positions 2 and 112 (Appendix C.2.1, Figure C-1). MON 87419 DMO shares sequence identity and many catalytic domain structural similarities with a wide variety of oxygenases present in bacteria and plants currently widely prevalent in the environment and consumed by humans or animals (Ferraro et al. 2005; Schmidt and Shaw 2001), establishing that animals and humans are extensively exposed to these types of enzymes with no adverse effects noted.

V.E.1.1.3. DMO Protein Catalyzes a Specific Enzyme Reaction

DMO converts dicamba to DCSA. This demethylation is very specific to dicamba. As described previously the active form of DMO is a trimer (Chakraborty et al. 2005; Dumitru et al. 2009). For MON 87419 DMO to be functionally active and confer dicamba tolerance to MON 87419, a trimeric structure is required. The activity of MON 87419 DMO was confirmed during characterization (Section V.B and Appendix C.2). The literature indicates the specificity of DMO for dicamba, as with many enzymes, is due to the specific interactions that occur at the catalytic site (D'Ordine et al. 2009; Dumitru et al. 2009). The catalytic site of DMO in MON 87419 is the same as the catalytic site of DMO in wild type because the differences on the amino acid sequence between these two forms of the protein occur at the N-terminus and position two, which are sterically distant from the catalytic site and consequently do not participate in substrate coordination (D'Ordine et al. 2009; Dumitru et al. 2009). Therefore, the amino acid differences between MON 87419 DMO and wild type DMO are not expected to have an effect on DMO activity and specificity. Dicamba interacts with amino acids in the catalytic site of DMO through both the carboxylate moiety and the chlorine atoms of dicamba, which are primarily involved in orienting the substrate in the catalytic site. These chlorine atoms are required for catalysis (D'Ordine et al. 2009;

Dumitru et al. 2009). Potential substrates (o-anisic acid, vanillic acid, syringic acid, ferulic acid and sinapic acid) based on structural similarity to dicamba which are found in plants, were not metabolized by a histidine-tagged *E. coli*-produced DMO. Given the limited existence of chlorinated compounds with structures similar to dicamba in plants and other eukaryotes (Wishart 2010; Wishart et al. 2009), it is unlikely that MON 87419 DMO will catalyze the conversion of endogenous compounds. Therefore, the activity of the enzyme is considered specific for dicamba (Appendix C.3)

V.E.1.2. DMO Protein in MON 87419 is Not Homologous to Known Allergens or Toxins

Bioinformatics analyses were performed to assess the allergenic potential, toxicity, or biological activity of MON 87419 DMO. The analysis demonstrated that MON 87419 DMO protein does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health (Section V.D).

V.E.1.3. DMO Protein in MON 87419 is Susceptible to Degredation in *in vitro* Digestion Assay

The susceptibility to degradation by pepsin and pancreatin of MON 87419-produced DMO was assessed using the *E. coli*-produced MON 87419 DMO, which was shown to be equivalent to the MON 87419-produced DMO (see Appendix C.2.). Results indicate that *E.coli*-produced MON 87419 DMO was readily degraded by pepsin or pancreatin. Rapid degradation of the MON 87419 DMO protein by pepsin or pancreatin makes it highly unlikely that intact or large peptide fragments MON 87419 DMO protein would be absorbed in the small intestine and have any adverse effects on human or animal health.

V.E.1.4. DMO Protein in MON 87419 is Not Acutely Toxic

An acute toxicology study with DMO protein was conducted previously in support of MON 88701 cotton. Results indicate that DMO protein did not cause any adverse effect in mice, with a No Observable Adverse Effect Level (NOAEL) at 283 mg/kg body weight, the highest dose tested, a dose that greatly exceeds anticipated human exposures. The DMO protein from MON 87419 has the same mode of action as the protein used in this acute toxicity assay, originally conducted in support of MON 88701, and the data are therefore applicable to MON 87419.

V.E.1.5. Human and Animal Exposure to the DMO Protein

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 dietary exposure. Since no evidence of mammalian toxicity has been reported for DMO, dietary risk assessment would normally not be considered necessary. In addition to the conservative exposure assumptions above, exposure to DMO is also overestimated because 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 DMO protein in MON 87419 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 87419. Thus, there are likely to be significantly lower exposures to the functionally active form of this protein through consumption of MON 87419 than the levels estimated above.

Nevertheless, a dietary risk assessment was conducted in order to provide further assurance of safety by calculating a MOE between the NOAEL for the DMO protein in mouse acute oral toxicity study (Section V.E.1.4) 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 2014c). DEEM-FCID utilizes food consumption data from the National Health and Nutrition Examination Survey (NHANES) conducted in 2005-2010. DEEM-FCID separates field maize into fourteen fractions, e.g. flour, meal, bran, starch, oil and syrup. However, corn oil and corn syrup were excluded from this assessment because they are essentially devoid of protein and thus would not contain significant amounts of DMO (Martín-Hernández et al. 2008). Starch was included in the assessment but, because of the very low protein content, any contribution from starch is expected to be minimal. Based on the mean DMO protein level in MON 87419 grain on a fresh weight basis (0.17 µg/g, Section V.C.1, Table V-1), 95th percentile exposure to DMO for the general U.S. population was estimated to be 0.35 µg/kg body weight (bw). For children 1-2 years of age in the U.S., the most highly exposed sub-population, 95th percentile exposure to DMO was estimated to be 0.81 µg/kg bw. The MOE for acute dietary intake of DMO was estimated to be 810,000 for the general U.S. population. The MOE for children 1-2 years of age, the highest exposed sub-population was estimated to be 350,000. Actual MOE will likely be much higher because: 1) the exposure estimates utilized are conservative (95th percentile of consumption, and all dietary sources of maize are presumed to be 100% MON 87419) and 2) DMO is rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin), further minimizing exposures. These very large MOEs indicate that there is no meaningful risk to human health from dietary exposure to the DMO protein produced by MON 87419.

The potential DMO protein exposure to animals from consumption of MON 87419 in feeds was evaluated by calculating an estimate of daily dietary DMO ingestion and relating that value to total daily dietary protein intake. Calculations were made for lactating dairy cows, poultry, and swine. The highest percentage of DMO protein per total protein consumed was estimated to occur in the lactating dairy cow, which was 0.004% (g/g) of the total daily dietary protein intake (0.00026 g of DMO/kg bw divided by 5.9 g/kg bw daily dietary protein for the lactating dairy cow). The percentage of the DMO 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 0.004% (g/g) or less of its total protein as DMO protein from MON 87419 maize.

In summary, there is no significant risk to human and animal health associated with dietary exposure to the DMO protein in food and feed products derived from MON 87419.

V.E.2. Safety Assessment of PAT Protein in MON 87419

The safety of PAT protein has been well established in the scientific literature based on protein safety literature (Hérouet et al. 2005) and GM crop safety literature (He et al. 2008; MacKenzie et al. 2007; Malley et al. 2007; Rhee et al. 2005), and by the tolerance exemption set by the EPA (U.S. EPA 1997). As a result, the safety of PAT protein has been favorably assessed following extensive reviews by regulatory agencies in at least 11 different countries for more than 38 biotechnology-derived events in eight different species (ILSI-CERA 2011). The lack of any documented reports of adverse effects of PAT-containing crops since their introduction in 1995 (Duke 2005) further demonstrates the safety of PAT protein.

PAT protein expressed in MON 87419 is 100% identical to the wild type PAT protein encoded by *S. viridochromogenes* except for the first methionine which is removed during post-translational processing in MON 87419. The PAT protein is expressed in several commercially available glufosinate tolerant soybean, canola and maize products including T25, TC1507 and DAS-59122-7 maize. EPA has issued a tolerance exemption for PAT protein regardless of the encoding gene or crop (U.S. EPA 1997). The safety of PAT proteins present in biotechnology-derived crops has been extensively assessed (Hérouet et al. 2005; ILSI-CERA 2011). Thus, these prior safety assessments for the PAT protein are directly applicable to the PAT protein expressed in MON 87419 and are not detailed further herein.

V.F. MON 87419 DMO and PAT Proteins Characterization and Safety Conclusion

MON 87419 DMO is a common class mono-oxygenase that catalyzes the O-demethylation of the herbicide dicamba and has homologs in bacteria and plants that share many of the typical structural and functional characteristics of these types of oxygenases, while maintaining specificity for its substrate. The physicochemical characteristics of the MON 87419-produced DMO protein were determined and equivalence between MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins was demonstrated. This equivalence justifies the use of the *E. coli*-produced MON 87419 DMO as a test substance in the protein safety studies (heat susceptibility and digestibility). Expression studies using immunoassay demonstrated that MON 87419 DMO was expressed at levels ranging from 0.19 to 26 µg/g dw, representing a low percentage of the total protein. An assessment of the allergenic potential of the MON 87419 DMO protein supports the conclusion that the MON 87419 DMO protein does not pose a significant allergenic risk. In addition, the donor organism for the MON 87419 DMO coding sequence, *S. maltophilia*, is ubiquitous in the environment and is not commonly known for allergenicity and human or animal pathogenicity. The MON 87419 DMO protein lacks structural similarity to allergens, toxins or other proteins known to have adverse effects on mammals. The MON 87419 DMO protein is rapidly digested by proteases found in the human gastrointestinal tract

(pepsin and pancreatin) and demonstrates no acute oral toxicity in mice at the level tested. Based on the above information, the consumption of the MON 87419 DMO proteins from MON 87419 or its progeny is considered safe for humans and animals.

PAT protein is an acetyltransferase that catalyzes the acetylation of the herbicide glufosinate. PAT proteins, including the PAT protein isolated from MON 87419, have been previously characterized, and the safety of crops expressing these proteins has been well established. Taken together, the consumption of the PAT protein from MON 87419 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 87419 or derived from MON 87419 are as safe as maize currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 87419

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. For maize, assessments are performed using the principles and analytes outlined in the OECD consensus document for maize composition (OECD 2002a).

A recent review of compositional assessments conducted according to OECD guidelines, encompassing 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 2002b). 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 control, i.e. a genetically similar conventional line, grown concurrently under similar field conditions, and 2) natural ranges from data published in the scientific literature or documented in the International Life Sciences Institute Crop Composition Database (ILSI-CCDB). The comparison to data published in the literature and the ILSI-CCDB places any potential differences between the assessed new crop variety and its conventional control 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 87419 treated with dicamba and glufosinate compared to that of a conventional control grown and harvested under similar conditions. The analyses of concentrations of key nutrients, anti-nutrients, and secondary metabolites of MON 87419 that was not treated with dicamba and glufosinate are not discussed, but are presented in Appendix E as supplemental information. The production of data for compositional analyses used a sufficient variety of field trial sites, a robust field design, and sensitive analytical methods to allow accurate assessments of compositional characteristics over a range of environmental conditions under which MON 87419 is expected to be grown.

VI.A. Compositional Equivalence of MON 87419 Grain and Forage to Conventional Maize

Grain and forage samples were collected from MON 87419 and a conventional control at five sites grown in the U.S. during 2013. The field sites were planted in a randomized complete block design with four blocks per site. MON 87419 and the conventional control were grown under agronomic field conditions typical for the different growing regions. MON 87419 plots were treated with dicamba and glufosinate to generate samples under conditions of the intended use of the product.

The evaluation of MON 87419 followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD 2002a). Grain samples were analyzed for levels of nutrients including proximates (protein, fat, ash, moisture), amino acids (18 components), fatty acids (22 components), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [A (β-carotene), B1, B2, B6, E (α-tocopherol), niacin, and folic acid]. The anti-nutrients analyzed in grain were phytic acid and raffinose. Secondary metabolites analyzed in grain were furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber (ADF, NDF), and minerals (calcium and phosphorus). In all, 78 different components were analyzed.

Of the 78 measured components, copper, furfural, and 13 fatty acids (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, heptadecanoic, heptadecenoic, gamma linolenic, eicosadienoic, eicosatrienoic, and arachidonic acids) had more than 50% of the observations below the assay limit of quantitation (LOQ) and were excluded from the statistical analyses. Moisture values for grain and forage were measured for conversion of components from fresh to dry weight, but were not statistically analyzed. Therefore, 61 components were statistically analyzed (53 in grain and eight in forage).

The statistical comparison of MON 87419 and the conventional control was based on compositional data combined across all field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). A statistically significant difference between MON 87419 and the conventional control does not necessarily imply biological relevance from a food and feed safety perspective. Therefore, statistically significant differences observed between MON 87419 and the conventional control were evaluated further to determine whether the detected difference indicated a biologically relevant compositional change or supported a conclusion of compositional equivalence, as follows:

Step 1 – Determination of the Magnitude of Difference between Test and Conventional Control Means

The difference in means between MON 87419 and the conventional control was determined for use in subsequent steps. For protein and amino acids only, the relative magnitude of the difference (percent change relative to the control) between MON 87419

and the conventional control was determined to allow an assessment of any observed difference in amino acids in relation to the difference in protein[3].

Step 2 – Assessment of the Difference in the Context of Natural Variation within the Conventional Control across Multiple Sites

The relative impact of MON 87419 was evaluated in the context of variation within the conventional control germplasm grown across multiple sites (i.e., variation due to environmental influence). This assesses the mean difference between MON 87419 and the conventional control in the context of the individual replicate values for the conventional control (maximum value minus the minimum value). When a mean difference is less than the variability seen due to natural environmental variation within the single, closely related germplasm, the difference is typically not a food or feed safety concern (Venkatesh et al. 2014).

Step 3 – Assessment of the Difference in the Context of Natural Variation Due to Multiple Sources

The relative impact of MON 87419 on composition was evaluated in the context of sources of natural variation such as environmental and germplasm influences. This assessment determined whether the mean value of MON 87419 was within the natural variability defined by the literature values or the ILSI Crop Composition Database (ILSI-CCDB) values. This naturally occurring variability is important in assessing the biological relevance of statistically significant differences in composition between MON 87419 and the conventional control.

These evaluations of natural variation are important as crop composition is known to be greatly influenced by environment and variety (Harrigan et al. 2010). Although used in the comparative assessment process, detection of statistically significant differences between MON 87419 and the conventional control mean values does not imply a meaningful contribution by MON 87419 to compositional variability. Only if the impact of MON 87419 on levels of components was large relative to natural variation inherent to conventional maize would further assessments be required to establish whether the change in composition would have an impact from a food and feed safety and nutritional perspective. The steps reviewed in this assessment, therefore, describe the process for determining whether the differences between MON 87419 and the conventional control are meaningful from a food and feed perspective or whether they support a conclusion of compositional equivalence.

The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients, and secondary metabolites in maize grain and

[3] Since total amino acids measured in a seed analysis are predominately derived from hydrolysis of protein, a change in protein levels would likely result in corresponding changes in amino acids levels. For this reason, the relative magnitudes of difference (percent change relative to the control) for amino acids and protein were determined to allow an assessment of the difference in amino acids in relation to a difference in protein. When the relative magnitudes of difference for amino acids are related to the relative magnitude of difference for protein, then steps 2 and 3 are not discussed for amino acids.

forage of MON 87419 and the conventional control (Tables VI-1 – VI-7). Of the 61 components statistically assessed, there were no significant differences in 60 components. Only one component (manganese in grain) showed a significant difference ($p<0.05$) between MON 87419 and the conventional control.

For manganese, the mean value was 6.03 mg/kg dw for MON 87419 and 5.51 mg/kg dw for the conventional control, a difference of 0.52 mg/kg dw (Table VI-4) (Step 1). This difference was evaluated in the context of the conventional control range value, 2.91 mg/kg dw, calculated from the minimum (4.50 mg/kg dw) and maximum (7.41 mg/kg dw) manganese values. The mean difference in manganese values between MON 87419 and the conventional control was less than the range value of the conventional control, indicating that MON 87419 does not impact levels of manganese more than natural variation within the conventional control grown at multiple locations (Step 2). Additionally, the MON 87419 mean manganese value was also within the range of values observed in the literature and the ILSI-CCDB (Table VI-8) (Step 3).

These results support the overall conclusion that MON 87419 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 to the conventional control in levels of these components. These data indicated that the statistically significant difference observed was not compositionally meaningful from a food and feed safety perspective.

Table VI-1. Summary of Maize Grain Protein and Amino Acids for MON 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value	% Relative[4]
Protein	11.52 (0.55) 9.14 - 14.60	11.07 (0.55) 9.22 - 14.04	4.82	0.45 (0.26)	0.120	4.10
Alanine	0.92 (0.057) 0.68 - 1.23	0.88 (0.057) 0.71 - 1.17	0.46	0.042 (0.026)	0.151	4.75
Arginine	0.46 (0.014) 0.38 - 0.55	0.45 (0.014) 0.39 - 0.52	0.13	0.010 (0.0076)	0.178	2.33
Aspartic Acid	0.73 (0.036) 0.58 - 0.94	0.70 (0.036) 0.59 - 0.88	0.30	0.029 (0.017)	0.127	4.21
Cystine/Cysteine	0.22 (0.0050) 0.18 - 0.26	0.22 (0.0050) 0.18 - 0.26	0.08	0.0013 (0.0043)	0.767	0.59
Glutamic Acid	2.43 (0.15) 1.80 - 3.26	2.32 (0.15) 1.88 - 3.12	1.24	0.11 (0.070)	0.160	4.69

Table VI-1 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value	% Relative[4]
Glycine	0.41 (0.011) 0.36 - 0.47	0.40 (0.011) 0.36 - 0.45	0.09	0.0095 (0.0060)	0.118	2.36
Histidine	0.33 (0.011) 0.27 - 0.40	0.32 (0.011) 0.28 - 0.37	0.09	0.0090 (0.0056)	0.114	2.81
Isoleucine	0.42 (0.024) 0.32 - 0.56	0.40 (0.024) 0.33 - 0.53	0.20	0.018 (0.011)	0.123	4.49
Leucine	1.59 (0.11) 1.15 - 2.18	1.51 (0.11) 1.20 - 2.08	0.89	0.078 (0.048)	0.144	5.18
Lysine	0.28 (0.0061) 0.25 - 0.33	0.28 (0.0061) 0.24 - 0.32	0.07	0.0043 (0.0054)	0.431	1.54
Methionine	0.23 (0.0074) 0.19 - 0.27	0.23 (0.0074) 0.18 - 0.28	0.10	0.0015 (0.0054)	0.788	0.64
Phenylalanine	0.63 (0.040) 0.47 - 0.87	0.61 (0.040) 0.48 - 0.79	0.31	0.028 (0.018)	0.161	4.61

Table VI-1 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value	% Relative[4]
Proline	1.08 (0.044) 0.87 - 1.33	1.04 (0.044) 0.89 - 1.27	0.37	0.041 (0.021)	0.084	3.94
Serine	0.59 (0.032) 0.47 - 0.77	0.57 (0.032) 0.48 - 0.72	0.25	0.023 (0.014)	0.108	4.01
Threonine	0.42 (0.018) 0.34 - 0.53	0.41 (0.018) 0.35 - 0.50	0.15	0.014 (0.0078)	0.074	3.51
Tryptophan	0.070 (0.0017) 0.058 - 0.083	0.069 (0.0017) 0.055 - 0.083	0.03	0.0011 (0.0018)	0.537	1.66
Tyrosine	0.31 (0.018) 0.22 - 0.41	0.30 (0.018) 0.24 - 0.39	0.15	0.0076 (0.0095)	0.429	2.53
Valine	0.54 (0.025) 0.43 - 0.69	0.52 (0.025) 0.44 - 0.65	0.22	0.020 (0.011)	0.077	3.87

[1]dw = dry weight.

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

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

[4]The relative magnitude of the difference in mean values between MON 87419 (Treated) and the control, expressed as a percent of the control.

Table VI-2. Summary of Maize Grain Total Fat and Fatty Acids for MON 87419 (Treated) and Conventional Control

Component	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Total Fat (% dw)[1]	3.40 (0.081) 2.89 - 3.81	3.49 (0.081) 2.80 - 3.98	1.18	-0.093 (0.084)	0.297
16:0 Palmitic[4]	14.51 (0.12) 13.62 - 15.25	14.51 (0.12) 13.80 - 15.56	1.77	0.0065 (0.14)	0.963
16:1 Palmitoleic	0.12 (0.0040) 0.097 - 0.13	0.12 (0.0040) 0.095 - 0.14	0.05	-0.0023 (0.0022)	0.318
18:0 Stearic	1.62 (0.028) 1.45 - 1.77	1.64 (0.028) 1.46 - 1.84	0.37	-0.021 (0.023)	0.366
18:1 Oleic	21.86 (0.20) 20.52 - 23.24	22.37 (0.20) 20.83 - 24.72	3.89	-0.50 (0.28)	0.078
18:2 Linoleic	60.08 (0.27) 58.17 - 62.44	59.52 (0.27) 57.68 - 61.91	4.23	0.56 (0.38)	0.150

Table VI-2 (continued). Summary of Maize Grain Total Fat and Fatty Acids for MON 87419 (Treated) and Conventional Control

Component	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
18:3 Linolenic	1.00 (0.027) 0.83 - 1.18	1.02 (0.027) 0.84 - 1.16	0.32	-0.021 (0.024)	0.397
20:0 Arachidic	0.40 (0.0079) 0.35 - 0.43	0.41 (0.0079) 0.37 - 0.45	0.08	-0.0072 (0.0057)	0.211
20:1 Eicosenoic	0.27 (0.0049) 0.24 - 0.29	0.27 (0.0049) 0.25 - 0.33	0.08	-0.0056 (0.0063)	0.381
22:0 Behenic	0.14 (0.0070) 0.065 - 0.17	0.15 (0.0070) 0.061 - 0.18	0.11	-0.0021 (0.0074)	0.781

[1]dw = dry weight.

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

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

[4]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, 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 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Carbohydrates by Calculation	83.57 (0.54) 80.87 - 86.28	84.04 (0.54) 81.36 - 85.93	4.57	-0.47 (0.36)	0.231
Acid Detergent Fiber	3.97 (0.12) 3.42 - 5.13	4.04 (0.12) 3.20 - 5.20	2.00	-0.068 (0.13)	0.608
Neutral Detergent Fiber	9.70 (0.11) 9.14 - 10.53	9.42 (0.11) 8.98 - 10.01	1.03	0.28 (0.15)	0.099
Total Dietary Fiber	9.18 (0.23) 7.15 - 11.78	8.97 (0.23) 7.21 - 10.64	3.43	0.21 (0.31)	0.514

[1]dw = dry weight.

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

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

Table VI-4. Summary of Maize Grain Ash and Minerals for MON 87419 (Treated) and Conventional Control

Component	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Ash (% dw)[1]	1.39 (0.021) 1.27 - 1.48	1.38 (0.021) 1.30 - 1.51	0.21	0.0066 (0.016)	0.686
Calcium (% dw)	0.0031 (0.00017) 0.0020 - 0.0042	0.0029 (0.00017) 0.0022 - 0.0054	0.003	0.00016 (0.00019)	0.427
Iron (mg/kg dw)	16.83 (0.54) 13.02 - 21.56	16.57 (0.55) 13.39 - 18.71	5.31	0.27 (0.43)	0.536
Magnesium (% dw)	0.13 (0.0019) 0.12 - 0.15	0.12 (0.0019) 0.086 - 0.14	0.05	0.0045 (0.0026)	0.092
Manganese (mg/kg dw)	6.03 (0.45) 4.81 - 8.72	5.51 (0.45) 4.50 - 7.41	2.91	0.52 (0.18)	0.019
Phosphorus (% dw)	0.36 (0.0059) 0.32 - 0.40	0.35 (0.0059) 0.25 - 0.40	0.15	0.0098 (0.0077)	0.204

Table VI-4 (continued). Summary of Maize Grain Ash and Minerals for MON 87419 (Treated) and Conventional Control

Component	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	Difference (MON 87419 minus Control) p-Value
Potassium (% dw)	0.36 (0.0081) 0.32 - 0.41	0.36 (0.0081) 0.33 - 0.40	0.07	0.0012 (0.0048)	0.802
Sodium (mg/kg dw)	5.45 (1.92) 0.36 - 24.28	5.63 (1.92) 0.36 - 35.05	34.69	-0.18 (2.65)	0.945
Zinc (mg/kg dw)	22.10 (1.13) 17.21 - 29.83	21.18 (1.13) 16.40 - 26.70	10.30	0.93 (0.67)	0.175

[1]dw = dry weight.

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

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

Table VI-5. Summary of Maize Grain Vitamins for MON 87419 (Treated) and Conventional Control

Component (mg/kg dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Folic Acid	0.65 (0.035) 0.41 - 1.03	0.66 (0.035) 0.48 - 0.89	0.41	-0.0063 (0.035)	0.859
Niacin	10.22 (0.41) 8.06 - 12.18	10.20 (0.41) 8.23 - 11.97	3.74	0.028 (0.46)	0.952
Vitamin A	5.44 (0.45) 3.67 - 11.11	5.47 (0.45) 3.66 - 8.19	4.53	-0.030 (0.48)	0.950
Vitamin B1	2.46 (0.12) 1.94 - 3.25	2.48 (0.12) 1.80 - 3.34	1.54	-0.018 (0.095)	0.850
Vitamin B2	2.18 (0.13) 1.52 - 3.47	2.16 (0.13) 1.54 - 3.43	1.89	0.018 (0.18)	0.917
Vitamin B6	5.42 (0.22) 3.45 - 6.62	5.43 (0.22) 2.82 - 7.61	4.79	-0.016 (0.32)	0.959

Table VI-5 (continued). Summary of Maize Grain Vitamins for MON 87419 (Treated) and Conventional Control

Component (mg/kg dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Vitamin E	11.56 (0.43) 9.28 - 13.37	11.07 (0.43) 8.65 - 12.76	4.11	0.49 (0.28)	0.085

[1]dw = dry weight.

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

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

Table VI-6. Summary of Maize Grain Anti-nutrients and Secondary Metabolites for MON 87419 (Treated) and Conventional Control

Component	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	Difference (MON 87419 minus Control) p-Value
Anti-nutrients (% dw[1])					
Phytic Acid	0.99 (0.031) 0.80 - 1.20	0.93 (0.031) 0.71 - 1.37	0.65	0.065 (0.038)	0.087
Raffinose	0.28 (0.010) 0.23 - 0.34	0.28 (0.010) 0.24 - 0.35	0.11	0.0039 (0.0070)	0.591
Secondary Metabolites (µg/g dw)					
Ferulic Acid	2352.80 (45.66) 2165.31 - 2652.33	2289.17 (45.66) 1882.22 - 2508.79	626.58	63.63 (37.49)	0.097
p-Coumaric Acid	196.51 (12.40) 149.01 - 282.91	187.70 (12.40) 132.56 - 254.88	122.32	8.81 (6.67)	0.194

[1]dw = dry weight.

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

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

Table VI-7. Summary of Maize Forage Proximates, Fiber and Minerals for MON 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Ash	3.86 (0.54) 2.28 - 5.34	3.89 (0.54) 2.27 - 5.70	3.43	-0.029 (0.10)	0.778
Carbohydrates by Calculation	87.12 (0.85) 83.54 - 89.84	87.15 (0.85) 83.47 - 90.85	7.38	-0.024 (0.29)	0.935
Protein	7.40 (0.36) 5.54 - 9.32	7.27 (0.36) 5.43 - 8.78	3.35	0.12 (0.19)	0.521
Total Fat	1.59 (0.17) 0.49 - 2.73	1.68 (0.17) 0.66 - 3.84	3.18	-0.091 (0.21)	0.664
Acid Detergent Fiber	26.52 (1.15) 20.80 - 33.33	26.72 (1.15) 20.79 - 40.90	20.10	-0.19 (1.13)	0.865
Neutral Detergent Fiber	41.28 (1.40) 36.10 - 56.57	41.16 (1.40) 32.32 - 47.52	15.20	0.12 (1.10)	0.917

Table VI-7 (continued). Summary of Maize Forage Proximates, Fiber, and Minerals for MON 87419 (Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (MON 87419 minus Control) Mean (S.E.)	p-Value
Calcium	0.21 (0.021) 0.12 - 0.28	0.22 (0.021) 0.13 - 0.33	0.20	-0.014 (0.012)	0.267
Phosphorus	0.20 (0.018) 0.093 - 0.37	0.21 (0.018) 0.13 - 0.32	0.18	-0.013 (0.015)	0.389

[1]dw = dry weight.

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

[3]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
16:1 Palmitoleic	0.059 – 0.23[a]	0.095 – 0.447
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)	0.0036 – 0.0068[a]; 0.0035 – 0.0070[b]	0.00127 – 0.02084
Copper (mg/kg dwt)	0.85-3.54[c]	0.73 – 18.50
Iron (mg/kg dwt)	14.17 – 23.40[a]; 15.90 – 24.66[b]	10.42 – 49.07
Magnesium (% dwt)	0.091 – 0.14[a]; 0.10 – 0.14[b]	0.0594 – 0.194
Manganese (mg/kg dwt)	4.83 – 8.34[a]; 4.78 – 9.35[b]	1.69 – 14.30
Phosphorus (% dwt)	0.24 – 0.37[a]; 0.27 – 0.38[b]	0.147 – 0.533
Potassium (% dwt)	0.29 – 0.39[a]; 0.36 – 0.43[b]	0.181 – 0.603
Sodium (mg/kg dwt)	ND	0.17 – 731.54
Zinc (mg/kg dwt)	16.78 – 28.17[a]; 18.25 – 30.44[b]	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]	0.14 – 11.27[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
Phosphorus	0.090 – 0.26[a]; 0.13 – 0.21[b]	0.09362 – 0.37041

[1]dw=dry weight; FA = fatty acids; ND = not detected.

[2]Literature range references: [a]U.S. and [b]Chile (Harrigan et al. 2009), [c](Ridley et al. 2011), [d](Egesel et al. 2003).

[3]ILSI range is from ILSI Crop Composition Database, 2011 [Accessed 9 May 2014] (ILSI 2011).

VI.B. Compositional Assessment of MON 87419 Conclusion

Compositional analysis was conducted on grain and forage of MON 87419 treated with dicamba and glufosinate and a conventional control grown at five sites in the U.S. during 2013. Of the 61 components statistically assessed, 60 showed no significant differences between MON 87419 and the conventional control. One component (manganese in grain) showed a significant difference between MON 87419 and the conventional control. For this one component, the mean difference in the component values between MON 87419 and the conventional control was less than the range value of the conventional control. The MON 87419 mean component value was also within the range of values observed in the literature and the ILSI-CCDB. These data indicated that the statistically significant difference for manganese in grain was not compositionally meaningful from a food and feed safety perspective.

These results support the overall conclusion that MON 87419 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 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 87419 compared to the conventional control. A subset of characteristics (field phenotypic measurements) were also evaluated for MON 87419 treated with dicamba and glufosinate to assess MON 87419 under an agronomic system that included application of these herbicides. The data support a conclusion that MON 87419 is not meaningfully different in plant pest risk from the conventional control. These conclusions are based on the results of multiple evaluations from laboratory and field assessments.

Phenotypic, agronomic, and environmental interaction characteristics of MON 87419 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 87419 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 87419, 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 including 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 87419, data were collected to provide a detailed description of the phenotypic, agronomic, and environmental interaction characteristics of MON 87419. 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 87419 encompassed five 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. 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 87419 not treated and/or treated with dicamba and glufosinate was compared to an appropriate conventional control that

had a genetic background similar to MON 87419 but did not possess the dicamba and glufosinate-tolerance trait. In addition, multiple commercial maize reference hybrids developed through conventional breeding and selection (see Appendices F-H and Tables F-1, G-1, G-2, and H-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 provide 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	Characteristic measured[1] (associated section where discussed)	Evaluation timing[2] (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 and 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[3])
	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[3])
	Early stand count (VII.C.2.1)	V2 – V5 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 growth stage (Field)	Rated on a 1-9 scale, where 1 = excellent vigor and 9 = poor vigor
	Stay green (VII.C.2.1)	Maturity (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 (Field)	Distance from the soil surface at the base of the plant to the primary ear attachment node on five plants per plot
	Plant height (VII.C.2.1)	R1 – Maturity (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	Characteristic measured[1] (associated section where discussed)	Evaluation timing[2] (Setting of evaluation)	Evaluation description (measurement endpoints)
Reproductive growth	Days to 50% pollen shed (VII.C.2.1)	Pollen shed (Field)	Days from planting until 50% of the plants have begun to shed pollen
	Days to 50% silking (VII.C.2.1)	Silking (Field)	Days from planting until 50% of the plants have multiple silks exposed
	Pollen viability (VII.C.3)	Pollen shed (Laboratory)	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen diameter (VII.C.3)	Pollen shed (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 (kilograms/hectoliter) 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)	An additional qualitative assessment of 5 plants per plot, with categorical scale of increasing severity (none, slight, moderate, severe)
	Ear/kernel rot disease (VII.C.2.2.1)	Harvest (Field)	An additional qualitative assessment of 5 plants per 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

Data category	Characteristic measured[1] (associated section where discussed)	Evaluation timing[2] (Setting of evaluation)	Evaluation description (measurement endpoints)
Environmental interactions	Corn earworm damage (VII.C.2.2.2)	R5 to onset of R6 growth stage (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 (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 per method during growing season (Field)	Quantitative assessment of arthropod abundance via sticky trap collections and visual counts

[1] All characteristics were measured for MON 87419 not treated with dicamba and glufosinate. With the exception of environmental interactions, characteristics evaluated in field settings were also measured for MON 87419 treated with dicamba and glufosinate.

[2] Plant growth stages followed the descriptions in Abendroth et al. (2011).

[3] 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 87419 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 87419 compared to a 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 87419 could be expected to pose an increased plant pest risk compared to conventional maize.

VII.B.1. Interpretation of Detected Differences Criteria

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. 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 plant 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.

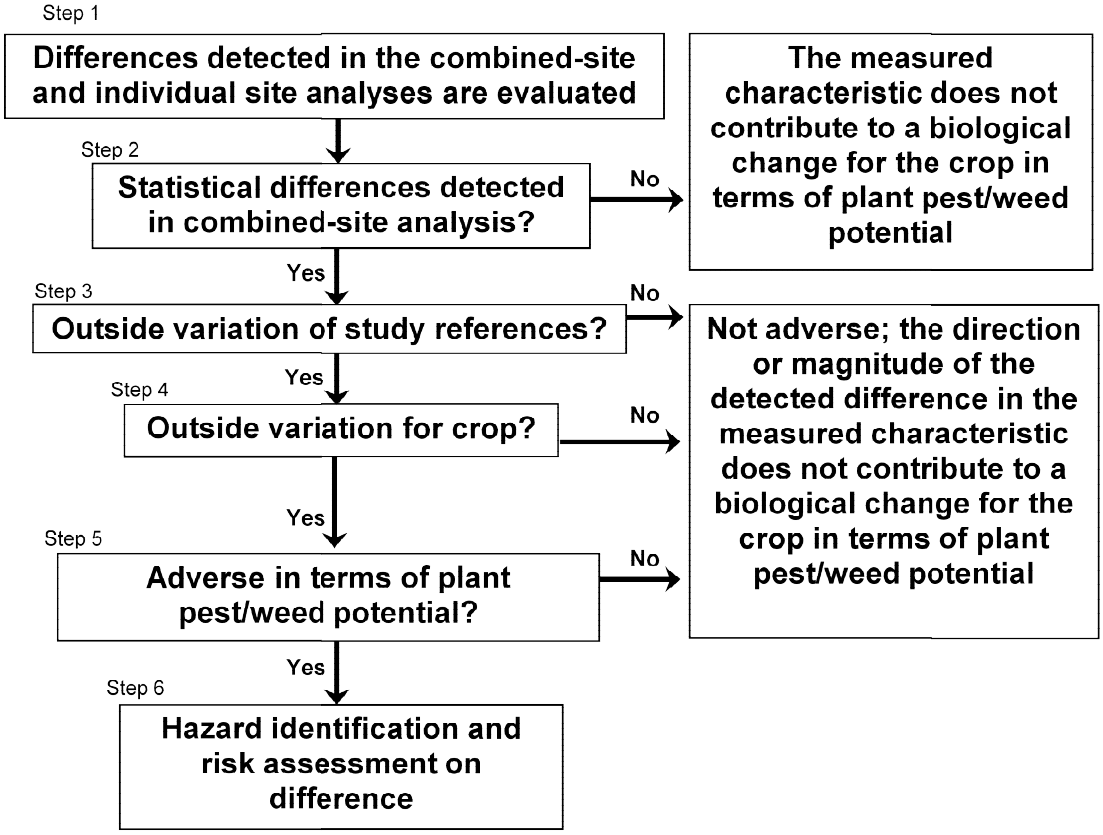

Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

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/weed 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 characteristics for a biotechnology-derived crop is outside the variation of the reference hybrids included 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 Difference to Plant Pest/Weed Potential

If the mean value of the characteristics 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, 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 vigor, the biotechnology-derived crop was considered different from the conventional control if the ranges of vigor did not overlap between the biotechnology-derived crop and the conventional control across all replications. Any observed differences between the biotechnology-derived crop and the conventional control were further assessed in the context of the range of the commercial reference hybrids and for consistency at other sites.

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

Quantitative assessments of corn earworm damage, European corn borer damage, and arthropod abundance were analyzed within each individual site. Statistically significant differences between the biotechnology-derived crop and conventional control were assessed for biological significance in the context of the range of the commercial reference hybrids, and for consistency with other collection methods and/or sites. Differences for which the biotechnology-derived crop mean value was within the reference range or that are not consistently detected in multiple collection methods or environments in which the same arthropod taxa occurred 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 87419

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 87419. The characteristics for MON 87419 evaluated in these assessments included: seed germination and dormancy 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 F, G, and H.

VII.C.1. Seed Germination and Dormancy 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 2013b; a). Additional temperature regimes were also evaluated.

A comparative assessment of seed germination and dormancy characteristics was conducted for MON 87419 and the conventional control. The seed lots were selfed F_2 grain from MON 87419, the conventional control, and the reference hybrids (four per site, eight unique across all locations) and were produced in replicated field trials during 2013 in Jackson County, Arkansas, York County, Nebraska, and Lehigh County, Pennsylvania. These geographic areas represent a broad range of environmental conditions for maize production. 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 F.

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 87419 and the conventional control for any characteristic at the AOSA temperature regime (alternating 20/30 °C). Furthermore, no statistically significant differences were detected between MON 87419 and the conventional control for any characteristic at the temperature regimes of 5 °C, 20 °C, or alternating 10/30 °C. In addition, no hard seed were observed at any temperature.

Six statistically significant differences were detected out of 17 statistical comparisons in the combined-site analysis between MON 87419 and the conventional control. MON 87419 had higher percent germinated seed than the conventional control at the 10 °C (99.0 vs. 97.3%), 30 °C (99.6 vs. 98.8%) and alternating 10/20 °C (99.7 vs. 97.9%) temperature regimes (Table VII-2). However, for the 30 °C and alternating 10/20 °C temperature regimes, the mean values of percent germinated seed for MON 87419 were within the respective reference ranges. The differences are therefore unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 3, "no" answer). The mean value of MON 87419 for percent germinated seed at the 10 °C temperature regime (99.0%) was slightly higher than the reference range (90.0 – 98.5%) in this study. In other studies of maize germination at 10 °C reference ranges have been as high as 100% germination (e.g. USDA Petition #06-298-01p, Section VII.A.3.a). Thus, the mean value for MON 87419 was within known values for maize and the difference is unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 4, "no" answer).

MON 87419 had lower percent dead seed compared to the conventional control at the 10 °C (0.4 vs. 1.8%), 30 °C (0.4 vs. 1.3%) and alternating 10/20 °C (0.3 vs. 2.1%) temperature regimes. For the 30 °C and alternating 10/20 °C temperature regimes, the mean values of percent dead seed for MON 87419 were within the respective reference ranges. The differences are therefore unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 3, "no" answer). The mean value of MON 87419 for percent dead seed at the 10 °C (0.4%) temperature regime was slightly lower than the reference range in this study (0.5 – 2.5%). Low levels of dead seed are common for maize and reference ranges in other studies of maize germination at 10 °C have included values as low as 0.0% dead seed (e.g., USDA Petition #06-298-01p, Section VII.A.3.b). Thus, the mean value for MON 87419 was within known values for maize and the difference is unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 4, "no" answer).

The germination and dormancy characteristics evaluated were used to assess MON 87419 in the context of plant pest/weed potential. The results of this assessment, including the lack of biologically meaningful differences and particularly the lack of increased hard seed, support the conclusion that the introduction of the dicamba and glufosinate-tolerance trait is not expected to result in increased plant pest/weed potential of MON 87419 compared to conventional maize.

Table VII-2. Combined-site Comparison of MON 87419 and the Conventional Control for Seed Germination and Dormancy Characteristics

Temperature		Mean % (S.E.)[1]		
(°C)	Characteristic	MON 87419	Control	Reference Range[2]
5	Germinated [3]	9.2 (1.78)	11.5 (3.26)	0.8 – 11.8
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	1.5 (0.53)	1.3 (0.33)	0.3 – 3.5
	Viable Firm-Swollen [3]	89.3 (1.82)	87.2 (3.05)	87.0 – 97.8
10	Germinated [4]	99.0 (0.40)*	97.3 (0.81)	90.0 – 98.5
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.4 (0.20)*	1.8 (0.43)	0.5 – 2.5
	Viable Firm-Swollen [4]	0.6 (0.34)	1.0 (0.48)	0.3 – 8.3
20	Germinated [4]	99.3 (0.37)	98.4 (0.53)	96.4 – 99.0
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.8 (0.37)	1.6 (0.53)	1.0 – 3.6
	Viable Firm-Swollen [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
30	Germinated [4]	99.6 (0.23)*	98.8 (0.43)	97.8 – 99.8
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.4 (0.23)*	1.3 (0.43)	0.3 – 2.3
	Viable Firm-Swollen [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
10/30	Germinated [4]	99.4 (0.19)	99.1 (0.29)	97.8 – 99.5
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.6 (0.19)	0.9 (0.29)	0.5 – 2.3
	Viable Firm-Swollen [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
10/20	Germinated [4]	99.7 (0.14)*	97.9 (0.56)	97.6 – 99.8
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.3 (0.14)*	2.1 (0.56)	0.3 – 2.4
	Viable Firm-Swollen [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3
20/30 (AOSA)	Normal Germinated [4]	98.8 (0.32)	98.2 (0.18)	97.0 – 99.5
	Abnormal Germinated [4]	0.5 (0.23)	0.6 (0.15)	0.0 – 1.0
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Dead [4]	0.7 (0.22)	1.2 (0.18)	0.5 – 2.3
	Viable Firm-Swollen [5]	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0

Note: The experimental design was a split-plot with four replications.

* Indicates a statistically significant difference between MON 87419 and the conventional control (α=0.05).

[1] MON 87419 and the conventional control values represent means with standard error (S.E.) in parentheses. n=12 with the following exceptions: n=11 for MON 87419 at the 10 °C temperature regime; n=11 for the control at the 20/30 °C temperature regime. Percentages do not always sum to 100% for a material within a temperature regime due to numerical rounding of the means.

[2] Reference range was calculated as the minimum and maximum mean values observed among the eight commercially available conventional reference hybrids

[3] Indicates statistical comparisons were performed using ANOVA.

[4] Indicates statistical comparisons were performed using Fisher's Exact Test.

[5] No statistical comparisons were made because of lack of variability in the data (all test and control values were 0 or 100).

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 87419. These data were developed to provide USDA-APHIS with a detailed description of MON 87419 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 87419 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 87419 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 87419 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 dicamba and glufosinate-tolerance trait did not meaningfully alter the plant pest/weed potential of MON 87419 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 dicamba and glufosinate-tolerance trait is not expected to result in increased plant pest/weed potential for MON 87419 compared to conventional maize.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

While phenotypic and agronomic characteristics from MON 87419 not treated with dicamba and glufosinate are most appropriate for the assessment, supplementary data under the expected use (e.g., herbicide-treated data) may be provided as well. Phenotypic and agronomic characteristics from MON 87419 both treated and not treated with dicamba and glufosinate are reported because of the intended amendment to dicamba use in MON 87419 maize.

Field trials were established to evaluate phenotypic and agronomic characteristics of MON 87419 compared to the conventional control. The trial sites provided a range of environmental and agronomic conditions representative of commercial maize production areas in North America. For MON 87419 not treated with dicamba and glufosinate, eight U.S. sites were established in 2013 (Table VII-3). For MON 87419 treated with dicamba and glufosinate, six U.S. sites were established in 2013 and two U.S. sites were established in 2014 (Table VII-4). The experimental design at each site was a randomized complete block with four replications. At each site, MON 87419, the conventional control, and four reference hybrids were evaluated. A total of 17 unique reference hybrids were evaluated among the eight sites for MON 87419 not treated with dicamba and glufosinate (Appendix G, Table G-1). A total of 14 unique reference

hybrids were evaluated among the eight sites for MON 87419 treated with dicamba and glufosinate (Appendix G, Table G-2). The planted plot dimensions varied between sites, due to variability in available planting equipment and the number of rows required for data collection (Appendix G, Tables G-3 and G-4). All plots of MON 87419, the conventional control, and the reference hybrids at each site were uniformly managed, except for intended application of dicamba and glufosinate to treated plots of MON 87419, in order to assess whether the introduction of the dicamba and glufosinate-tolerance trait altered the phenotypic and agronomic characteristics of MON 87419 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 G (Tables G-7 and G-8). The results of the combined site analyses are summarized below.

Field Phenotypic and Agronomic Characteristics for MON 87419 Not Treated with Dicamba and Glufosinate

In the combined-site analysis, no statistically significant differences were detected between MON 87419 not treated with dicamba and glufosinate and the conventional control for any of the 13 assessed characteristics: early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield (Table VII-5). Thus, the phenotypic and agronomic characteristics of MON 87419 not treated with dicamba and glufosinate were not altered in terms of pest/weed potential compared to conventional maize (Figure VII-I, step 2, “no” answer).

Plant vigor data were summarized as ranges within individual sites. MON 87419 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 87419 not treated with dicamba and glufosinate and the conventional control in plant vigor at any site (Appendix G, Table G-7).

Field Phenotypic and Agronomic Characteristics for MON 87419 Treated with Dicamba and Glufosinate

In the combined-site analysis, no statistically significant differences were detected between MON 87419 treated with dicamba and glufosinate and the conventional control for any of the 13 assessed characteristics: early stand count, days to 50% pollen shed, days to 50% silking, stay green rating, ear height, plant height, dropped ears, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight, and yield (Table VII-6). Thus, the phenotypic and agronomic characteristics of MON 87419 treated with dicamba and glufosinate were not altered in terms of pest/weed potential compared to conventional maize (Figure VII-I, step 2, “no” answer).

Plant vigor data were summarized as ranges within individual sites. MON 87419 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 87419 treated with dicamba and glufosinate and the conventional control in plant vigor at any site (Appendix G, Table G-8).

The phenotypic and agronomic characteristics evaluated in this study were used to provide a detailed description of MON 87419 compared to the conventional control. A subset of these characteristics was used to assess the weediness of MON 87419. The results of the agronomic and phenotypic assessment demonstrate that there were no unexpected changes in the phenotype of MON 87419, whether treated or not treated with dicamba and glufosinate, compared to the conventional control. Thus, the introduction of the dicamba and glufosinate-tolerance trait is not expected to result in increased plant pest/weed potential of MON 87419 compared to conventional maize.

Table VII-3. Field Phenotypic Evaluation Sites for MON 87419 Not Treated with Dicamba and Glufosinate during 2013

Site Code	County, State
ARNE	Jackson, Arkansas
ILMN	Warren, Illinois
KSLA	Pawnee, Kansas
NCBD	Perquimans, North Carolina
NEYO	York, Nebraska
PAGR	Lehigh, Pennsylvania
PAHM	Berks, Pennsylvania
WIDL	Walworth, Wisconsin

Table VII-4. Field Phenotypic Evaluation Sites for MON 87419 Treated with Dicamba and Glufosinate during 2013 and 2014

Site Code	Year	County, State
ARNE	2013	Jackson, Arkansas
ILMN	2013	Warren, Illinois
INKI	2013	Clinton, Indiana
KSLA	2013	Pawnee, Kansas
NEYO–2013	2013	York, Nebraska
NEYO–2014	2014	York, Nebraska
OHTR	2014	Miami, Ohio
PAGR	2013	Lehigh, Pennsylvania

Table VII-5. Combined-Site Comparison of MON 87419 Not Treated with Dicamba and Glufosinate to the Conventional Control for Phenotypic and Agronomic Characteristics During 2013

Phenotypic Characteristic (units)	Mean (S.E.)[1]		Reference Range[2]
	MON 87419	Control	
Early stand count	83.9 (0.86)	82.7 (0.77)	77.5 – 87.0
Days to 50% pollen shed	63.6 (0.87)	63.2 (0.74)	54.0 – 67.5
Days to 50% silking	64.3 (0.90)	64.1 (0.81)	54.8 – 68.8
Stay green rating (1-9 scale)	6.1 (0.36)	5.9 (0.34)	2.8 – 7.5
Ear height (cm)	106.9 (2.30)	110.6 (2.36)	93.3 – 126.3
Plant height (cm)	216.0 (3.76)	220.4 (3.83)	196.9 – 250.6
Dropped ears	0.2 (0.10)	0.0 (0.03)	0.0 – 0.5
Stalk lodged plants	0.9 (0.31)	1.4 (0.31)	0.0 – 5.0
Root lodged plants	0.3 (0.16)	0.1 (0.05)	0.0 – 3.8
Final stand count	75.1 (0.68)	74.1 (0.72)	61.3 – 79.0
Grain moisture (%)	17.8 (0.79)	17.7 (0.77)	14.6 – 21.7
Test weight (kg/hl)	73.2 (0.56)	73.3 (0.58)	68.1 – 79.6
Yield (Mg/ha)	12.5 (0.45)	12.4 (0.46)	8.8 – 15.9

Note: The experimental design was a randomized complete block with four replications per site. No statistically significant differences were detected between MON 87419 and the conventional control (α=0.05) using ANOVA for all characteristics except dropped ears and root lodged plants and nonparametric analysis for dropped ears and root lodged plants.

[1] MON 87419 and the conventional control values represent means with standard error in parentheses. n=32, except where noted in Table G-5 (Appendix G).

[2] Reference range was calculated as the minimum and maximum mean values from among 17 unique reference hybrids.

Table VII-6. Combined-Site Comparison of MON 87419 Treated with Dicamba and Glufosinate to the Conventional Control for Phenotypic and Agronomic Characteristics During 2013 and 2014

	Mean (S.E.)[1]		
Phenotypic Characteristic (units)	MON 87419	Control	Reference Range[2]
Early stand count	82.2 (0.97)	81.2 (0.84)	65.9 – 86.3
Days to 50% pollen shed	62.0 (0.78)	62.2 (0.82)	54.0 – 66.0
Days to 50% silking	62.8 (0.78)	62.9 (0.78)	54.8 – 66.0
Stay green rating (1-9 scale)	6.6 (0.34)	6.7 (0.34)	2.8 – 7.4
Ear height (cm)	105.1 (1.59)	106.7 (1.68)	91.0 – 123.0
Plant height (cm)	216.6 (3.44)	220.7 (3.77)	196.9 – 250.6
Dropped ears	0.1 (0.10)	0.0 (0.03)	0.0 – 0.7
Stalk lodged plants	1.3 (0.30)	1.0 (0.26)	0.4 – 9.7
Root lodged plants	0.4 (0.20)	0.3 (0.16)	0.0 – 2.1
Final stand count	74.0 (0.82)	73.6 (0.71)	62.0 – 77.5
Grain moisture (%)	18.4 (0.88)	18.1 (0.86)	14.6 – 23.8
Test weight (kg/hL)	73.1 (0.65)	73.1 (0.59)	68.8 – 79.6
Yield (Mg/ha)	13.7 (0.31)	13.5 (0.36)	11.3 – 15.9

Note: The experimental design was a randomized complete block with four replications per site. No statistically significant differences were detected between MON 87419 and the conventional control (α=0.05) using ANOVA.

[1] MON 87419 and the conventional control values represent means with standard error in parentheses. n=32, except where noted in table G-6 (Appendix G).

[2] Reference range was calculated as the minimum and maximum mean values from among 14 unique reference hybrids.

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 87419. In the 2013 U.S. field trials conducted to evaluate the phenotypic and agronomic characteristics of MON 87419, 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-7 through VII-8 and Tables G-9 through G-14). 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 dicamba and glufosinate-tolerance trait did not unexpectedly alter the assessed environmental interactions of MON 87419 compared to the conventional control. The lack of biologically meaningful differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance supports the conclusion that the introduction of the dicamba and glufosinate-tolerance trait is not expected to result in increased plant pest potential from MON 87419 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 87419 to the range of responses for the conventional control across all observation times and sites are reported.

Descriptions of the evaluated qualitative 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 G (Tables G-9 through G-11).

In an assessment of plant response to abiotic stress, disease damage, and arthropod damage, no differences were observed between MON 87419 and the conventional control for any of the 291 comparisons (including 93 abiotic stress response, 107 disease damage, and 91 arthropod damage comparisons) among all observations at eight sites. (Table VII-7).

The lack of differences observed between MON 87419 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 dicamba and glufosinate-tolerance 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-7. Summary of Qualitative Environmental Interactions Assessments for MON 87419 during 2013

Stressor	Number of observations across all sites	Number of observations with no differences between MON 87419 and the conventional control across all sites
Abiotic stressors	93	93
Disease damage	107	107
Arthropod-related damage	91	91
Total	291	291

Note: The experimental design was a randomized complete block with four replications per site. No differences were observed between MON 87419 and the conventional control during any observation for damage caused by any of the assessed stressors.

[1]MON 87419 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 87419 and the conventional control.

VII.C.2.2.2. Quantitative Environmental Interaction Assessments for MON 87419

Quantitative arthropod assessments on corn earworm (CEW: *Helicoverpa zea*) damage, European corn borer (ECB: *Ostrinia nubilalis*) damage, and arthropod abundance were conducted at three sites (Table VII-3; NCBD, PAHM, and WIDL). Damage by CEW and ECB was assessed once during the growing season at each site. Arthropod abundance was assessed from collections performed using sticky traps and visual counts for a total of five collections per method during the growing season at each site.

Descriptions of the quantitative environmental interactions characteristics are listed in Table VII-1. The materials and methods, additional details concerning the specific arthropod damage and arthropod abundance assessments, and detailed results are presented and discussed in Appendix G (Tables G-12 through G-14).

In the assessments of damage by CEW and ECB, no statistically significant differences were detected between MON 87419 and the conventional control for eight out of nine comparisons at three sites (Tables VII-8 and G-12). A single difference was observed where MON 87419 had less damage from corn earworm infestation compared to the conventional control at one site. However, the mean damage rating for MON 87419 was within the range of the commercial reference hybrids at this site and no differences were detected at other 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 plant pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

In the assessment of arthropod abundance data from sticky traps, no statistically significant differences were detected between MON 87419 and the conventional control for 21 out of 23 comparisons (Tables VII-8 and G-13). Significant differences were detected between MON 87419 and the conventional control for corn rootworm beetles (less abundant on MON 87419) and spiders (more abundant on MON 87419). The mean abundance values of MON 87419 for these arthropods were slightly outside of the respective ranges of the reference hybrids. However, these differences were not consistently detected across collection methods (i.e., in visual counts; Table G-14) and/or sites. Thus, these differences were not indicative of consistent responses associated with the trait and are not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

In the assessment of arthropod abundance data from visual counts, no statistically significant differences were detected between MON 87419 and the conventional control for 10 out of 11 comparisons (Tables VII-8 and G-14). A significant difference was detected between MON 87419 and the conventional control for minute pirate bugs (less abundant on MON 87419). However, the mean abundance value for MON 87419 was within the range of the reference hybrids. Additionally, this difference was not consistently detected across collection methods (i.e., in sticky traps; Table G-13) or sites. Thus, this difference was not indicative of a consistent response associated with the trait and is not considered biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

Table VII-8. Summary of Quantitative Arthropod Assessments and Detected Differences for MON 87419 during 2013

Summary of Statistical Comparisons[1]				Summary of detected differences[2]			
Arthropod assessments	Number of sites	Total number of comparisons	Number of comparisons where no differences were detected	Arthropod	Site	Within reference range?	Consistently detected across collection methods and/or sites?
Specific arthropod damage (Corn earworm and European corn borer)	3	9	8	Corn earworms	NCBD	Yes	No
Abundance (sticky traps)	3	23	21	Corn rootworm beetles	WIDL	No	No
				Spiders	NCBD	No	No
Abundance (visual counts)	3	11	10	Minute pirate bugs	PAHM	Yes	No

Note: the experimental design was a randomized complete block with four replications per site.
[1] Quantitative arthropod damage and abundance assessments were statistically analyzed within individual sites at α=0.05 using ANOVA.
[2] Four statistically significant differences were detected. These differences are further assessed following Section VII.B.2.

VII.C.3. Pollen Characteristics

USDA-APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait(s) 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 87419. In addition, morphological characterization of pollen produced by MON 87419 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87419 compared to the conventional control.

The viability and morphology of pollen collected from MON 87419 compared to that of the conventional control were assessed. Pollen was collected from MON 87419, the conventional control, and four commercial references grown under similar agronomic conditions at a field site in Clinton County, Indiana, a geographic area that represents environmentally relevant conditions for maize production for this product. The study 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 H.

No statistically significant differences (α=0.05) were detected between MON 87419 and the conventional control for percent viable pollen or pollen grain diameter (Table VII-9). Furthermore, no visual differences in general pollen morphology were observed between MON 87419 and the conventional control (Appendix H, Figure H-1).

The pollen characterization data contribute to the detailed phenotypic description of MON 87419 compared to the conventional control. Based on the assessed characteristics, the results support a conclusion that neither pollen viability nor morphology of MON 87419 were altered compared to conventional maize.

Table VII-9. Pollen Characteristics of MON 87419 Compared to the Conventional Control during 2013

Pollen Characteristic (unit)	Mean (S.E.)[1]		Reference Range[2]
	MON 87419	Control	
Viability[3] (%)	99.4 (0.20)	99.7 (0.09)	98.7 – 99.6
Diameter[4] (µm)	79.7 (0.78)	80.2 (1.00)	77.5 – 85.7

Note: No significant differences were detected between the MON 87419 and the conventional control (α=0.05) using ANOVA.

[1] MON 87419 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 subsamples per replication.

[4] Evaluated from 10 representative viable pollen grains per replication.

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 dicamba and glufosinate-tolerance trait altered the plant pest potential of MON 87419 compared to the conventional control, considered within the context of the variation among the reference hybrids. These assessments included five 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; and 5) plant response to abiotic stress and interactions with diseases and arthropods. All field-measured characteristics from categories 1 through 4 were also evaluated for MON 87419 treated with dicamba and glufosinate. 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 87419 compared to conventional maize.

Results from these assessments comparing MON 87419 and the conventional control demonstrate that MON 87419, whether treated or not treated with dicamba and glufosinate, does not possess: 1) increased weediness characteristics; or 2) characteristics that would confer a plant pest risk compared to conventional maize. Furthermore, the results demonstrate that MON 87419 not treated with dicamba and glufosinate does not possess increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods. Therefore, based on the results of multiple assessments discussed above and presented in the appendices, the weight of evidence indicates that MON 87419 is not meaningfully different from conventional maize with the exception of the dicamba and glufosinate-tolerance 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 to 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 field maize and is included in this petition as a baseline to assess whether there is likely to be a significant change in agricultural practices due to the cultivation of MON 87419 and whether such changes are likely to exacerbate plant pests or diseases associated with maize. Discussions include maize production, plant growth and development, general management practices during the season, management of insects, diseases and weeds, crop rotation, and volunteer management. Information presented in Section VII demonstrated that MON 87419 is no more susceptible to diseases or pests than commercially cultivated maize. Additionally data presented in Section VIII.C show that, with the exception of tolerance to the herbicides dicamba and glufosinate, MON 87419 is phenotypically equivalent to conventional maize and is not expected to pose a plant risk compared to conventional maize. Thus, there are no expected changes to the inputs needed for MON 87419, and no expected impacts to most of the agronomic practices employed for production of maize compared to the current practices.

Maize is planted in almost every state demonstrating its wide adaptation to soils and climate. However, the majority of maize is produced in the Midwest states because the fertile soils and climate are favorable for maize production. Proper seedbed preparation, good genetics, proper planting dates and plant population, and good integrated pest management practices are important to optimize the yield potential and economic returns of maize.

Annual and perennial weeds are considered to be the greatest pest problem in maize production (Aref and Pike 1998). Weeds compete with maize for water, nutrients, and light resulting in substantial yield losses when left uncontrolled. Weed species in maize vary from region to region and state to state. Economic thresholds for controlling weeds in maize require some form of weed management practice on all maize acreage. Weed management practices include mechanical practices (e.g., tillage), cultural practices (e.g., crop rotation, variety selection, optimizing planting data), and chemical practices (e.g., herbicide application). Numerous herbicides are available for preplant, preemergence, and postemergence control of annual and perennial weeds in maize, and approximately 98% of the maize acreage in the U.S. receives a herbicide application (USDA-NASS 2010b).

As shown in Sections VI and VII, with the exception of the dicamba and glufosinate tolerance traits, no phenotypic, compositional, or environmental interactions differences between MON 87419 and conventional maize have been observed. Moreover, herbicide-tolerant maize is currently grown on 89% of U.S. maize acres (USDA-NASS 2014c). Dicamba and glufosinate herbicides are currently labeled for use as preplant and postemergence applications in maize. As proposed to EPA, MON 87419 will allow the 0.5 lb a.e. postemergence application window for dicamba to be extended from the V5 to the V8 growth stage or 30-inch height of maize, whichever occurs first, without reducing

the application rate of dicamba. Glufosinate is currently labeled for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on glufosinate-tolerant hybrids. Glufosinate use with MON 87419 will not change from current labeled uses of glufosinate. MON 87419 will likely be combined with Roundup Ready® Corn 2 utilizing traditional breeding techniques. The combination of herbicide-tolerance traits will allow the preemergence and postemergence use of dicamba, glufosinate and glyphosate herbicides in an integrated weed management program with multiple modes of action to control a broad spectrum of grass and broadleaf weed species, including herbicide-resistant and tough to control weed species. Therefore, it is not anticipated that commercialization of MON 87419 in the U.S. would have a notable impact on current maize cultivation practices, beyond the intended benefits of effective management of common, troublesome weeds, and/or herbicide-resistant weeds.

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

The U.S., China, Brazil, European Union, and Ukraine are the top five countries/regions producing maize globally (USDA-FAS 2014). The U.S. is the largest producer of maize (*Zea mays*), producing approximately 36% of the world maize production in 2013/14 (USDA-FAS 2014). China follows with 22% of the maize production.Maize for all purposes was planted on approximately 95.4 million acres in 2013 in the U.S., more than any other field crop, exceeding soybean and wheat with acreages of 76.5 and 56.2 million acres, respectively (Table VIII-1, USDA-NASS, 2014b and 2014c). Much of that production occurs in the upper Midwest States (Figure VIII-1). The 2013 maize acreage was down 1.8 million acres from 2012 (Table VIII-1). Approximately 87.7 million acres were harvested for grain and 6.2 million acres were harvested for silage (USDA-NASS 2014b). Total maize grain production was approximately 13.9 billion bushels in 2013 with an average yield of 158.8 bushels per acre (Table VIII-1). The value of maize production reached $62.72 billion in the U.S. in 2013 (USDA-NASS 2014a). The value of maize production in the U.S. has ranged from $18.50 to $76.94 billion in the past 14 years. The principal uses of maize are feed, food, seed, ethanol fuel, and export, and high-fructose maize syrup (Capehart et al. 2012).

Table VIII-1. Field Maize Production in the U.S., 2000-2013[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2013	95,365	87,668	158.8	13,925,147	62.72
2012	97,155	87,375	123.4	10,780,296	74.33
2011	91,936	83,989	147.2	12,359,612	76.94
2010	88,192	81,446	152.8	12,446,865	64.64
2009	86,382	79,490	164.7	13,091,862	46.73
2008	85,982	78,570	153.9	12,091,648	49.31
2007	93,527	86,520	150.7	13,037,875	54.67
2006	78,327	70,638	149.1	10,531,123	32.08
2005	81,779	75,117	147.9	11,112,187	22.19
2004	80,929	73,631	160.3	11,805,581	24.38
2003	78,603	70,944	142.2	10,087,292	24.47
2002	78,894	69,330	129.3	8,966,787	20.88
2001	75,702	68,768	138.2	9,502,580	18.88
2000	79,551	72,440	136.9	9,915,051	18.50

[1]Source: (USDA-NASS 2010a)

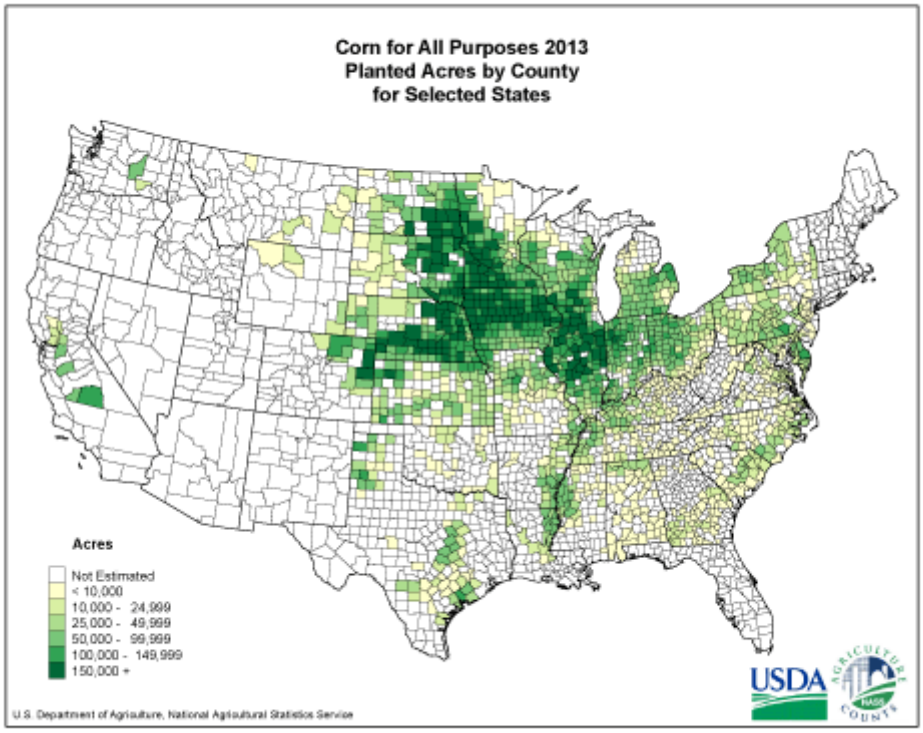

Figure VIII-1. U.S. Maize Acreage by County in 2013
Source: (USDA-ERS 2009)

VIII.C. Production Management Considerations

Other than the insertion of the *dmo* and *pat* genes to confer tolerance to additional herbicides (dicamba and glufosinate, respectively), MON 87419 is similar to several other events present in herbicide tolerant maize hybrids being grown in the U.S. (e.g., Genuity® SmartStax®, Genuity® VT Double PRO®, LibertyLink®, etc). With the widespread use of herbicide tolerant maize hybrids since 1997 (currently 89% of planted acreage), Monsanto anticipates no specific changes in production management practices beyond the intended benefits of more effective and improved management of common, troublesome and/or herbicide-resistant weeds (USDA-NASS 2014a).

VIII.D. 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 (USDA-APHIS Petition #13-290-01p). MON 87419 does not contain insect protection traits, therefore the information on this subject is incorporated here by reference. In brief, insect pests continue to cause damage to maize and are commonly addressed by biotechnology-derived insect-tolerant traits, 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. The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates the distribution, sale, use and testing of pesticidal substances (including those produced in plants), that are intended to control insect pests.

MON 87419 was developed to improve the management of weeds and has no unique pest control attributes. Thus, no changes to insect pest control practices are expected from use of MON 87419.

Environmental observations in field studies have demonstrated no apparent impact of MON 87419 on arthropods (Section VII.C.). Therefore, no changes in current insect management practices are anticipated from the introduction of MON 87419.

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VIII.E. 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 Petition for nonregulated status for Corn Rootworm-Protected MON 87411 (USDA-APHIS Petition #13-290-01p). MON 87419 does not contain disease protection traits, therefore the information on this subject is incorporated here by reference (USDA-APHIS Petition #13-290-01p). 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.

MON 87419 was developed to improve the management of weeds and has no unique pest control attributes. Thus, no changes to insect pest control practices are expected from use of MON 87419.

Environmental observations in field studies have demonstrated no apparent impact of MON 87419 on diseases of maize (Section VII.C.). Therefore, no changes in current disease management practices are anticipated from the introduction of MON 87419.

VIII.F. Weed Management

VIII.F.1. Methods of Weed Control in Maize

Annual and perennial weeds are considered to be the greatest pest problem in maize production (Aref and Pike 1998). Weed control in maize is essential for optimizing yield because weeds compete with maize for light, nutrients, and moisture and can lead to reductions in yield (Knake et al. 1990). The duration of competition from weeds is important to determine the potential loss of yield in maize and the critical time period can vary with the density and species of the weed and environmental factors (Hall et al. 1992). Early weed competition studies indicated that weeds must be removed by a certain time and maize heights to avoid yield losses in maize (Carey and Kells 1995; Hall et al. 1992; Knake and Slife 1965; Tapia et al. 1997). Weed control the first several weeks after maize emergence is the most critical period to avoid yield losses in maize (Bosnic and Swanton 1997; Carey and Kells 1995; Hall et al. 1992). Some weeds can tolerate cold, wet conditions better than maize, and can gain an advantage prior to planting. Annual weed species such as giant foxtail (*Setaria* spp.), barnyardgrass (*Echinochloa crus-galli* (L.) *Beauv*.) and Palmer pigweed (*Amaranthus* spp.) can reduce maize yields by up to 13%, 35% and 74%, respectively (Bosnic and Swanton 1997; Fausey et al. 1997; Gianessi et al. 2002; Knake and Slife 1965). In a study of mixed weed populations competing with maize, yields were reduced by up to 20% when the weeds reached a height of eight inches (Carey and Kells 1995; Gianessi et al. 2002).

A survey of Extension Service weed scientists solicited estimates of the percent of maize acreage infested with individual weed species by state or region, as well as the potential impact on maize yields if the species were left uncontrolled. In this survey, twelve annual broadleaf, nine annual grass, and seven perennial species were identified as troublesome weeds (Table VIII-3) (Gianessi et al. 2002). Estimates of yield loss ranged from a low of 15% due to wirestem muhly and sandburs to a high of 48% from burcucumber.

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in maize. Foxtail spp., pigweed, velvetleaf (*Abutilon theophrasti*), lambsquarters (*Chenopodium album*), and cocklebur (*Xanthium strumarium*) are common weeds in Midwest maize and soybean fields. The most frequently reported common weeds in the Southeast region are morningglory (*Ipomoea* spp.), pigweeds, crabgrass (*Digitaria* spp.), nutsedge (*Cyperus* spp.) and broadleaf signalgrass (*Brachiaria platyphylla*) (Webster et al. 2009). Morningglory, pigweed, nutsedge, johnsongrass (*Sorghum halepense*) crabgrass, and broadleaf signalgrass are the most frequently mentioned troublesome weeds in the Southeast region (Webster et al. 2009).

In a recent survey of growers utilizing glyphosate-tolerant crops, pigweed, morningglory, Johnsongrass, ragweed spp., foxtail, and velvetleaf were mentioned as the most problematic weeds, depending on the state and cropping system (Kruger et al. 2009). With the exception of morningglory and pigweed, these problematic weed species were present before the introduction of glyphosate-tolerant crops, and some improvement in weed control was realized after the implementation of glyphosate-tolerant cropping systems (Kruger et al. 2009). Common waterhemp (*Amaranthus rudis*) and ragweed were

the most frequently mentioned problematic weeds in glyphosate-tolerant crops in Illinois, Indiana and Iowa. Velvetleaf and common waterhemp were the most frequently mentioned in Nebraska while morningglory, sicklepod (*Cassia obtusifolia*), and pigweed species were the most commonly mentioned in Mississippi and North Carolina.

Until the early 1950s, tillage and cultivation practices were primarily used for weed control in maize, but they have been largely replaced by the use of herbicides. Herbicide use in maize became widespread by the end of the 1970s. In 2010, herbicides were applied to 98% of the planted maize acreage (USDA-NASS 2010c). Glyphosate is the most widely applied herbicide in maize being applied on 83% of the planted acreage (Table VIII-3). Triazines (atrazine, metribuzin, simazine) are the second most widely used herbicides at 67%. The chloracetamide herbicides (acetochlor, alachlor, dimethenamid, metolachlor) are applied to 64% of the planted maize acreage.

The introduction of Roundup Ready® maize, a biotechnology-derived herbicide-tolerant maize, in 1998 offered growers an alternative and effective solution for the control of weeds in maize. In 2014, approximately 89% of the total maize acreage in the U.S. was planted with hybrids possessing herbicide-tolerance traits (USDA-NASS 2009). Maize hybrids possessing both herbicide-tolerance and insect-protected traits were planted on 76% of the maize acreage in 2014. Although glyphosate alone provides broad-spectrum control of numerous annual and perennial weed species, preemergence residual herbicides are a key component of weed control programs for Roundup Ready® Corn 2. This is very apparent with the widespread use of chloracetamide and atrazine herbicides in maize (Table VIII-3). Preemergence residual herbicides provide early season weed control to reduce early weed competition, improve control of certain hard to control broadleaf weed species (morningglory spp.), and help provide control of some glyphosate-resistant weeds.

Tables VIII-4 and VIII-5 provide a summary of the crop tolerance ratings of herbicides applied in maize production and the efficacy of these herbicides on 26 common weed species. These tables list only the most commonly used herbicides in maize production. Seldom would one field or farm have all 26 weed species, but they generally have a mixture of grass and broadleaf weed species. These ratings can be used by growers to facilitate the selection of a herbicide program in maize, which offers the best overall control of the weed species present. Generally, a mixture or premixture of two or more herbicide active ingredients is needed to achieve broadspecturm control of both grasses and broadleaf weed species. However, glyphosate, glufosinate, tembotrione, and topramezone each provide control of numberous grass and broadleaf weed species.

Table VIII-2. Troublesome Weeds in Maize Production[1]

Weed Species ***Latin name***	**Area Infested State/Region[2]**	**Acreage Infested (%)**	**Potential Yield Loss (%)**
Annual Broadleaves			
Bur Cucumber (*Sicyos angulatus*)	PA/OH/TN/SE	5-10	48
Cocklebur (*Xanthiums strumarium*)	MW/NP/SE	20-60	33
Jimsonweed (*Datura stramonium*)	MW/CO	5-20	17
Kochia (*Kochia scopari*)	NP/NW	10-70	33
Lambsquarters (*Chenopodium album*)	MW/SE/NE/CA	15-80	33
Morningglory (*Ipomoea purpurea*)	MW/SE/SP	20-75	33
Nightshade (*Solanum nigrum*)	MW/NP/CA	25-50	26
Pigweeds/Waterhemp (*Amaranthus* spp.)	US	30-90	36
Ragweed, Common (*Ambrosia artemisiifolia* L.)	MW/SE/NE	20-70	30
Ragweed, Giant (*Ambrosia trifida*)	MW/NP	10-45	28
Smartweeds (*Polygonum* spp.)	MW/SD/NE/SE	30-70	22
Velvetleaf (*Abutilon theophrasti*)	MW/NE/NP	25-70	28
Annual Grasses			
Barnyardgrass (*Echinochloa crus-galli* (L.) *Beauv*.)	SP/NW/CA	80-90	23
Bermudagrass (*Cynodon dactylon*)	MD/SE/UT/CA	10-20	47
Crabgrass spp. (*Digitaria* spp.)	MW/SE/NE	20-80	29
Cupgrass, Woolly (*Eriochloa villosa*)	IA/WI	15-20	29
Foxtail spp. (*Setaria* spp.)	MW/NE/NP	50-90	31
Millet, Wild-Proso (*Panicum miliaceum*)	UT/WY/CO/ID	15-40	31
Panicum, Fall (*Panicum dichotomiflorum*)	MW/SE/NE/NP	15-80	30
Sandburs (*Cenchrus* spp.)	NP/UT/WY	5-30	15
Shattercane (*Sorghum bicolor*)	MW/SP	5-40	33
Perennials			
Bindweed, Field (*Convolvulus arvensis*)	ND/SW/CA	40-80	18
Dogbane, Hemp (*Apocynum cannabinum* L.)	IL/MO	2-20	21
Johnsongrass (*Sorghum halepense*)	MW/SE/SW/CA	20-60	45
Muhly, Wirestem (*Muhlenbergia frondosa*)	PA	2	15
Nutsedge, Yellow (*Cyperus esculentus*)	MW/SE/NE/NP/ CA	10-70	21
Quackgrass (*Elytrigia repens*)	MW/NE/UT	10-70	27
Thistle, Canada (*Cirsium arvense*)	NE/MW/NP/CO	5-25	26

[1]Source: (Gianessi et al. 2002).

[2]Regions: MW = Midwest, NE = Northeast, NP = Northern Plains, NW = Nothwest, SE = Southeast, SW = Southwest, SP = Southern Plains.

Table VIII-3. Herbicide Applications in Maize in 2013 in the U.S.[1]

Herbicide	Chemical Family	Mode-of-Action (MOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per MOA
Glyphosate	Glycine	EPSPS inhibitor	83.7	83.7
Atrazine	Triazine	PSII inhibitor	58.7	61.5
Metribuzin	Triazine		0.4	
Simazine	Triazine		2.4	
Acetochlor	Chloroacetamide	Long-chain fatty acid inhibitor	26.6	61.4
Alachlor	Chloroacetamide		0.2	
Dimethenamid	Chloroacetamide		6.3	
Metolachlor	Chloracetamide		28.3	
Pyroxasulfone	Isoxazoline		0.3	
Isoxaflutole	Isoxazole	HPPD inhibitor	9.4	44.6
Mesotrione	Triketone		25.5	
Tembotrione	Triketone		6.7	
Topramezone	Triketone		3.0	

Table VIII-3 (continued). Herbicide Applications in Maize in 2013 in the U.S.[1]

Herbicide	Chemical Family	Mode-of-Action (MOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per MOA
2,4-D	Phenoxy	Synthetic Auxin	14.3	40.5
Clopyralid	Carboxylic acid		11.9	
Dicamba	Benzoic acid		13.9	
Fluroxpyr	Caryridine Carboxylic acid		0.4	
Flumetsulam	Imidazolinone	ALS inhibitor	11.5	29.4
Halosulfuron	Sulfonylurea		0.3	
Nicosulfuron	Sulfonylurea		1.1	
Primisulfuron	Sulfonylurea		0.2	
Prosulfuron	Sulfonylurea		0.2	
Rimsulfuron	Sulfonylurea		4.6	
Thifensulfuron	Sulfonylurea		2.7	
Thiencarbazone	Triazolones		8.5	
Tribenuron	Sulfonylurea		0.3	

Table VIII-3 (continued). Herbicide Applications in Maize in 2013 in the U.S.[1]

Herbicide	Chemical Family	Mode-of-Action (MOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per MOA
Diflufenzopyr	Semicarbazone	Auxin transport	7.9	7.9
Fluthiacet	Thiadiazole	PPO inhibitor	0.9	6.1
Carfentrazone	Aryl triazone		0.5	
Saflufenacil	Pyrimidinedione		4.0	
Flumioxazin	N-phenylphthalimide		0.7	
Paraquat	Bipyridylium	Photosystem-I-electron diverter	1.4	1.4
Glufosinate	Phosphinic acid	Glutamine Synthase Inhibitor	1.2	1.2
Pendimethalin	Dinitroanaline	Microtubule inhibitor	0.7	0.7
Total			**99**	

[1]Source: Monsanto Company, 2014.

Table VIII-4. Crop Tolerance and Grass Weeds Responses to Herbicides Applied in Maize Production

Herbicide/Application	CT[3]	Common Grass Weeds[1,2]										
		BY	BS	CG	FP	FT	GG	SC	JGr	JGs	IR	NSy
					Preplant or Preemergence							
Acetochlor	1	9	NA	9	8	9	NA	-	NA	NA	NA	8+
Acetochlor/atrazine	1	9	8	9	8	9	9	-	0	7	8	8+
Acetochlor/flumetsulam	2	8	NA	8	8	8	NA	-	NA	NA	NA	7
Atrazine	0	8	5	-	-	7	6	-	0	4	NA	7
Dimethenamid	1	8	6	8+	8	8+	8	-	4	7	5	8
Flmetsulam	2	-	NA	-	-	-	NA	-	NA	NA	NA	-
Flumetsulam/clopyralid	2	-	NA	-	-	-	NA	-	NA	NA	NA	-
Flumioxazin	1	-	NA	-	-	-	NA	-	NA	NA	NA	-
Flumioxazin/pyroxasulfone	1	8	8	8	8	8	8	-	3	9	9	-
Isoxaflutole	1	8	NA	7	8	8	NA	6	NA	NA	NA	-
Mesotrione	1	-	NA	6	-	-	NA	-	NA	NA	NA	-
Metolachlor	1	8	NA	9	8+	9	NA	-	NA	NA	NA	8+
Metolachlor/atrazine	1	9	8	9	8	9	9	-	0	7	8	8
Metolachlor/mesotrione	1	8	NA	9	8+	9	NA	-	NA	NA	NA	8+
Metolachlor/mestrione/atr	1	9	8	9	8+	9	9	-	2	8	7	8
Pyroxasulfone	1	8	8	8	8	9	9	-	4	6	9	-
Pyroxasultone/fluthiacet	1	8	8	8	8	9	9	-	4	7	9	-
Pyroxasultone/fluthiacet/atr	1	8	8	8	8	9	9	-	4	7	9	6
Rimsulfuron	1	7	NA	6	6	7	NA	-	NA	NA	NA	-
Rimsulfuron/isoxaflutole	2	8	NA	7	8	8	NA	6	NA	NA	NA	-
Rimsulfuron/mesotrione	1	7	NA	6	6	7	NA	-	NA	NA	NA	-
Saflufenacil	1	-	1	-	-	-	1	-	NA	1	1	-
Saflufenacil/Dimethenamid/ clopyralid	1	8	6	8	8	8	8	-	NA	NA	NA	-
Simazine	0	8	5	7	7	8	7	-	0	4	NA	-
Thiencarbazone/isoxaflutole	1	8	8	8+	8+	8+	9	7	NA	NA	NA	7
					Preemergence							
Pendimethalin	2	8	6	8	8	8	8	6	4	7	5	-

Table VIII-4 (continued). Crop Tolerance and Grass Weeds Responses to Herbicides Applied in Maize Production

Herbicide/Application	CT[3]	Common Grass Weeds[1,2]										
		BY	BS	CG	FP	FT	GG	SC	JGr	JSs	IR	NSy
		Postemergence										
2,4-D	2	-	0	-	-	-	1	-	0	0	0	-
2,4-D/atrazine	2	-	NA	-	-	6	NA	-	NA	NA	NA	-
Atrazine	1	7	7	-	-	8	6	-	0	3	NA	7
Bentazon	0	-	0	-	-	-	1	-	0	0	0	8
Bromoxynil	1	-	NA	-	-	-	NA	-	NA	NA	NA	-
Carfentrazone	2	-	NA	-	-	-	NA	-	NA	NA	NA	-
Clopyralid	0	-	NA	-	-	-	NA	-	NA	NA	NA	-
Dicamba	2	-	1	-	-	-	1	-	0	0	0	-
Dicamba/diflufenzopyr	1	6	4	6	6	6	3	-	0	5	0	-
Flroxypyr	1	-	NA	-	-	-	NA	-	NA	NA	NA	-
Flumiclorac	2	-	NA	-	-	-	NA	-	NA	NA	NA	-
Fluroxypyr/clopyralid	1	-	NA	-	-	-	NA	-	NA	NA	NA	-
Fluroxypyr/colpyralid	1	-	NA	-	-	-	NA	-	NA	NA	NA	-
Fluthiacet	2	-	NA	-	-	-	NA	-	NA	NA	NA	-
Glufosinate	0*	7	8	8	8	8+	5	8	7	8	6	-
Glyphosate	0*	8	9	8	8	9	9	9	7	9	6	7
Halosulfuron	1	-	2	-	-	-	2	-	1	2	NA	9
Mesotrione	1	-	7	7	-	-	NA	-	0	0	NA	-
Nicosulfuron	1	8+	8	4	8+	9	NA	9	8	9	6	6
Nicosulfuron/rimsulfuron	1/2	8	NA	-	8	9	NA	9	NA	NA	NA	-
Primisulfuron	2	-	NA	-	8	7	NA	9	NA	NA	NA	6
Primisulfuron/dicamba	2	-	NA	-	7	6	NA	9	NA	NA	NA	-
Prosulfuron/primisulfuron	2	-	NA	-	7	6	NA	9	NA	NA	NA	-
Rimsulfuron	1	7	NA	-	7	7	NA	7	NA	NA	NA	-
Rimsulfuron/meostrione	1	7	7	-	7	7	8	7	7	9	4	-
Tembotrione	0	8	8	6	-	7	7	8	5	6	NA	-
Thiencarbazone/tembotrione	1	8	8	8	8	8+	NA	8	5	7	NA	-
Topramezone	0	7	6	7+	6	7+	7	6	4	7	0	-

[1]All weed control ratings except for BS, GG, JGr, JGs and IR are from the 2014 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University (Loux et al. 2014). Ratings for BS, GG, JGr, and IR are from the 2015 Weed Control Guidelines for Mississippi, Mississippi State University (MSU 2015). Weed control rating for weeds, except BS, GG, JGr. JGs and IR, are: 9 = 90% to 100%, 8 = 80% to 90%, 7 = 70% to 80%, 6 = 60% to 70%, - = less than 60% control, not recommended. Weed control ratings for BS, GG, JGr, JGs and IR are: 9-10 = excellent, 7-8 = good, 4-6 = fair, 0-3 = none to slight. Ratings assume the herbicides are applied in the manner suggested in the guidelines and according to the label under optimum growing conditions.

[2]Weed species: BY = barnyardgrass, BS = broadleaf signalgrass, CG = crabgrass, FP = fall panicum, FT = giant and yellow foxtail, GG = goosegrass, SC = shattercane, JGr = rhizome johnsongrass, JGs = seedling johnsongrass, IR = Italian ryegrass, and NSy = yellow nutsedge.

[3]Crop tolerance (CT) rating: 0 = excellent, 1 = good, 2 = fair, 3 = poor.

(-) denotes data not available. *Rating based on glufosinate to Liberty Link® maize and glyphosate applied to Roundup Ready® Corn 2 maize.

Table VIII-5. Broadleaf Weeds Responses to Herbicides Applied in Maize Production

	Common Broadleaf Weeds[1,2]													
Herbicide/Application	BN	CB	CR	GR	LQ	MG	HS	PA	PW	PS	SP	SW	VL	WH
					Preplant or Preemergence									
Acetochlor	8+	-	7	-	7+	NA	NA	NA	8+	NA	NA	-	-	8
Acetochlor/atrazine	9	8	9	8	9	8	6	9	9	8	6	9	8	9
Acetochlor/flumetsulam	8+	8	8+	7+	9	6	NA	NA	9	NA	NA	8+	8+	8
Atrazine	9	8	9	8	9	8	7	9	9	8	8	9	8	9
Dimethenamid	8+	-	-	-	6	NA	NA	NA	8	NA	NA	-	-	8
Flmetsulam	8	7	7	-	9	NA	NA	NA	9	NA	NA	8	8+	-
Flumetsulam/clopyralid	8+	8	8+	7+	9	6	NA	NA	9	NA	NA	8+	9	-
Flumioxazin	9	-	7	-	9	7	NA	NA	9	NA	NA	7	7	7
Flumioxazin/pyroxasulfone	9	-	8	-	9	7	8	9	9	8	7	7	7	8
Isoxaflutole	9	-	9	6	9	NA	NA	NA	9	NA	NA	8	9	8
Mesotrione	9	7	7	6	9	6	NA	NA	9	NA	NA	9	9	9
Metolachlor	8	-	-	-	6	NA	NA	NA	8	NA	NA	-	-	8
Metolachlor/atrazine	9	8	9	8	9	8	6	9	9	8	6	9	8	9
Metolachlor/mesotrione	9	7	7	6	9	6	NA	NA	9	NA	NA	9	9	9
Metolachlor/mestrione/atr	9	8	9	8	9	8	8	9	9	9	8	9	9	9
Pyroxasulfone	8	-	7	-	8	NA	3	9	8	7	NA	-	7	8
Pyroxasultone/fluthiacet	8		7	-	8	NA	3	9	8	7	NA	-	7	8
Pyroxasultone/fluthiacet/atr	9	7	9	6	9	7	7	9	9	8	8	9	8	9
Rimsulfuron	-	-	7	-	7	7	NA	NA	7	NA	NA	7	6	-
Rimsulfuron/isoxaflutole	8	-	8	6	9	7	NA	NA	9	NA	NA	8	9	8
Rimsulfuron/mesotrione	9	7	8	6	9	6	NA	NA	9	NA	NA	9	9	9
Saflufenacil	8	8	8	8	9	8	6	9	9	7	5	8	8	8
Saflufenacil/Dimethenamid/ clopyralid	9	8	9	8	9	8	6	9	9	7	5	9	8	9
Simazine	9	7	9	7	9	7	NA	9	9	9	8	8+	7	-
Thiencarbazone/isoxaflutole	9	8	9	8	9	7	9	NA	9	NA	NA	9	9	9
					Preemergence									
Pendimethalin	-	-	-	-	8	NA	0	7	9	0	0	-	-	8

Table VIII-5 (continued). Broadleaf Weeds Responses to Herbicides Applied in Maize Production

Herbicide/Application	Common Broadleaf Weeds[1,2]													
	BN	CB	CR	GR	LQ	MG	HS	PA	PW	PS	SP	SW	VL	WH
	Postemrgence													
2,4-D	7	9	9	9	9	9	8	8	9	8	8	6	8	8
2,4-D/atrazine	9	9	9	9	9	9	NA	NA	9	NA	NA	9	8+	9
Atrazine	9	9	9	8	9	9	7	9	9	9	8	9	8	9
Bentazon	-	9	7	6	6	NA	4	3	-	8	1	9	8+	-
Bromoxynil	9	9	9	8	9	8	NA	NA	7	NA	NA	8	8	6
Carfentrazone	8	-	6	-	7	8	NA	NA	8+	NA	NA	-	9	7
Clopyralid	8	9	9	9	-	NA	NA	NA	-	NA	NA	-	-	-
Dicamba	8	9	9	9	8	9	9	9	8	8	9	8	7+	8
Dicamba/diflufenzopyr	8	9	9	9	9	9	9	9	9	9	9	8+	8	8
Fluroxypyr	7	8	9	-	-	9	NA	NA	-	NA	NA	7	8	-
Flumiclorac	-	7	7	-	7	NA	NA	NA	9	NA	NA	-	9	7
Fluroxypyr/clopyralid	7	9	9	9	7+	7	NA	NA	7+	NA	NA	9	8+	-
Fluroxypyr/colpyralid	7	9	9	9	-	9	NA	NA	-	NA	NA	7	8	-
Fluthiacet	-	-	-	-	7	7	NA	NA	8	NA	NA	-	9	7
Glufosinate	9	9	9	9	8	8	9	8	8	9	9	9	8	8
Glyphosate	8	9	8+	8+	8+	6	6	9	9	7	8	8	8	8
Halosulfuron	-	9	8	8	-	6	8	6	9	7	5	7	8	-
Mesotrione	9	7+	7	8	9	7	NA	9	8	9	5	9	9	9
Nicosulfuron	-	-	-	-	-	8	7	6	9	4	5	8	-	7
Nicosulfuron/rimsulfuron	-	6	-	-	-	6	NA	NA	9	NA	NA	7	-	-
Primisulfuron	8	9	9	9	-	6	NA	NA	9	NA	NA	8	8	-
Primisulfuron/dicamba	9	9	9	9	9	8	NA	NA	9	NA	NA	9	8+	8
Prosulfuron/primisulfuron	8	9	9	9	6	7	NA	NA	9	NA	NA	8+	8+	-
Rimsulfuron	-	6	6	-	7		NA	NA	8	NA	NA	6	7	-
Rimsulfuron/meostrione	9	8	8	8	9	7	NA	8	9	9	7	9	9	9
Tembotrione	9	8	8	8	9	7	NA	9	9	7	7	8	9	9
Thiencarbazone/tembotrione	9	8	8	8	9	7	NA	9	9	7	7	8	9	9
Topramezone	9	8	7	7	9	7	7	8	9	9	6	8	9	9

[1]All weed control ratings except for HS, PA, PS, and SP are from the 2014 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University (Loux et al. 2014). Ratings for HS, MG, PA, PS, and SP are from the 2015 Weed Control Guidelines for Mississippi, Mississippi State University (MSU 2015). Weed control ratings for weeds, except HS, MG, PA, PS, and SP, are: 9 = 90% to 100%, 8 = 80% to 90%, 7 = 70% to 80%, 6 = 60% to 70%, - = less than 60% control, not recommended. Weed control ratings for HS, MG, PA, PS, and SP are: 9-10 = excellent, 7-8 = good, 4-6 = fair, 0-3 = none to slight. Ratings assume the herbicides are applied in the manner suggested in the guidelines and according to the label under optimum growing conditions.

[2]Weed species: BN = black nightshade, CB = cocklebur, CR = common ragweed, GR = giant ragweed, LQ = lambsquarters, MG = morningglory spp., HS = hemp sesbania, PA = palmer and spiny amaranth, PW = pigweed, PS= prickly sida, SP = sicklepod, SW = smartweed, VL = velvetleaf, and WH = waterhemp.

(-) denotes data not available. *Rating based on glyphosate applied to Roundup Ready® Corn 2 maize.

VIII.F.2. Herbicide Resistant Weeds in Maize

Table VIII-6 provides a summary of the common weeds in maize that have biotypes reported resistant to the various herbicide mechanisms-of-action in the U.S. To date there are only two weed species with biotypes confirmed to be resistant to dicamba in the U.S. after over 40 years of use – kochia and prickly lettuce (Heap 2014b). Additionally, a population of lambsquarters has been confirmed as resistant to dicamba in New Zealand, and in Canada, common hempnettle and wild mustard have been confirmed as resistant, for a total of five species worldwide with confirmed resistance to dicamba (Heap 2014b). Currently in the U.S., six grass species and eight broadleaf species have been confirmed to have resistance to glyphosate . Dicamba provides good to excellent control of all eight of the broadleaf species. None of these broadleaf weed biotypes have been shown to have populations that are resistant to both glyphosate and dicamba. The first species in the U.S. with a biotype resistant to glufosinate was recently confirmed in a glyphosate-resistant Italian Ryegrass population (Avila-Garcia and Mallory-Smith 2011). Additionally, a population of goosegrass from Malaysia has been confirmed resistant to glufosinate (Seng et al. 2010). Thus, there are a total of two species worldwide with biotypes that have resistance to glufosinate. A discussion regarding the usefulness of MON 87419 in management of herbicide resistant weeds can be found in Section VIII.F.4. The potential for development of weeds resistant to dicamba or glufosinate resistance can be found in Appendix I.

Table VIII-6. Common Weeds in Maize and Weed Resistance to Herbicide Sites of Action in the U.S.[1]

	Site of Action											
Weed Species[2]	**ACCase Inhibitors**	**ALS Inhibitors**	**Long Cbain Fatty Acit Inhibitors**	**Microtubule Inhibitors**	**EPSPS Inhibitors**	**Photosystem II Inhibitors –C1**	**HPPD Inhibitors**	**PSII Inhibitors – C2**	**Synthetic Auxins**	**PS1 Electron Diverter**	**PPO Inhibitors**	**Glutamine Synthase Inhibitors**
Grasses												
Barnyardgrass	X	X				X		X	X			
Crabgrass spp. (large, smooth)	X								X			
Foxtail spp. (giant, green, yellow)	X	X		X		X						
Italian ryegrass	X	X	X		X							X
Goosegrass				X	X	X				X		
Johnsongrass	X	X		X	X							
Broadleaves												
Black nightshade (Eastern)		X				X						
Common chickweed		X										
Common cocklebur		X										
Common purslane						X		X				
Common ragweed		X			X	X					X	
Giant ragweed		X			X							
Horseweed (marestail)		X			X	X		X		X		
Jimsonweed						X						
Kochia		X			X	X			X			
Lambsquarters		X				X						
Palmer amaranth		X		X	X	X						
Prickly sida		X										
Pigweed spp. (redroot, smooth, Powell, waterhemp)		X			X	X		X	X		X	
Russian thistle		X										
Shattercane		X										
Smartweed spp. (Pennsylvania, ladysthumb)						X						
Sunflower		X										
Velvetleaf						X						
Yellow nutsedge		X										

[1](Heap 2014a)
[2]Weed species listed are only those common in maize

VIII.F.3. Introduction of Dicamba and Glufosinate-Tolerant Maize – MON 87419

Monsanto has developed a new herbicide-tolerant maize, MON 87419, that can provide growers additional options for an effective and sustainable weed management system. MON 87419 contains dicamba-tolerant and glufosinate-tolerant traits. MON 87419, with the dicamba-tolerant trait, will provide improved crop tolerance and facilitate more effective use rates of dicamba for preemergence and in-crop postemergence applications compared to currently labeled uses of dicamba in conventional maize hybrids. Dicamba and glufosinate herbicides are currently labeled for use as preplant and postemergence applications in maize. MON 87419 with the dicamba-tolerance trait will provide improved crop tolerance and provide more effective preemergence and postemergence control of problem weed species compared to currently labeled applications of dicamba in conventional maize hybrids. MON 87419 will likely be combined with Roundup Ready® Corn 2 technology utilizing traditional breeding techniques. The combination of herbicide-tolerance traits will allow the preemergence and postemergence use of dicamba, glufosinate and glyphosate herbicides in an integrated weed management program to control a broad spectrum of grass and broadleaf weed species (Johnson et al. 2010). Dicamba will improve the control of hard to control broadleaf weeds in combination with glyphosate (e.g., hemp sesbania, morningglory species, prickly sida¸ and wild buckwheat) and also offer an effective control option for glyphosate-resistant broadleaf weed species, namely marestail, common ragweed, giant ragweed, palmer pigweed, and waterhemp spp. (Johnson et al. 2010). Dicamba and glufosinate will also offer an effective control option for broadleaf species resistant to acetolactate synthatse (ALS) and protoporphyrinogen oxidase (PPO) chemistries. With the introduction of MON 87419, growers will have the ability to continue to use established maize production practices including crop rotation, tillage systems, labeled herbicides, and row spacing, thereby using the same planting and harvesting machinery currently being utilized. As MON 87419 will likely be stacked with Roundup Ready® Corn 2 technology, growers will also continue to have the flexibility and simplicity in weed control provided by glyphosate that will allow growers to continue to reap the environmental benefits associated with the use of conservation-tillage that is facilitated by the use of glyphosate for postemergence weed control in the Roundup Ready® Corn 2 maize system (CDMS 2015).

Glufosinate is currently authorized by U.S. EPA and labeled for preplant applications prior to planting or prior to emergence on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on glufosinate-tolerant hybrids only (CDMS 2015). Glufosinate use in MON 87419 will not change from current labeled uses of glufosinate.

Monsanto will petition U.S. EPA to increase the maximum use rate of dicamba in maize from 0.5 to 1.0 lbs. a.e. of dicamba per acre for preemergence applications and up to two applications of 0.5 lbs. a.e. of dicamba per acre for postemergence applications through the V8 growth stage or maize height of 30 inches, whichever comes first. The combined maximum annual application rate of dicamba on MON 87419 would be 2.0 lbs. a.e. dicamba per acre per year.

As previously noted, glufosinate is currently authorized by U.S. EPA and labeled for preplant and in-crop postemergence applications in maize hybrids designated as glufosinate-tolerant (CDMS 2015). Glufosinate can also be applied as a burndown treatment prior to planting or prior to emergence of any conventional or non-glufosinate herbicide-tolerant maize hybrids. Once MON 87419 is available, growers will be able to apply glufosinate alone or tank-mixed with dicamba for preplant or postemergence in-crop applications on MON 87419. Over-the-top postemergence application rates and timings for glufosinate alone would be the same as currently labeled for glufosinate use in glufosinate-tolerant hybrids (i.e., from emergence up to the V7 growth stage at up to 0.402 lbs. a.i./acre, seasonal maximum of 0.80 lbs. a.i. per acre) (CDMS 2015). MON 87419 with the glufosinate tolerance trait will provide an additional mechanism-of-action to manage glyphosate- and other herbicide-resistant weed populations.

MON 87419 will likely be stacked with Roundup Ready® Corn 2 maize technology (e.g., NK603, MON 88017, and MON 87411). Following authorization by U.S. EPA, the anticipated use patterns for dicamba on MON 87419 will vary slightly across U.S. maize growing regions. This variability will be dictated by variations in weed spectrum, tillage systems, and environment across these regions. The general recommendations for all the regions in the U.S. are shown in (Table VIII-7). A preemergence residual herbicide is recommended regardless of tillage system to 1) reduce early weed competition and 2) to ensure that at least two effective herbicide mechanisms-of-action are used in maize and to provide protections against additional resistance development to existing maize herbicides. This is consistent with Monsanto and academics recommendations for a comprehensive weed resistance management program. Conventional and conservation tillage (reduced or no-till) planted acres with hard-to-control weed species and no glyphosate-resistant weeds are expected to receive a single in-crop application per season of dicamba at 0.5 lbs. a.e. per acre. All acres with glyphosate-resistant weedspecies present regardless of tillage system are expected to receive up to two applications of dicamba (one preplant application at 0.5 lbs. a.e. per acre and one in-crop application at 0.5 lbs. a.e. per acre). These recommendations are a high-end estimate of anticipated dicamba use associated with MON 87419 combined with glyphosate-tolerant maize. Dicamba use preemergence may not be necessary depending on the weed species, the degree of infestation, and/or the preemergence soil residual.

Table VIII-7. Anticipated Weed Management Recommendations for MON 87419 Combined with Glyphosate-Tolerant Maize Systems[1]

Application Timing	Conventional Tillage		Conservation Tillage (No-till or reduced till)	
	Hard To Control Weeds[2]	GR[3] Weeds and Hard to Control Weeds[3]	Hard To Control Weeds[2]	GR Weeds and Hard to Control Weeds[3]
Preemergence (burndown, at planting)	Residual	Residual + Dicamba	Glyphosate + Residual	Glyphosate + Residual + Dicamba
Postemergence	Glyphosate + Dicamba	Glyphosate + Dicamba	Glyphosate + Dicamba	Glyphosate + Dicamba

[1] The anticipated use patterns represent a high-end estimate for potential dicamba use associated with MON 87419 combined with glyphosate-tolerant maize. Actual weed control practices by growers will vary depending on the specific weed spectrum and agronomic situation of the individual maize field, specifically dicamba use could be lower especially for the preemergence applications. MON 87419 allows glufosinate as another viable option available to growers for in-crop post-emergence applications.
[2] Hard to control weeds namely, morningglory species, hemp sesbania, prickly sida¸ and wild buckwheat.
[3] GR = glyphosate resistant
[4] Recommendations for all fields will assume GR weeds are present.

Upon integration of MON 87419 into the Roundup Ready® Corn 2 system, growers will have the ability to continue use of established maize production practices including tillage systems; the same planting and harvesting machinery; traditional management of insects, diseases, and other pests; and many of the current herbicides used for weed control, including glyphosate with its established environmental benefits.

VIII.F.4. MON 87419 as a Component in Weed Resistance Management

Although herbicide resistance may eventually occur in a weed species when an herbicide is widely used, resistance can be delayed, contained, and managed through good management practices, research and education. The addition of dicamba and glufosinate tolerance to glyphosate-tolerant maize will facilitate the utilization of additional herbicide mechanisms-of-action in a grower's weed control system, and reduce the potential for further resistance development to glyphosate, dicamba, and glufosinate, as well as other important maize herbicides. Current research results conducted by Monsanto indicate that the application of a soil-active residual herbicide followed by an in-crop early postemergence application of dicamba that may be tank-mixed with glyphosate can optimize weed control and be consistent with best weed resistance management practices. Such a program would ensure the use of two or more mechanisms-of-action against the targeted weeds. In areas with glyphosate-resistant and hard to control broadleaf weed

populations, dicamba may also be tank mixed with the preplant applicaton of residual herbicide. This is not expected to increase selection pressure against either herbicide since the preplant weed spectrum is generally different from the in-crop spectrum.

Stewardship of dicamba and glufosinate to preserve their usefulness for growers is an important aspect of Monsanto's stewardship commitment, as is discussed in Appendix I. Specifically, Monsanto has implemented and will continue to develop and proactively provide weed resistance management practices[4], and will utilize multiple methods to distribute technical and stewardship information to growers, academics, and grower advisors through a variety of communication tools. Monsanto's Technology Use Guide (TUG) will set forth the requirements and best practices for the cultivation of MON 87419 including recommendations on weed resistance management practices. Growers purchasing products containing MON 87419 are required by the Monsanto Technology Stewardship Agreement (MTSA) to read and follow the TUG. Furthermore, Monsanto is committed to actively evaluate herbicide performance and weed efficacy on a continuing basis, and develop additional mitigation plans as necessary to manage resistance development for glyphosate, dicamba, and glufosinate. In addition, U.S. EPA regulates the use of all herbicides used in maize and will review new herbicide uses on MON 87419 maize. As part of the U.S. EPA review process additional conditions may be applied by EPA.

[4] Weed resistance management guidelines available at http://www.weedtool.com and http://www.monsanto.com/weedmanagement/Pages/default.aspx

VIII.F.5. Introduction of Dicamba and Glufosinate-Tolerant Maize - MON 87419 - Conclusion

Integration of MON 87419 into glyphosate-tolerant maize hybrids will allow the use of dicamba, glufosinate, and glyphosate herbicides in an integrated weed management program to control a broad spectrum of grass and broadleaf weed species in maize. These herbicides will also provide three distinct mechanisms-of-action for an effective proactive (to delay selection of additional herbicide resistant weeds) and reactive (to manage weed populations that have developed resistance) weed resistance management program in maize. Due to the crop safety of dicamba and glufosinate when used with dicamba and glufosinate tolerant MON 87419, growers will be afforded two effective herbicide mechanisms-of-action for in-crop control of glyphosate's hard to control and resistant broadleaf weeds that are present in U.S. maize production.

Furthermore, the integration of MON 87419, along with the glyphosate-tolerant maize trait, will provide growers with the ability to continue use of established maize production practices including tillage systems; the same planting and harvesting machinery; traditional management of insects, diseases, and other pests; and many of the current herbicides used for weed control, including glyphosate with its established environmental and grower benefits. Therefore, it is anticipated that the commercialization of MON 87419 in the U.S. is not likely to impact current maize agronomic practices, cultivation or seed production practices, beyond the intended benefits of more effective and improved management of common and troublesome weeds, including herbicide-resistant weeds.

VIII.G. 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). While there are tangible benefits from crop rotations, many other factors such as 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. Approximately 30% of the U.S. maize acres are rotated back to maize and 57% are rotated to soybean the following year (Table VIII-8). Wheat and cotton are other significant rotational crops with approximately 5% and 2%. Table VIII-8 provides an assessment of the dicamba, glufosinate and glyphosate herbicide use in each of the rotational crops following maize at the U.S. country level. For the purpose of this assessment, a 50% adoption rate in U.S. maize production was assumed for MON 87419 and all these acres would receive an application of dicamba. The adoption rate for glyphosate-tolerant alfalfa was assumed to be 50% also since it has

only been available a short time. Since MON 87708 soybean and MON 87701 cotton also contain the dicamba-tolerance trait and received a determination of nonregulated status (USDA-APHIS Petitions #10-188-01p and #12-185-01p), the 50% adoption rate was assumed for these products also and all these acres would receive an application of dicamba. With these adoption rate assumptions and the current useage of dicamba in other rotational crops, the usage of dicamba in rotational crop acres following maize is approximately 45% for the U.S. In comparison the usage of glyphosate in rotational crop acres is approximately 83%.

Introduction of MON 87419 is not, however, expected to impact crop rotation practices any more so than current biotechnology-derived herbicide tolerant products available to growers.

Table VIII-2. Rotational Practices in the U.S. Following Maize Production

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
United States 95,365	Corn	28,291	29.7	50	14146	1	302	82	23310			
	Soybean	54,451	57.1	50	27226	2	1349	95	51586			
	Wheat	4,527	4.7	8	355	NL		17	751			
	Cotton	1,870	2.0	50	935	21	397	85	1597			
	Alfalfa[8]	1,303	Herbi1.4	NL		NL		50	652			
	Other Hay	1,118	1.2	NL		NL		NA	0			
	Sorghum	799	0.8	10	82	NL		42	336			
	Oats	469	0.5	0	0	NL		0	0			
	Sugar Beets	455	0.5	NL		0	0	100	455			
	Sunflower	453	0.5	11	52	NL		85	386			
	Barley	320	0.3	3	8	NL		12	39			
	Peanut	281	0.3	NL		NL		28	78			
	Vegetables[9]	283	0.3	NL		NL		NA	0			
	Dry Beans	273	0.3	NL		NL		25	69			
	Potatoes	213	0.2	NL		NL		12	25			
	Tobacco	140	0.1	NL		NL		5	7			
	Millet	99	0.1	NL		NL		0	0			
	Rice	16	0.02	NL		NL		50	8			
	Safflower	6	0.01	NL		NL		0	0			
		Total[10]: 95,365			**Total: 42804**		**Total: 2048**		**Total: 79214**	**44.9**	**2.1**	**83.1**

This table was developed by compiling the data from all four regional summaries. All acreages are expressed as 1000s of acres.
NL indicates not labeled for use. NA indicates not available

1 Maize acreage based on 2013 planting data (USDA-NASS 2014a).

2 Column C is obtained by compiling the data from the four regional summaries.

3 Column D is obtained by dividing Column C by Column A.

[4] Column E is obtained by dividing Column F by Column C; Column F is obtained by compiling the data from all five regional summaries.
[5] Column G is obtained by dividing Column H by Column C; Column H is obtained by compiling the data from all five regional summaries.
[6] Column I is obtained by dividing Column J by Column C; Column J is obtained by compiling the data from all five regional summaries
[7] Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total; Column M is obtained by dividing Column J Total by Column C Total.
[8] Newly seeded alfalfa.
[9] Vegetables: chili peppers, cantaloupe, watermelon, tomatoes, onions, snap beans, sweet corn, cabbage, lima beans cucumbers, bell peppers, squash, green peas, carrots.
[10] Totals may not be exact due to rounding.

VIII.H. Maize Volunteer Management

Volunteer maize is defined as a plant that germinates and emerges unintentionally in a subsequent crop. Volunteer maize commonly occurs in rotational crops in the season following cultivation of maize. Viable grain is not produced on the approximately 6.2 million of U.S. maize acres that is 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, the occurrence of 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 the Northern maize-growing regions, volunteer maize does not always occur in the rotational crop because of seed decomposition over the winter, efficient harvest procedures, and tillage prior to planting rotational crops.

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 maize grain lost in the field; 2) planting maize hybrids that reduce the extent of ear drop; 3) choosing maize hybrids with superior stalk strength and reduced lodging; and 4) practicing no-till production to significantly reduce the potential for volunteer growth in the rotational crop. If volunteer maize does occur in subsequent crops, preplant tillage and in-crop cultivation are very effective management tools. In addition, several postemergence herbicides also are available to control volunteer maize (conventional or glyphosate-tolerant maize, and by extension dicamba-tolerant maize) in each of the major maize rotational crops. Because of these control measures and field evaluations which confirmed that MON 87419 has equivalent volunteer potential as other maize, the introduction of MON 87419 plus Roundup Ready® Corn 2 will not pose new concerns about managing volunteer maize nor will it result in any greater dependence on preplant or in-crop tillage and cultivation because there are adequate alternative herbicide options. Table VIII-9 provides a summary of labeled selective postemergence herbicides for the effective control of volunteer maize in specific crops and include Assure II® (quizalofop), Fusilade® DX (fluazifop), Fusion® (fluazifop + fenoxaprop), Poast5® (sethoxydim), and Select® 2EC (clethodim). These herbicides are labeled for use in 12 vegetable rotation crops and 10 field crops that include soybean, cotton, sugar beet and alfalfa.

The availability of multiple herbicidal and cultivation methods for controlling volunteers, as well as the demonstrated lack of difference in germination of MON 87419 compared to conventional maize (see Section VII.C.1), the introduction of MON 87419 into the Roundup Ready® Corn 2 system is not expected to impact the management of maize volunteer plants.

® Assure II is a trademark of E.I. DuPont de Nemours, Inc.
® Fusilade and Fusion are trademarks of Syngenta Group Company.
® Poast is a trademark of BASF Corporation.
® Select is a trademark of Valent U.S.A. Corporation.

Table VIII-3. Herbicides Labeled for Control of Volunteer Maize in Labeled Rotational Crops[1]

Crop	Assure II	Fusilade DX	Fusion	Poast	Select 2EC
Soybeans	x	x	x	x	x
Hay				Alfalfa, Clover	Alfalfa, Clover
Cotton	x	x	x	x	x
Sugar Beets	x	x		x	x
Sunflower	x			x	x
Peanuts		x		x	x
Dry Beans	x	x		x	x
Lentils	x			x	
Potatoes				x	x
Sweet Potatoes				x	x
Vegetables					
Cabbage					x
Cantaloupe					x
Carrots		x			x
Cucumbers					x
Leaf Lettuce				x	x
Peas, green	x			x	
Peppers, Chili				x	x
Peppers, Tabasco					x
Onions		x			Bulbs only
Snap Beans	x				
Tomatoes				x	x
Watermelon					x

[1]Source: (CDMS 2015)

VIII.I. Stewardship of MON 87419

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). These 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 communicate stewardship requirements and best practices.

As an integral action of fulfilling this stewardship commitment, Monsanto will meet applicable regulatory requirements for MON 87419 in the country of intended production and for key import countries identified in the trade assessment process that have functioning regulatory systems to assure global compliance and support the flow of international trade. These actions will be consistent with the ETS Guide for Product Launch Stewardship of Biotechnology-Derived Plant Products (ETS 2013), the BIO Product Launch Stewardship policy (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 re-evaluate its stewardship plans and make appropriate modifications to minimize the potential for trade disruption.

Monsanto also commits to industry best practices on seed quality assurance and control to ensure the purity and integrity of MON 87419 maize hybrid seed. As with all of Monsanto's products, before commercializing MON 87419 in any country, a MON 87419 detection method will be available to maize producers, processors, and buyers.

The dicamba and glufosinate-tolerant maize system will enable a higher use rate of dicamba herbicide in maize production. Monsanto is seeking regulatory approvals with the U.S. EPA for the higher use rate of dicamba herbicide as a weed control tool in mazie. Furthermore, Monsanto will establish appropriate dicamba Maximum Residue Levels (MRLs) for key maize import countries where necessary. No additional regulatory approvals with U.S. EPA will be required for glufosinate products for use in MON 87419.

Stewardship of dicamba and glufosinate, to preserve their usefulness for growers, is also an important aspect of Monsanto's stewardship commitment. Detailed information regarding dicamba and glufosinate weed resistance and the usefulness of dicamba and glufosinate-tolerant maize in combination with glyphosate-tolerant maize to address herbicide-resistance issues is presented in Section VIII-F and Appendix I.

VIII.J. Impact of the Introduction of MON 87419 on Agricultural Practices

MON 87419 has been developed to faciliate greater choices for growers implementing effective weed management including tough to control and herbicide resistant broadleaf weeds. The ability to use dicamba and glufosinate herbicides with two unique mechanisms-of-action can be part of an effective weed management system for maize production in the U.S. As dicamba and glufosinate are already labelled for use in maize (Clarity®: EPA Reg No. 7969-137, Liberty®: EPA Reg No. 264-660), the introduction of MON 87419 is not expected to have adverse 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 87419.

MON 87419 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 87419 is expected to continue to provide benefits to growers, that include reduced use of insecticides, increased yield protection and opportunity, soil conservation, and increased worker safety.

IX. PLANT PEST ASSESSMENT

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 87419 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 fungas; (F) a virus or viroid; or (G) an infectious agent or other pathogens (7 U.S.C. § 7702[14]).

The regulatory endpoint under the USDA-APHIS 7 CFR §340 regulations 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 potential for 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 87419 is not expected to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR § 340.

IX.B. Plant Pest Assessment of MON 87419 and Expressed Protein

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the DMO and PAT proteins expressed in MON 87419 used to evaluate the food, feed, and environmental safety of MON 87419.

IX.B.1. Characteristics of the Genetic Insert and Expressed Protein

IX.B.1.1. Genetic Insert

As described in Section III, MON 87419 was developed by *Agrobacterium*-mediated transformation of maize embryos using plasmid vector PV-ZMHT507801. Characterization of the DNA insert in MON 87419 was conducted using a combination of sequencing, PCR, and bioinformatics methods. The results of this characterization demonstrate that MON 87419 contains one copy of the intended transfer DNA containing the *dmo* and *pat* expression cassettes that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple breeding generations. These

methods also confirmed that no vector backbone or other unintended plasmid sequences are present in MON 87419. Additionally, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87419 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 87419 upon DNA integration.

IX.B.1.2. Mode-of-Action

MON 87419 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide and *pat* gene from *Streptomyces viridochromogenes* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate herbicide. DMO protein rapidly demethylates dicamba to the herbicidally inactive metabolite 3,6-dichlorosalicylic acid (DCSA), a well known metabolite of dicamba in conventional cotton, soybean, livestock, and soil (FAO-WHO 2011b; a; U.S. EPA 2009). PAT protein acetylates the free amino group of glufosinate to produce non-herbicidal N-acetyl glufosinate, a well known metabolite in glufosinate-tolerant plants (OECD 2002c).

IX.B.1.3. Protein Safety and Expression Levels

The safety and expression of the DMO and PAT proteins are detailed in Section V. Expression levels were determined from four tissue types from trials conducted in 2013 in the U.S. and are presented in Section V.C. The expression in the various tissues ranged from 0.14 µg/g dw to 37 µg/g dw for DMO and 0.56 µg/g dw to 17 µg/g dw for PAT. Both the DMO and PAT proteins have been assessed in multiple products by USDA-APHIS and U.S. FDA in past years. DMO protein is produced in both MON 87708 soybean and MON 88701 cotton that were granted nonregulated status in 2015. Additionally, starting in 1996 with Bayer's T25 maize, a number of glufosinate tolerant crops (canola, cotton, maize, soybean, sugar beet) containing PAT protein have been granted nonregulated status by USDA-APHIS. After either extensive testing and/or wide scale commercial cultivation, in no instance have adverse impacts to NTOs been associated with exposure to DMO or PAT proteins from these biotechnology-derived crops. PAT protein has an established histories of safe use, having been assessed by USDA, FDA and U.S. EPA on multiple occasions (Section V.E.). Neither protein originates from an organism known to be a source of allergens, a bioinformatic assessment of each shows no shared amino acid sequence similarities to known allergens (Section V). Taken together, the results of these analyses support a determination that MON 87419 is no more likely to pose a plant pest risk than conventional maize.

IX.B.2. Compositional Characteristics

Compositional comparisons of MON 87419 followed considerations relevant to the compositional quality of maize as defined by the OECD consensus document (OECD 2002a) were presented in Section VI. Grain samples were analyzed for levels of nutrients including proximates (protein, fat, ash, moisture), amino acids (18 components), fatty acids (22 components), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [A (β-

carotene), B1, B2, B6, E (α-tocopherol), niacin, and folic acid]. The anti-nutrients analyzed in grain were phytic acid and raffinose. Secondary metabolites analyzed in grain were furfural, ferulic acid, and p-coumaric acid. Forage samples were analyzed for levels of proximates, carbohydrates by calculation, fiber (ADF, NDF), and minerals (calcium, and phosphorus). In all, 78 different components were analyzed.

Of the 78 measured components, copper, furfural, and 13 fatty acids (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, heptadecanoic, heptadecenoic, gamma linolenic, eicosadienoic, eicosatrienoic, and arachidonic acids) had more than 50% of the observations below the assay limit of quantitation (LOQ) and were excluded from the statistical analyses. Moisture values for grain and forage were measured for conversion of components from fresh to dry weight, but were not statistically analyzed. Therefore, 61 components were statistically analyzed (53 in grain and eight in forage).

Of the 61 components statistically assessed, 60 showed no significant differences between MON 87419 and the conventional control. One component (manganese in grain) showed a significant difference between MON 87419 and the conventional control. For this one component, the mean difference in the component values between MON 87419 and the conventional control was less than the range value of the conventional control. The MON 87419 mean component value was also within the range of values observed in the literature and the ILSI-CCDB. These data indicated that the statistically significant difference for manganese in grain was not compositionally meaningful from a food and feed safety perspective.

These results support the overall conclusion that MON 87419 was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 to the conventional control in levels of these components.

IX.B.3. Phenotypic, Agronomic, and Environmental Interaction Characteristics

An extensive set of comparative plant characterization data were used to assess whether the introduction of dicamba and glufosinate herbicide tolerance traits altered the plant pest potential of MON 87419 compared to the conventional control (Section VII). Phenotypic, agronomic, and environmental interaction characteristics of MON 87419 were evaluated and compared to those of the conventional control. As described previously, these assessments included: seed germination and dormancy 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 87419 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 87419 is no more likely to pose a plant pest risk than conventional maize.

IX.B.3.1. Seed Germination and Dormancy

A comparative assessment of seed germination and dormancy characteristics was conducted on MON 87419 and the conventional control. The results of this assessment, including the lack of biologically meaningful differences and particularly the lack of increased hard seed, support the conclusion that the introduction of the dicamba and glufosinate-tolerance trait is not expected to result in increased plant pest/weed potential of MON 87419 compared to conventional maize.

IX.B.3.2. 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 and yield were assessed. In the combined-site analysis, no statistically significant differences were detected between MON 87419 (both treated and not treated with dicamba and glufosinate) and the conventional control for any of the assessed characteristics not treated with dicamba and glufosinate. Thus, the phenotypic and agronomic characteristics of MON 87419 not treated and treated with dicamba and glufosinate were not altered in terms of pest/weed potential compared to conventional maize.

IX.B.3.3. 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 87419 and the conventional control for percent viable pollen or pollen grain diameter. Additionally, no visual differences in general pollen morphology were observed between MON 87419 and the conventional control. Based on the assessed characteristics, the results support a conclusion that neither pollen viability nor morphology of MON 87419 were altered and therefore do not contribute to an increased pest/weed potential compared to conventional maize.

IX.B.3.4. Interactions with Non-target Organisms Including Those Beneficial to Agriculture

Evaluation of MON 87419 for potential adverse impacts on NTOs is a component of the plant pest risk assessment. Since MON 87419 is a product with no pesticidal activity, all organisms that interact with MON 87419 are considered to be NTOs. In 2013 U.S. phenotypic and agronomic assessment, observational data on environmental interactions were collected for MON 87419 and the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each characteristic. The environmental interactions assessment (Section VII.C.2.2.2) included data collected on plant-arthropod and plant-disease interactions. The results of this assessment indicated that the presence of the dicamba and glufosinate tolerance traits did not meaningfully alter plant-arthropod interactions,

including beneficial arthropods and arthropod pests, nor did it alter disease susceptibility of MON 87419 compared to conventional maize. The lack of biologically meaningful differences in disease damage, arthropod-related damage, and pest and beneficial arthropod abundance demonstrate that the introduction of the dicamba and glufosinate tolerance traits is unlikely to be biologically meaningful in terms of increased plant pest potential.

The potential for MON 87419 to harm NTOs was evaluated using a combination of biochemical information and experimental data. The biochemical information and experimental data included molecular characterization, the MON 87419 DMO safety assessments, the history of environmental exposure to mono-oxygenases (the class of enzymes to which DMO belongs), results from the environmental assessment described above, and the demonstration of compositional, agronomic and phenotypic equivalence to conventional maize. Taken together, the data support the conclusion that MON 87419 is unlikely to adversely affect NTOs, or pose an additional risk to threatened or endangered species or their designated critical habitat above those posed by the cultivation of conventional maize.

According to USDA-APHIS (2014), "Corn possesses few of the characteristics of successful weeds, and has been cultivated around the globe without any report that it is a serious weed or that it forms persistent feral populations." USDA-APHIS (2014) also concluded that none of the listed threatened or endangered plant species or plants proposed for listing in the states where maize is grown are in the same genus or are known to cross pollinate with species of the genus *Zea*, and therefore, maize would not be sexually compatible with any of these listed plant species. Because MON 87419 has been shown to be agronomically and phenotypically equivalent to conventional maize without increased weediness potential, the planting of MON 87419 is not expected to affect listed threatened or endangered plant species or designated critical habitat for listed plant or animal species.

The potential for maize to be a host plant (required by a listed species to complete a portion of its lifecycle) has also been considered. USDA-APHIS (2014) indicates that none of the listed species in states where maize is grown require maize as a host plant. Furthermore, according to USDA-APHIS (2014) and U.S. EPA (2014b; a), there are only a limited number of threatened or endangered species that may be found in maize fields, and there is an even more limited number of species that might feed on maize plants or maize grain. The safety of the MON 87419 DMO, and the compositional, agronomic and phenotypic equivalence of MON 87419 to conventional maize, support a conclusion that no biologically significant changes to the habitat or diet of threatened or endangered species are expected. Consequently, the planting of MON 87419 is not expected to affect listed threatened or endangered species.

IX.C. Weediness Potential of MON 87419

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 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 87419 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 87419 and the conventional control for traits potentially associated with weediness. Furthermore, comparative field observations between MON 87419 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 87419 has no increased weed potential compared to conventional maize and it is no more likely to become a weed than conventional maize.

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

Pollen-mediated gene flow (cross pollination) is the first step towards introgression which is the transfer of one or more genes from one plant population to another. Pollen-mediated gene flow and introgression are natural biological processes and do not constitute inherent environmental risks. Gene introgression must be considered in the context of the trait in the biotechnology-derived plant, and the likelihood that the presence of the trait and its subsequent transfer to recipient plants will result in increased plant pest potential. The potential for pollen-mediated gene flow from MON 87419 to other cultivated maize and the potential for introgression of the MON 87419 trait to species that can outcross with maize are discussed below.

IX.D.1. Hybridization with Cultivated Maize

Maize is a wind pollinated species with plant morphology that facilitates cross pollination. Therefore, relatively high levels of pollen-mediated gene flow can occur in this species at short distances (Jones and Brooks 1950). Some biotic and abiotic factors that may influence the amount of pollen-mediated gene flow in maize include: (1) wind direction and speed; (2) distance between the pollen-source and pollen-recipient plants; (3) environmental factors that may impact pollen viability and dispersal (e.g. temperature and relative humidity); (4) duration of pollen shed and (5) floral synchrony between pollen donor and pollen recipient.

The results from several studies conducted on the extent of pollen-mediated gene flow between maize fields demonstrate consistent trends regardless of the experimental design, world region, or detection method. The amount of pollen-mediated gene flow is greatest within the first few meters and decreases sharply with increasing distance from the pollen source (Table IX-1). The distance >200 m (660 feet) is used for managing gene flow during breeding, seed production, identity preservation or other applications; in addition, it forms the basis for the USDA-APHIS performance standards for maize. All U.S.

testing and production of regulated MON 87419 seed or grain have been conducted under USDA notification according to these standards. Since no meaningful differences were observed for MON 87419 in nutritional value, composition analysis, or in pest/weed potential in field evaluations, gene flow from commercial production of MON 87419 to other maize is not different than conventional maize in terms of concern or hazard.

Table IX-1. Summary of Published Literature on Maize Outcrossing (Cross Pollination) Relative to Distance between Pollen Source and Pollen Recipient

Distance (m)	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 over three years. Pollen source was a yellow dent and the pollen recipient 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 over two years. A purple gene marker was utilized to measure pollen mobility.	México	(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	Frequencies of outcrossing by distance over three years and three sites. Single male and female per location.	Canada	(Ma et al. 2004)
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	Frequencies of outcrossing by distance over two years and two sites was quantified by measuring cross pollination of a conventional grain production field by a transgenic hybrid plot. A combination of three marker genes was utilized to detect outcrosses: y1 (seed color gene), *Bt* and glyphosate tolerance.	USA	(Goggi et al. 2006)

Table IX-1 (continued). Summary of Published Literature on Maize Outcrossing (Cross Pollination) Relative to Distance between Pollen Source and Pollen Recipient

Distance (m)	Outcrossing (%)	Comments	Country	Reference
~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	Frequencies of outcrossing by distance at one year and one site. A comparison of a PCR based method to phenotypic method to measure outcrossing. Four *Bt* hybrids and a single non-*Bt* hybrid were used as a pollen donor and recipient, respectively.	Spain	(Pla et al. 2006)
2 5 10 20 25 50 150 200	34.9 9.9 12.2 8.2 4 5.9 5.4 0.24	Frequencies of outcrossing were conducted on a large farm scale evaluation across the UK. Outcrossing was quantified by using a quantitative PCR assay specific to the HT (pat) gene. Values reported 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	Frequencies of outcrossing by distance by the frequency of yellow kernels in 13 neighboring white kernel maize fields.	Switzerland	(Bannert and Stamp 2007)

IX.D.2. Hybridization with Annual Teosinte

For gene flow to occur by typical sexual transmission, the following conditions must exist: (1) the two parents must be sexually compatible; (2) there must be flowering synchrony between the pollen source and pollen recipient; (3) the plants must be within sufficient proximity to each other; and (4) suitable environmental factors, such as relative humidity, temperature, or wind, must be present.

Maize is sexually compatible with certain subspecies of annual teosinte (e.g., *Zea mays* subsp. *mexicana*). Teosinte and maize can cross pollinate when growing in close proximity to each other, e.g. in areas of Mexico (Wilkes 1972). For example, in experiments where maize and teosinte (*Zea mays* subsp. *mexicana*) were planted side by side, very low hybridization rates were observed (1-2% or less) (Baltazar et al. 2005; Ellstrand et al. 2007). Hybrids between teosinte and maize are not expected in the U.S. because teosinte does not naturally grow in the U.S. Therefore, natural outcrossing between teosinte and maize in the U.S. is highly unlikely.

IX.D.3. Hybridization with *Tripsacum*

Tripsacum is a genus with 16 recognized species (Gómez Montiel et al. 2008). There are three species of *Tripsacum* that occur naturally in the U.S.: *T. floridanum* (Florida gamagrass) (USDA-NRCS 2014a; Wunderlin and Hansen 2014), *T. lanceolatum* (Mexican gamagrass) (USDA-NRCS 2014c), and *T. dactyloides* (Eastern gamagrass) (USDA-NRCS 2014b).

Tripsacum floridanum (Florida gamagrass), naturally grows in the extreme southern Florida counties of Miami-Dade, Collier, Martin, and Monroe (Wunderlin and Hansen 2014). Florida gamagrass has been described as rare and occurring in shallow soils in low rocky crevices in pinelands (Blakey et al. 2007) and it has been categorized as a threatened species by the state of Florida (USDA-NRCS 2014a). *Tripsacum lanceolatum* (Mexican gamagrass) has been reported in Arizona and New Mexico (USDA-NRCS 2014c) and is found on moist escarpments and stream banks (de Wet and Harlan 1978). *Tripsacum dactyloides* (Eastern gamagrass) is found primarily throughout the eastern U.S. It has been categorized as endangered in Massachusetts and Pennsylvania and as threatened in the state of New York (USDA-NRCS 2014b).

To our knowledge, hybrids between maize and *Tripsacum* do not occur in nature. The formation of hybrids between maize and *Tripsacum* species requires human intervention under specific controlled laboratory conditions, and the hybrids are male sterile even after several backcrosses to maize (Russell and Hallauer 1980). Thus, no species of *Tripsacum* is expected to form viable hybrid progeny with maize under natural conditions.

In summary, although hybrids between maize and *Tripsacum* have been produced using specialized laboratory techniques, there is no documentation that hybrids form in nature. Therefore, gene flow from maize to any *Tripsacum* species is extremely unlikely. Under natural conditions, as is the case with conventional maize, pollen-mediated gene flow from MON 87419 to any species of *Tripsacum* is not expected.

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

Monsanto is not aware of any reports confirming the transfer of genetic material from maize to sexually-incompatible plant species in the environment. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk associated with horizontal gene flow from MON 87419 due to the presence of the dicamba and glufosinate-tolerance trait is not expected. Even if it were to occur, the consequence of horizontal gene flow of the dicamba and glufosinate-tolerance trait into other plants that are sexually-incompatible is expected to be negligible because, as data presented in this petition confirm, the gene and trait confer no increased plant pest potential to maize. Therefore, any plants receiving the trait through horizontal gene flow would also not be expected to exhibit any increased plant pest potential. In the highly unlikely event that horizontal gene transfer were to occur, the presence of the dicamba and glufosinate-tolerance trait would not be expected to increase pest potential in the recipient species.

IX.E. Potential Impact on Maize Agronomic Practices

An assessment of current maize agronomic practices was conducted to determine whether the cultivation of MON 87419 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 *dmo* and *pat* coding sequence that provides tolerance to dicamba and glufosinate herbicides, MON 87419 is similar to other maize hybrids available commercially in the U.S.

The data presented demonstrate that MON 87419 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 87419 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.F. 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 87419 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, including products with DMO (MON 87708 and MON 88701) and many products with PAT proteins. 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 biotechnology-derived maize product, is based upon several facts. Namely: 1) stability of the genetic inserts is confirmed in each approved biotechnology-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 biotechnology-derived products (Pilacinski et al. 2011).

Therefore, based on the considerations above and the conclusion that MON 87419 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 87419 and conventional maize or deregulated biotechnology-derived maize are no more likely to pose a plant risk than commercially cultivated maize.

IX.G. 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 fungas; (F) a virus or viroid; (G) an infectious agent or other pathogens; or (H) any article similar to or alied with any of the articles specified in the preceeding subparagraphs (7 U.S.C. § 7702[14]). Characterization data presented in Sections III through VII of this petition confirm that MON 87419, with the exception of dicamba and glufosinate tolerance, 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 87419 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 87419 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 *dmo* and *pat* genes; 2) characterization and safety of the expressed product; 3) compositional equivalence of MON 87419 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, including land use, 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 87419 is not expected to be a plant pest. Results also support a conclusion of no

increased weediness potential of MON 87419 compared to conventional maize. Therefore, Monsanto Company requests a determination from APHIS that MON 87419 and any progeny derived from crosses between MON 87419 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 87419 indicating that there would be adverse consequences from its introduction. MON 87419 produces DMO and PAT proteins, which have been fully characterized, and its safety has been thoroughly assessed in this and previous submissions. As demonstrated by field test results and laboratory tests, the only phenotypic differences between MON 87419 and conventional maize are dicamba and glufosinate tolerance.

The data and information presented in this petition demonstrate that MON 87419 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 *dmo* and *pat* expression cassettes 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 87419 demonstrate that MON 87419 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 87419 is unchanged compared to conventional maize. There is no indication that MON 87419 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 87419 are negligible.

The introduction of MON 87419 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 crop rotational practices, weed control practices and/or volunteer control measures that consider the presence of the herbicide tolerant traits while providing the desired agronomic practice(s).

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APPENDICES

Appendix A: USDA Notifications and Permits

Field trials of MON 87419 have been conducted in the U.S. since 2011. 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 87419 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, from the 2013 - 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.

Table A-1. USDA Notifications and Permits Approved for MON 87419 and Status of Trials Planted

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2011	11-013-103n	2/11/2011	Submitted	HI	1
	11-045-103n	3/16/2011	Submitted	IL	1
	11-045-109n	3/16/2011	Submitted	MS	1
	11-123-104rm	9/1/2011	Submitted	HI	1
	11-152-104n	7/1/2011	Submitted	HI	1
	11-153-103n	7/2/2011	Submitted	PR	1
2012	11-123-104rm	9/1/2011	Submitted	HI	1
	11-291-111rm	2/15/2012	Submitted	IA	1
				IL	1
				KS	1
	11-305-105rm	3/1/2012	Submitted	HI	3
	11-320-105rm	3/15/2012	Submitted	IL	3
				IN	1
	11-326-108rm	3/22/2012	Submitted	IA	5
				IL	6
	12-059-109n	3/28/2012	Submitted	HI	1
	12-059-120n	3/28/2012	Submitted	IL	1
				NE	1
	12-125-106rm	9/1/2012	Submitted	HI	3
				PR	1
2013	12-214-109rm	12/1/2012	Submitted	HI	2
	12-312-103n	12/7/2012	Submitted	HI	1
	12-312-109rm	3/7/2013	Submitted	HI	2
				PR	1
	12-320-109rm	3/15/2013	Submitted	IA	13
	12-320-114rm	3/15/2013	Submitted	IL	22
				KS	5
	12-320-125rm	3/15/2013	Submitted	NE	5
	13-037-106rm	6/1/2013	Submitted	HI	3
	13-044-101rm	3/7/2013	Submitted	HI	2
	13-059-103n	3/30/2013	Submitted	IA	2
				IL	2
	13-064-123n	4/4/2013	Submitted	IA	1
	13-066-105n	4/6/2013	Submitted	AR	1
				IA	1
				IL	2

Table A-1 (continued). USDA Notifications and Permits Approved for MON 87419 and Status of Trials Planted

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2013				IN	1
				KS	1
				MO	1
				NC	1
				NE	1
				PA	2
				WI	1
	13-066-107n	4/6/2013	Submitted	IA	1
				IL	1
				MI	1
				NE	2
				WI	1
	13-119-103n	5/29/2013	Submitted	HI	1
	13-120-107rm	9/1/2013	Submitted	HI	1
	13-120-108rm	9/1/2013	Submitted	HI	1
				PR	1
	13-213-106rm	12/1/2013	In Progress	PR	1
2014	13-213-106rm	12/1/2013	In Progress	HI	1
	13-297-108rm	3/1/2014	In Progress	HI	2
				PR	1
	13-297-109rm	3/1/2014	In Progress	PR	1
	13-301-101n	12/4/2013	In Progress	HI	2
	13-305-105rm	3/1/2014	In Progress	AL	1
				IL	14
				MO	1
	13-305-109rm	3/1/2014	In Progress	MS	1
				NE	3
	13-305-116rm	3/1/2014	In Progress	IA	5
				NE	1
	14-065-101n	4/5/2014	In Progress	AZ	1
				IL	2
				LA	1
				NE	1
				OH	1
	14-065-103n	4/5/2014	In Progress	CA	1
	14-114-101n	5/24/2014	In Progress	HI	1
Grand Total					160

Appendix B: Overview, Materials, Methods, and Supplementary Results for Molecular Analyses of MON 87419

B.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 ^{32}P 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).

B.1.1. 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 B-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 B-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; 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). The 75× coverage used in this method is predicted, based on established and accepted methods (Clarke and Carbon, 1976; Lander and Waterman, 1988) to provide genome coverage that would be expected to not miss a single base pair in complex genomes (Kovalic et al., 2012). Furthermore, even with 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 spiked into conventional control DNA at ~0.03% concentration was sampled using bioinformatic tools at 1 and 1/10 copy genome equivalent ratios. This sampling analysis demonstrated that any portion of the plasmid may be detected at a single copy per genome level and 99.43% coverage at 100% identity at 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 (Table B-1). 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).

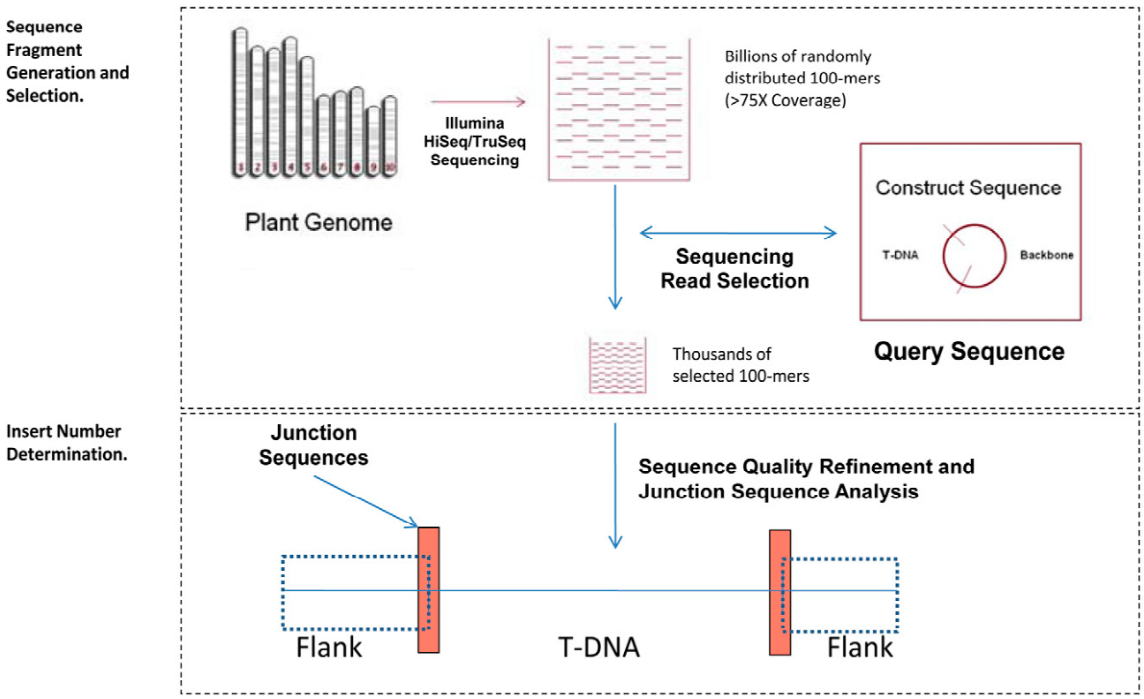

Figure B-1. Sequencing and Sequence Selection

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 (Kovalic et al., 2012) and an example is shown in Figure B-2 below. 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 (Kovalic et al., 2012, Figure 2). By evaluating the number and the sequences of all 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 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, represented 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 in the transformation event.

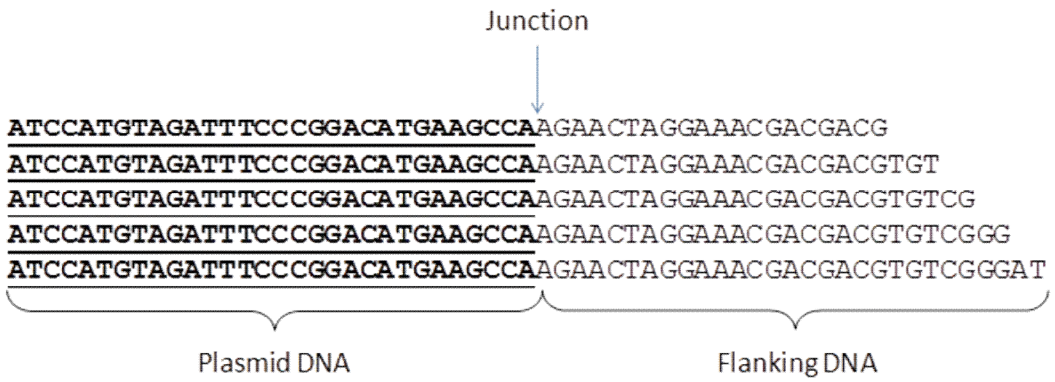

Figure B-2. Junctions and Junction Sequences

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 insert copy number, integrity of the insert, lack of backbone or other unintended plasmid sequences, 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 determines if the sequence of the insert is identical to the corresponding sequence in plasmid vector, if each genetic element in the insert is intact, if the plasmid vector sequence is inserted as a single copy, and establishes no vector backbone or other unintended plasmid sequences were inserted in the event. This comparison allows a determination of whether the T-DNA elements are present in the intended order. 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 B-2), with one each originating from either end of the insert (Figure B-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.

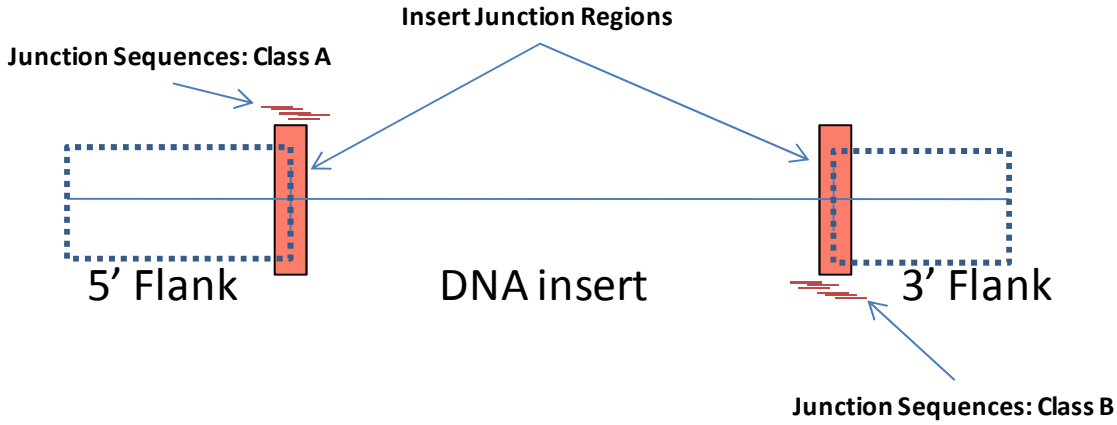

Figure B-3. Two Unique Junction Sequence Classes are Produced by the Insertion of a Single Plasmid Region
A schematic representation of a single DNA insertion within the genome showing the inserted DNA, the 5' and 3' flanks (depicted as areas bounded by dotted lines), and the two distinct regions spanning 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.

B.2. Materials and Methods

B.2.1. Test Substance

The test substance in this study was MON 87419. Five breeding generations of MON 87419 were used to assess the stability of the T-DNA I insert. Genomic DNA for use in this study was extracted from tissue listed in the table below.

MON 87419 Breeding Generation	Seed ORION[6] ID
R_3	11372765
R_4	11356841
R_5	11372740
R_3F_1	11356837
R_4F_1	11372737

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

B.2.2. Control Substance

The control substances were the conventional maize varieties which have similar genetic background as the generations as shown in the table below. Genomic DNA for use in this study was extracted from seed tissue listed in the table below.

Control Substance	Breeding Generations	Seed ORION ID
LH244	R_3, R_4, R_5	11264747
HCL645 × LH244	R_3F_1 (hybrid), R_4F_1 (hybrid)	11320031

B.2.3. Reference Substance

The reference substance was plasmid vector PV-ZMHT507801, which was used to develop MON 87419. 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.

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

The seed for MON 87419 and the conventional controls used in this study were obtained from Monsanto Trait Development. The synthesis records for these materials are located in the ORION[1] system. The identities of the MON 87419 and the conventional control substances 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 MON 87419 and the conventional control substances prior to the use of these materials in the study.

MON 87419, conventional 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.

B.2.5. Genomic DNA Isolation

For sequencing library construction and PCR reactions, genomic DNA was isolated from seed tissues of MON 87419 and conventional 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 75°C-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 Ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 1.05 M NaCl, and 0.75% (w/v) PVP) and RNase A was added to ~6 g ground seed tissue. The samples were incubated at 60°C-70°C for 25-35 minutes with intermittent mixing. The samples were cooled to room temperature and subjected to multiple 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 precipitate 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.

B.2.6. DNA Quantification

PV-ZMHT507801 DNA and extracted genomic DNA were quantified using a Qubit® Fluorometer (Invitrogen) or a Nanodrop™ Spectrophotometer (Thermo Scientific).

B.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.

B.2.8. Shearing of DNA

Approximately one µg of DNA from the test, control and reference substances were sheared using a Covaris S-220 ultrasonicator. The DNA was diluted to ~18-20 ng/µl in Buffer EB (Qiagen, Inc.) 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 ~ 3-10°C for 80 seconds for MON 87419 and conventional control DNA or 60 seconds for reference DNA.

B.2.9. Bioanalyzer Analysis

One microliter of sheared genomic DNA was diluted 1:10 in Buffer EB or TE buffer and run on a DNA High Sensitivity chip on an Agilent 2100 Bioanalyzer to check the quality of the shearing. After preparing the chip, 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.

B.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, Inc.).

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.) 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 DNA 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 DNA Size Selection system (Sage Science, Inc.) using 2% gel cassettes. 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 Primer Cocktail and 25 µl of Illumina PCR Master Mix 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.

B.2.11. Next Generation Sequencing

The library samples described above were sequenced by Monsanto's Sequencing Technologies using Illumina HiSeq technology that produces short sequence reads (~100 bp long). Sufficient numbers of these sequence fragments were obtained (≥75 × genome coverage) to comprehensively cover the entire genomes of MON 87419 and the conventional control (Kovalic et al., 2012).

B.2.12. Junction Sequence Analysis Bioinformatics

High-throughput sequence reads were enriched by mapping to the PV-ZMHT507801 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 artifacts 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 MON 87419 and the conventional 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.

B.2.12.1. Sequencing Read Enrichment

The transformation plasmid PV-ZMHT507801 sequence was used as reference to find all reads that were either fully matched to the insert plasmid fragments or partially matched with junction sequences. The sequence used was obtained from the MEGA[7] system. A junction sequence is characterized by a combination of transformation plasmid sequence and flanking sequence that is likely to be host genome flanking sequence or any other co-inserted sequence. Local alignment with BlastAll (v.2.2.21) was performed to collect all sequencing reads with an e-Score (expectation 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.

B.2.12.2. 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.

B.2.12.3. Computer software

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.

B.2.12.4. Mapping and Junction Detection

Enriched and quality refined reads of both MON 87419 and conventional control samples were aligned against the whole PV-ZMHT507801 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

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

30 bases match and 96.7% identity were collected as potential junction sequences (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 the sequence reads relative to the transformation plasmid 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.

B.2.12.5. Effective Sequencing Depth Determination

A single copy locus from the native plant genome (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.

B.2.12.6. Positive Control

To produce the positive control sample for sequencing, a plasmid DNA library was created as described in sections B.2.8 - B2.10 and then diluted to approximately 0.03% of the concentration of the genomic DNA libraries (to gain sufficient depth of coverage) and pooled with samples produced from the conventional control materials (as described above).

Table B-1. Sequencing (NGS) Conducted for MON 87419 and Control Genomic DNA

Sample	Total Nucleotides (Gb)	Effective Median Depth of Coverage (x-fold)
LH244	281.7	118×
HCL645 × LH244	359.9	123×
R_3	324.5	135×
R_3F_1	283.3	103×
R_4	352.4	141×
R_4F_1	347.5	98×
R_5	340.1	144×

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 single copy locus within the maize genome (*pdc3*). The median effective depth of coverage is shown for all samples.

B.2.12.7. Positive Spike-in Controls and Experimental Limit of Detection

In order to confirm the method's ability to detect any sequences derived from the transformation plasmid, and to demonstrate adequate experimental sensitivity, a positive control was generated as described as above and pooled with samples produced from the conventional control materials. The plasmid sequence reads were mapped to the known plasmid sequence at various depth-of-sequencing rates and the resultant experimental data were fit to the known response model (Clarke and Carbon, 1976; Waterman, 1995). The mapped sequence showed 100% coverage of the known PV-ZMHT507801 sequence at one genome equivalent (Table B-2). This demonstrates that all bases of the transformation plasmid are observed by the sequencing and bioinformatics performed in this study when present at one genome equivalent. Also, observed coverage was adequate at a level of at least 1/10th genome equivalent (99.43% coverage at 100% identity).

Table B-2. Summary of NGS Data for the Conventional Control DNA Sample Spiked with PV-ZMHT507801

	12x coverage	122x coverage
Extent of coverage[1] of PV-ZMHT507801	99.43%	100%
Percent identity of coverage[2] of PV-ZMHT507801	100%	100%

[1] Extent of coverage is calculated as the percent of all PV-ZMHT507801 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-ZMHT507801 bases observed in the sequencing of the spike-in samples:

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

B.2.13. PCR and DNA Sequence Analyses to Examine the Insert and Flanking Sequences in MON 87419

Overlapping PCR products, denoted as Product A, Product B, Product C, Product D, Product E and Product F were generated that span the insert and adjacent 5′ and 3′ flanking DNA sequences in MON 87419. 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 87419, as well as that of the DNA flanking the 5′ and 3′ ends of the insert.

The PCR analyses for Product A, Product B, Product C, Product D, Product E and Product F were each conducted using 100 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 1× concentration of Phusion High Fidelity PCR Master Mix with HF Buffer (NEB).

The amplification of Product A, was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 30 cycles at 98°C for 10 seconds, 69 °C for 20 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

The amplification of Product B, was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 30 cycles at 98°C for 10 seconds, 70°C for 20 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

The amplification of Product C and Product E, were performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 30 cycles at 98°C for 10 seconds, 71°C for 20 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

The amplification of Product D was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 35 cycles at 98°C for 10 seconds, 71°C for 20 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

The amplification of Product F was performed under the following cycling conditions: 1 cycle at 98°C for 30 seconds; 30 cycles at 98°C for 10 seconds, 72°C for 2 minutes 20 seconds; 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, 1.2 units of Exonuclease I (Affymetrix) and 1.2 units of Shrimp Alkaline Phosphatase (Affymetrix) were added to 30 µl of each verified PCR product and incubated at 37°C for 15 minutes followed by 80°C for 15 minutes. The treated PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Monsanto's Sequencing Technologies using BigDye terminator chemistry (Applied Biosystems).

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-ZMHT507801 sequence to determine the integrity and organization of the integrated DNA and the 5′ and 3′ insert to flank DNA junctions in MON 87419.

B.2.14. PCR and DNA Sequence Analyses to Examine the Integrity of the DNA Insertion Site in MON 87419

To examine the MON 87419 T-DNA I insertion site in conventional 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 87419. 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 100 ng of genomic DNA template in a 50 µl reaction volume. The reaction contained a final concentration of 0.2 µM of each primer and 1.25 units/reaction of PrimeSTAR GXL Polymerase (Takara Bio, Inc.). The amplification was performed under the following cycling conditions: 8 cycles at 98°C for 10 seconds, 70°C, decreasing 1°C per cycle for 30 seconds, 72°C for 2 minutes; 27 cycles at 98°C for 10 seconds, 63°C for 30 seconds, 72°C for 2 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. Prior to sequencing, 1.2 units of Exonuclease I (Affymetrix) and 1.2 units of Shrimp Alkaline Phosphatase (Affymetrix) were added to 30 µl of each verified PCR product and incubated at 37°C for 15 minutes followed by 80°C for 15 minutes. The treated PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Monsanto's Sequencing Technologies 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 87419 insert to determine the integrity and organization of the insertion site.

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Appendix C: Protein Reaction Products, Materials, Methods and Results for Characterization of MON 87419 DMO Protein Produced in MON 87419 and Substrate Specificity

C.1. DMO Reaction Products

MON 87419 when treated with dicamba herbicides will yield the reaction products DCSA and formaldehyde during demethylation of the herbicide. These products have either been previously deemed safe (DCSA) or are commonly produced in nature and at sufficiently low levels in this MON 87419 cropping system (formaldehyde) so as to not raise concerns with regard to the plant pest risk assessment for MON 87419.

C.1.1. DCSA in MON 87419

DCSA is a metabolite generated when dicamba herbicide is sprayed on MON 87419 maize, MON 87708 soy, and MON 88701 cotton and is also produced by livestock and soil whose safety has been evaluated by the Environmental Protection Agency (U.S. EPA, 2009; FAO-WHO, 2011). DCSA residue levels were measured in dicamba-treated MON 87419 to support Monsanto's registration request for the inclusion of DCSA in the maize grain dicamba residue definitions. DCSA is structurally similar to salicylic acid (SA). Numerous studies have reported on the stress defense activities of SA, although most studies have looked at the protective effects of exogenously applied SA (Janda et al., 2007).

C.1.2. Formaldehyde in the Environment

Formaldehyde is ubiquitous in the environment; plants and animals are constantly exposed to low levels already present in the environment and the atmosphere from a variety of biogenic (e.g., plant and animal) and anthropogenic (e.g., automotive or industrial emissions) sources (WHO-IPCS, 1989). In water, formaldehyde dissipates through biodegradation to low levels in a few days (USHHS-ATSDR, 1999). Aerobic biodegradation half-lives are estimated to be 1-7 days for surface water and 2-14 days for ground water (U.S. EPA, 2008). The half-life of formaldehyde in air is dependent on a number of factors (light intensity, temperature, and location). Through reaction with hydroxyl radical, the half-life of formaldehyde in air varies from 7 to 70 hours (U.S. EPA, 2008). The photolytic half-life of formaldehyde in air (e.g., in the presence of sunlight) is estimated to be 1.6-6 hours (U.S. EPA, 2008; USHHS-ATSDR, 1999). Formaldehyde is rapidly consumed in the atmosphere through direct photolysis or by oxidation with hydroxyl or nitrate radicals (USHHS-ATSDR, 1999).

Humans are constantly exposed to low levels of formaldehyde. Human exposure to formaldehyde is primarily due to indoor air exposures (USHHS-ATSDR, 1999). Formaldehyde is found in a variety of consumer products such as cosmetics and paints, often as an antimicrobial agent, and is used extensively in urea-formaldehyde "slow-release" fertilizer formulations and adhesives (USHHS-ATSDR, 1999). Indoor formaldehyde air concentrations are generally significantly higher than outdoor air concentrations (USHHS-ATSDR, 1999) as a result of combustion (cooking, heating, tobacco use) and the emission of formaldehyde from a variety of construction materials

(e.g., particle board, plywood or foam insulation) as well as permanent press fabrics (e.g., clothing or draperies) (U.S. CPSC, 1997). Formaldehyde present in outdoor air results from a number of sources, and levels of formaldehyde are generally higher in urban areas than in rural areas (USHHS-ATSDR, 1999). Direct contributions of formaldehyde to the atmosphere (i.e., those in the form of formaldehyde itself) from man-made sources are present, but are generally considered to be small relative to natural sources or indirect production of formaldehyde in the atmosphere (WHO, 2002).

C.1.3. Formaldehyde in MON 87419

Formaldehyde is a metabolite when dicamba is sprayed on MON 87419 maize. However, formaldehyde is not considered a relevant metabolite in the demethylation of dicamba by U.S. EPA. According to the guidelines published by Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (U.S. EPA, 1996), the methoxy side chain that is cleaved from dicamba to form formaldehyde would specifically not be chosen to be labeled in a metabolism study (U.S. EPA, 1996). This is because it is not metabolically stable and would not be considered a significant moiety as it would be readily metabolized and incorporated into the 1-carbon pool of the plant through known pathways. Therefore, formaldehyde was not measured in the residue study when dicamba was applied to MON 87419.

Plants have a large capacity to metabolize formaldehyde naturally produced from internal processes (Hanson and Roje, 2001), and any additional amount of formaldehyde that could be theoretically produced in the plant by dicamba treatment in MON 87419 would be metabolized very quickly. Thus the incremental increase in formaldehyde over and above the levels already presumed to be present in the maize plant would be small and transient and associated with an outdoor application of dicamba herbicide. Further, since current literature supports that formaldehyde is only emitted from foliage under certain conditions (Cojocariu et al., 2005; Cojocariu et al., 2004; Nemecek-Marshall et al., 1995) and that emission rates are low (Nemecek-Marshall et al., 1995), little opportunity exists for formaldehyde to be released from MON 87419 after dicamba treatment. Therefore human safety concerns of formaldehyde released from dicamba-treated MON 87419 are considered to be negligible and the most relevant route of exposure is from repeated inhalation of concentrated levels associated with indoor or occupational environments. USHHS-NTP has already stated that there is no evidence to suggest that dietary intake of formaldehyde is important, despite NTP's 12th Report on Carcinogens reclassifying formaldehyde as a known human carcinogen (USHHS-NTP, 2011). Therefore, the potential for human exposure to any formaldehyde in dicamba-treated MON 87419 maize is highly unlikely.

C.1.4. Conclusion

Data from dicamba-treated and not treated MON 87419 compared to a conventional control are available from multiple sites across the U.S., where composition, agronomic, phenotypic and environmental interaction data were collected. The results of this assessment demonstrate no biologically meaningful difference between MON 87419 treated with and without dicamba and the conventional control, and support a conclusion that the formation of DCSA and formaldehyde does not alter the weedy characteristics or increase susceptibility or tolerance to diseases, insect pests or abiotic stresses.

MON 87419 was also determined not to be a major contributor to variation in compositional component levels in maize grain and forage, confirming the compositional equivalence of MON 87419 to the conventional control in levels of these components. Therefore, MON 87419, as cultivated, is no more likely to be a plant pest risk or have a biologically meaningful change in environmental impact than conventional maize.

C.2. Characterization of MON 87419 DMO Protein in MON 87419

C.2.1. Forms of DMO

Various forms of the DMO protein (Figure C-1) were used to establish enzyme structure, activity, substrate specificity and safety of the proteins in MON 87419. The wild-type DMO was first isolated and characterized from *S. maltophilia* (Herman et al., 2005). The MON 87419 DMO+12 or +7 proteins are identical to the wild-type DMO protein, except for the amino acids derived from the *CTP4* (12 or 7) and an additional leucine at position two (Figure C-1). The *E. coli*-produced N-terminal histidine-tagged DMO is identical to the wild-type DMO, but with a histidine-tag on the N-terminus (Figure C-1), was used for specificity experiments. The *E. coli*-produced C-terminal histidine-tagged DMO is identical to the wild-type DMO, but with a histidine-tag on the C-terminus and the exception of an alanine added at position 2 (Figure C-1), was used for crystallography experiment. The differences in the amino acid sequence or the addition of N- or C-terminal histidine tag did not appear to have an effect on mode-of-action, structure of the catalytic site, functional activity, or specificity of DMO as demonstrated by the crop tolerance to both dicamba and glufosinate sprays in the agronomic and phenotypic assessments and these amino acids are sterically distant from the catalytic domain centers involved in electron transport (Rieske and non-heme iron centers) and the catalytic centers for the dicamba substrate (D'Ordine et al., 2009; Dumitru et al., 2009).

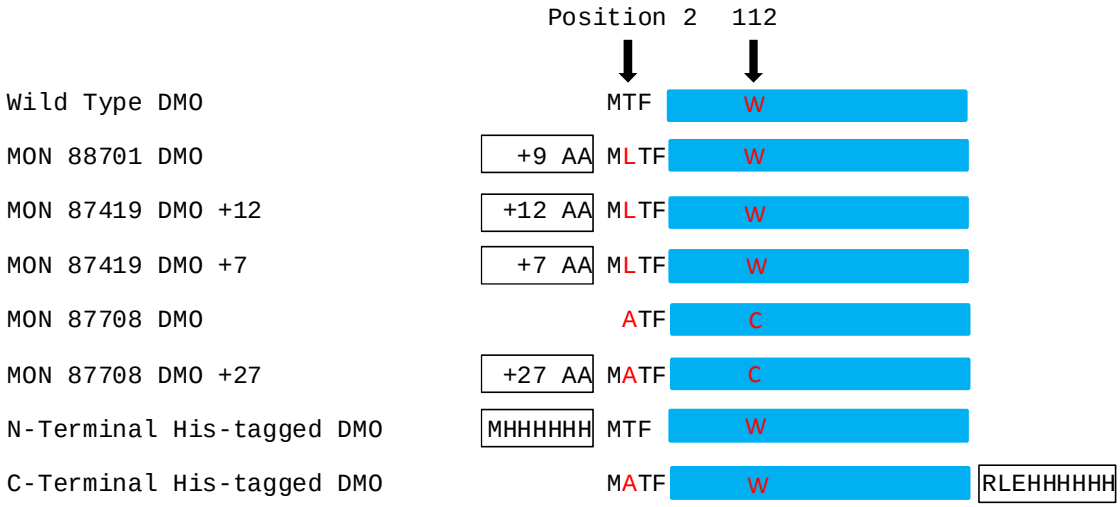


Figure C-1. Forms of DMO Protein and Their Relation to the Wild-Type DMO Protein

The diagram represents the various DMO forms described in this petition. The blue regions indicate regions of 100% amino acid identity. The wild-type DMO form isolated from *S. maltophilia* was the first form sequenced (Herman et al., 2005). The MON 88701 DMO protein previously reviewed by USDA-APHIS (Petition #12-185-01p) is identical to wild type DMO, except for an insertion of a leucine at position 2, and an addition of 9 amino acids from CTP2 at the N-terminus. The MON 87419 DMO+12 or +7 proteins are also identical to wild type DMO, except for an insertion of a leucine at position 2 and an addition of 12 or 7 amino acids, respectively, from CTP4 at the N-terminus. The MON 87419 DMO+12 or +7 proteins are identical to MON 87708 DMO (fully processed), except for an addition of 12 or 7 amino acids, respectively, from CTP4, and a methionine that remained at the N-terminus from the *dmo* gene, and two single amino acid changes at positions 2 and 112. MON 87708 DMO protein was previously reviewed by USDA-APHIS (Petition # 10-188-01p). The N-terminal histidine-tagged DMO was produced in *E. coli* and was used for *in vitro* specificity studies (Appendix C.3). The C-terminal histidine-tagged DMO was produced in *E. coli* and was used for crystallography studies (Appendix C.3.2.1.) (D'Ordine et al., 2009; Dumitru et al., 2009). Position refers to amino acid residues as wild type DMO and boxed regions correspond to CTP or a histidine-tag, except for MON 87708 DMO+27 that is encoded by the *RbcS* coding region.

C.2.2. Materials

The MON 87419-produced DMO protein (lot 11391916) was purified from maize grain of MON 87419 (lot 11371533). The MON 87419-produced DMO protein was stored in a -80ºC freezer in a buffer solution containing 50 mM potassium phosphate, pH 8.0, 1.0 mM dithiothreitol (DTT), 1.0 mM benzamidine hydrochloride, 0.1 M sodium chloride and 10% glycerol.

The *E. coli*-produced DMO protein (lot 11383588) was used as the reference substance. The MON 87419 DMO protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON290351 expression plasmid. The *dmo* gene coding sequence contained on the expression plasmid (pMON290351) was confirmed prior to and after fermentation. The *E. coli*-produced MON 87419 DMO protein was previously characterized.

C.2.3. Description of Assay Control

Protein MW standards (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were used to calibrate some SDS-PAGE gels and verify protein transfer to polyvinylidene difluoride (PVDF) and nitrocellulose membranes. Broad Range SDS-PAGE MW standards (Bio-Rad) were used to generate a standard curve for the apparent MW estimation. The *E. coli*-produced MON 87419 DMO reference substance was used to construct a standard curve for the estimation of total protein concentration using a Bio-Rad protein assay. A phenylthiohydantoin (PTH) amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the Applied Biosystems 494 Procise Sequencing System for each analysis. A peptide mixture (Sequazyme Peptide Mass Standards kit, Applied Biosystems) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin (Sigma-Aldrich, St. Louis, MO) was used as positive control for glycosylation analysis.

C.2.4. MON 87419-produced DMO Protein Purification

The DMO protein was purified from maize grain of MON 87419. The purification procedure was not performed under a Good Laboratory Practices (GLP) - compliant protocol, however, all procedures were documented on worksheets and, where applicable, Standard Operating Procedures were followed. The purification procedure is briefly described below; a detailed description of the purification procedure was archived under lot 11391916 at Monsanto Company.

DMO was extracted from approximately 10 kg of MON 87419 maize flour with a 50 mM tris buffer, pH 8.0 containing 1.0 mM DTT, 1.0 mM benzamidine-HCl, and protease inhibitor cocktail (extract buffer). The maize flour was suspended at a 10:1 ratio; 10 liters extraction buffer per kg of maize flour. The suspension was stirred at room temperature for 2 hours. After incubation for 1 hour, Celpure P100 was added to the suspension as a filter aid at a final concentration of 5% (w/v). An Ertel Alsop filter press was set up at room temperature with a complete set of filter pads. Fifty liters of extraction slurry was pumped through the press and the extract was washed out of the press with 100 liters of the extract buffer without the protease inhibitors. The remaining 50 liters of the slurry was filtered in the same manner as described above. In both cases,

only the first half of the wash contained DMO and was therefore retained while the latter half was collected and discarded. A combined volume of 200 liters of DMO containing filtrate was then concentrated at room temperature to 15 liters using a hollow fiber cartridge (GE Healthcare, Piscataway, NJ) with a 3.2 m^2 total surface area and a 100 kDa molecular weight cutoff. The concentrate was incubated overnight at 4°C in order to allow insoluble substances to precipitate. The concentrated sample was then filtered with a fiber filter precoated with Celpure P100 using a benchtop Buchner funnel at room temperature. After filtration, solid sodium chloride was added to the concentrate to a final concentration of 0.5 M sodium chloride. The sample was then diafiltered against extract buffer supplemented with 0.5 M sodium chloride. The diafiltration buffer was exchanged seven times to facilitate buffer exchange and remove some contaminant proteins. Following diafiltration, the appropriate amount monobasic and dibasic solid salts was added to the diafiltrate to bring it to 10 mM phosphate buffer at pH 8.0.

Following sample preparation, a ceramic hydroxyapatite (CHT) (Bio-Rad) was packed at a protein to resin ratio of 10 mg/ml. The diafiltrate was loaded on a CHT (Bio-Rad) column at 4°C and washed with the extract buffer supplemented with 10 mM phosphate, pH 8.0 and 0.5 M NaCl. The flowthrough and wash were combined for a total volume of 23 liters and concentrated at room temperature, using the same hollow fiber cartridge utilized for the initial sample preparation, to a final volume of 3.25 liters in preparation for immunoaffinity chromatography.

For immunoaffinity chromatography, 9.5 ml of protein A sepharose fast flow resin (GE Healthcare) was cross-linked with a monoclonal mouse antibody raised against *E. coli*-produced DMO protein. The resin was equilibrated with extract buffer supplemented with 10 mM sodium phosphate, pH 8.0 and 0.5 M NaCl (equilibration buffer). The protein was loaded onto the immunoaffinity column by recirculation at a flow rate of ~ 2.0 ml/min. The column was then washed with equilibration buffer until the optical density at 280 nm (OD_{280}) of the wash returned to baseline followed by washing with 15 column volumes of 1.0 M ammonium sulfate, buffered with 50 mM Tris, pH 8.0. Finally, the column was washed with 15 column volumes of extract buffer supplemented with 0.5 M NaCl. The protein was eluted from the column with immunoaffinity elution buffer (50 mM Tris, pH 8.0, 0.75 M ammonium sulfate and 40% propylene glycol). Fractions containing the MON 87419 DMO proteins were identified by SDS-PAGE and pooled, resulting in a final sample volume of 40 ml. The sample was then concentrated to 4 ml with two 10 kDa molecular weight cutoff spin concentrators (Corning, Tewksbury, MA) passivated with 10% PEG 3000 and equilibrated with immunoaffinity elution buffer. Following concentration, the sample was dialyzed at 4 °C against the final storage buffer (50 mM potassium phosphate, pH 8.0, 1.0 mM DTT, 1.0 mM benzamidine-HCl, 0.1 M sodium chloride and 10% glycerol). The purified MON 87419-produced DMO protein was aliquoted, assigned lot 11391916, and stored at -80°C.

C.2.5. N-Terminal Sequencing

C.2.5.1. Methods

Approximately 6 µg of the MON 87419-produced DMO protein was coupled to a PVDF membrane in a ProSorb™ sample preparation cartridge (Applied Biosystems) and

subjected to Edman degradation chemistry for N-terminal sequence analysis. The analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and a Perkin Elmer Series 200 Ultraviolet/Visible Spectrum (UV/VIS) Absorbance Detector and Procise were used. Chromatographic data were collected using SequencePro software. Phenylthiohydantoin (PTH)-AA Standard Solution (Applied Biosystems) was used to chromatographically calibrate the instrument for the analysis. A control protein (10 picomole β-lactoglobulin, Applied Biosystems) was analyzed before and after the analysis of the MON 87419-produced DMO protein.

C.2.5.2. Results of the N-terminal Sequence Analysis

Fifteen cycles of N-terminal sequencing was performed on MON 87419-produced DMO protein. The expected sequence for the DMO protein deduced from the *dmo* expression cassette present in maize of MON 87419 was observed. The experimentally determined sequence corresponds to the deduced DMO protein beginning at the initial serine (MON 87419 DMO+12, Table C-1). In addition, a shorter form of the DMO protein that does not contain the first five amino acids of MON 87419 DMO+12, MON 87419 DMO+7 was observed. Hence, the sequence information identified the N-terminal sequences of the DMO proteins isolated from the grain of MON 87419.

Table C-1. N-Terminal Sequence of the MON 87419-produced DMO Protein

Amino Acids Residue # from the N-terminus	→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Expected Sequence	→	S	F	R	I	S	A	S	V	A	T	A	C	M	L	T
Experimental Sequence(+12)	→	S	F	R	I	S	A	S	V	A	T	A	X	X	X	X
Experimental Sequence(+7)	→						A	S	V	A	T	A	X	X	X	X

The experimental sequences obtained from the MON 87419-produced DMO were compared to the expected sequence deduced from the *dmo* expression cassette present in MON 87419. The N-terminal sequences of two forms of DMO proteins identified corresponded to the CTP. The amino acid at position S of the deduced sequence is shown as position 1 of the observed protein. The single letter International Union of Pure and Applied Chemistry - International Union of Biochemistry (IUPAC-IUB) amino acid code is A, alanine; C, cysteine; F, phenylalanine; I, isoleucine; L, leucine; M, methionine; R, arginine; S, serine; T, threonine; V, valine. X's refer to undesignated calls.

C.2.6. MALDI-TOF Tryptic Mass Map Analysis

C.2.6.1. Methods

The MON 87419-produced DMO protein (~10 µg) was dried to completion using vacuum centrifugation and resuspended in 30 µl of 40% (v/v) 2,2,2, trifluoroethanol (TFE) in 25 mM ammonium bicarbonate. The sample was vortexed vigorously, sonicated for 5 min in a water bath (20-25°C) and incubated at 37°C for 30 min. The sample was treated with 2 µl of 75 mM Tris (2-carboxyethyl) phosphine (TCEP) for 30 min at ~37°C and then incubated in the dark for 25 min at room temperature with 2 µl of 150 mM iodoacetic acid. To quench the reaction, 2 µl of 75 mM TCEP was added. The sample was then brought to 100 µl with 25 mM ammonium bicarbonate, 2.5 µl of 0.2 µg/µl trypsin was added, and incubated at 37°C. After 12-16 hours the digestion was quenched with 1 µl of formic acid. The sample was dried to completion using vacuum centrifugation and then solubilized in 100 µl of 50% acetonitrile/0.1% trifluoroacetic acid (TFA) with sonication. Aliquots of the sample were spotted to wells on an analysis plate and mixed with α-cyano-4-hydroxycinnamic acid (α-Cyano, Thermo Fisher Scientific Inc., Waltham, MA). Mass range for this analysis was from 800 to 4000 Da. The analysis was performed and data processed on an AB Sciex™ 5800 Mass Spectrometry System using TOF/TOF Series Explorer Software (AB Sciex, Foster City, CA). A plate model/default calibration was performed using TOF/TOF calibration mixture standards (AB Sciex). Protonated peptide masses were isotopically resolved in reflector positive ion mode (Aebersold, 1993; Billeci and Stults, 1993). Only monoisotopic ions were assigned in a mass list. The mass spectra were searched against DMO protein sequence using Mascot (Matrix Science Inc, London, UK) and ProteinPilot (AB Sciex) 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.

C.2.6.2. 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 87419-produced DMO protein was confirmed by MALDI-TOF MS analysis of peptide fragments produced by the trypsin digestion of the MON 87419-produced DMO protein.

There were 37 unique peptides identified that corresponded to the expected masses (Table C-2). The identified masses were used to assemble a peptide map of the DMO protein. The experimentally determined coverage of the DMO protein was 77% (Figure C-2, 272 out of 352 amino acids). This analysis confirms the identity of MON 87419-produced DMO protein.

Table C-2. Summary of the Tryptic Masses Identified for the MON 87419-produced DMO Using MALDI-TOF MS

Experimental Mass	Calculated Mass[2]	Difference[3]	Fragment[4]	Sequence[5]
1626.7737	1626.8109	-0.0372	4-18	ISAS.....TFVR
2142.0752	2142.1109	-0.0357	19-37	NAWY.....PLGR
1274.6915	1274.7234	-0.0320	38-48	TILD.....ALYR
3017.5625	3017.6008	-0.0383	38-64	TILD.....CPHR
1760.8528	1760.8880	-0.0351	49-64	QPDG.....CPHR
832.4121	832.4443	-0.0322	111-117	SFPVVER
1469.6354	1469.6245	0.0109	149-161	TVGG.....CNYK
3444.5726	3444.6343	-0.0618	149-178	TVGG.....YVHR
1992.9860	1993.0204	-0.0343	162-178	LLVD.....YVHR
3480.7068	3480.7320	-0.0252	162-191	LLVD.....RLER
1107.4616	1107.4945	-0.0329	179-188	ANAQ.....AFDR
1505.6868	1505.7222	-0.0354	179-191	ANAQ.....RLER
2988.4524	2988.4975	-0.0450	179-205	ANAQ.....ALMK
1898.9833	1899.0135	-0.0302	189-205	LERE.....ALMK
1500.7408	1500.7858	-0.0450	192-205	EVIV.....ALMK
1169.6055	1169.6478	-0.0423	206-217	IPGG.....LMAK
1585.8609	1585.9014	-0.0405	206-220	IPGG.....KFLR
1427.6513	1427.6793	-0.0280	221-233	GANT.....NDIR
1855.8617	1855.8965	-0.0348	221-236	GANT.....RWNK
3581.7244	3581.8089	-0.0845	221-253	GANT.....GTPK
3009.5040	3009.5494	-0.0455	234-260	WNKV.....IHSR
1743.8305	1743.9229	-0.0924	237-253	VSAM.....GTPK
2581.2884	2581.3322	-0.0439	237-260	VSAM.....IHSR
855.3948	855.4199	-0.0250	254-260	EQSIHSR
2397.0259	2397.0696	-0.0436	261-281	GTHI.....GSSR
1576.6827	1576.7192	-0.0365	282-295	NFGI.....GVLR
1029.5247	1029.5607	-0.0360	296-304	SWQAQALVK
1401.6680	1401.7252	-0.0572	296-307	SWQA.....KEDK
2297.1993	2297.2379	-0.0387	296-315	SWQA.....AIER
1285.6536	1285.6878	-0.0341	305-315	EDKV.....AIER
1441.7338	1441.7889	-0.0551	305-316	EDKV.....IERR
913.4900	913.5233	-0.0333	308-315	VVVEAIER
1069.5792	1069.6244	-0.0452	308-316	VVVEAIERR
2449.1089	2449.1842	-0.0752	317-338	RAYV.....AAVR
2293.0455	2293.0831	-0.0376	318-338	AYVE.....AAVR
1613.8257	1613.8624	-0.0367	339-352	VSRE.....LEAA
1271.6128	1271.6608	-0.0480	342-352	EIEK.....LEAA

[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] Position refers to amino acid residues within the predicted MON 87419-produced DMO sequence as depicted in Figure C-2.
[5] For peptide matches greater than nine amino acids in length the first 4 residues and last 4 residues are show separated by dots (.....).

```
  1 SFRISASVAT ACMLTFVRNA WYVAALPEEL SEKPLGRTIL DTPLALYRQP
 51 DGVVAALLDI CPHRFAPLSD GILVNGHLQC PYHGLEFDGG GQCVHNPHGN
101 GARPASLNVR SFPVVERDAL IWIWPGDPAL ADPGAIPDFG CRVDPAYRTV
151 GGYGHVDCNY KLLVDNLMDL GHAQYVHRAN AQTDAFDRLE REVIVGDGEI
201 QALMKIPGGT PSVLMAKFLR GANTPVDAWN DIRWNKVSAM LNFIAVAPEG
251 TPKEQSIHSR GTHILTPETE ASCHYFFGSS RNFGIDDPEM DGVLRSWQAQ
301 ALVKEDKVVV EAIERRRAYV EANGIRPAML SCDEAAVRVS REIEKLEQLE
351 AA
```

Figure C-2. MALDI-TOF MS Coverage Map of the MON 87419-produced DMO
The amino acid sequence of the MON 87419-produced DMO protein was deduced from the *dmo* expression cassette present in MON 87419. Boxed regions correspond to peptides that were identified from the MON 87419-produced DMO protein sample using MALDI-TOF MS. In total, 77% coverage (272 out of 352 amino acids) of the expected protein sequence was identified.

C.2.7. Western Blot Analysis-Immunoreactivity

C.2.7.1. Methods

Western blot analysis was performed as follows to confirm the identity of the MON 87419-produced DMO protein and to compare the immunoreactivity of the MON 87419-produced DMO protein and *E. coli*-produced MON 87419 DMO protein. MON 87419-produced DMO protein and *E. coli*-produced MON 87419 DMO protein were diluted in 5 × Loading Buffer (5 × LB, 0.31 M Tris-Cl, pH 7.5, 10% SDS, 50% glycerol, 25% (v/v) 2-mercaptoethanol, 0.025% (w/v) Bromophenol blue) and Storage Buffer (50 mM potassium phosphate, pH 8.0, 1.0 mM DTT, 1.0 mM benzamidine-HCl, 0.1 M sodium chloride and 10% glycerol) to obtain a final concentration of 1 × Loading Buffer (1 × LB) and heated to 95-105°C for 3-5 min. Three amounts (~ 1, ~ 2, and ~ 3 ng) of the intact test substance (total protein concentration × purity of the intact DMO protein) and the intact reference substance (total protein concentration × purity of the intact DMO protein) were loaded in duplicate onto a pre-cast Tris-glycine 4-20% polyacrylamide mini-gel (Invitrogen). Pre-stained molecular weight standards (Precision Plus Protein Standards™, Bio-Rad) were loaded on the gel 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 (Bio-Rad).

The western blotting procedure was performed using an iBind™ Western System apparatus (Life Technologies, Grand Island, NY). The membrane was blocked with 1 × iBind™ Solution (Life Technologies) and incubated with goat anti-DMO antibody (lot G884602) at a dilution of 1:1000 in 1 × iBind™ Solution. After washing with 1 × iBind™ Solution, the membrane was next incubated with horseradish peroxidase conjugated horse anti-goat IgG (Vector Laboratories, Burlingame, CA) at a dilution of 1:1000 in 1 × iBind™ Solution and washed again with 1 × iBind™ Solution. Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL) detection system (GE Healthcare) and exposed to Hyperfilm ECL high performance chemiluminescence film (GE Healthcare). The film was developed using a Konica SRX-101A automated film processor (Konica, Tokyo, Japan).

Quantification of the bands on the blot was performed on a Bio-Rad GS-800 densitometer with the supplied QuantityOne software using the volume tool. The signal intensities of the immunoreactive bands migrating at the expected position for the DMO protein were quantified as adjusted volume values. The immunoreactivity was reported in OD × mm^2.

C.2.7.2. Results of MON 87419 DMO Protein Immunoreactivity Equivalence

Western blot analysis was conducted using goat anti-DMO polyclonal antibody as additional means to confirm the identity of the DMO protein isolated from the grain of MON 87419 and to assess the equivalence of the immunoreactivity of the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins.

The results showed that immunoreactive bands with the same electrophoretic mobility were present in all lanes loaded with the MON 87419-produced and *E. coli*-produced

MON 87419 DMO proteins (Figure C-3). For each amount loaded, comparable signal intensity was observed between the MON 87419-produced and *E. coli*-produced MON 87419 DMO protein bands. As expected, the signal intensity increased with increasing load amounts of the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins, thus, supporting identification of MON 87419-produced DMO protein.

To compare the immunoreactivity of the MON 87419-produced and the *E. coli*-produced MON 87419 DMO proteins, densitometric analysis was conducted on the bands that migrated at the expected apparent MW for DMO proteins (~ 40 kDa). The signal intensity (reported in $OD \times mm^2$) of the band of interest in lanes loaded with MON 87419-produced and the *E. coli*-produced MON 87419 DMO proteins was measured (Table C-3). Because the mean signal intensity of the MON 87419-produced DMO protein band was within 35% of the mean signal of the *E. coli*-produced MON 87419 DMO protein, the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins were determined to have equivalent immunoreactivity.

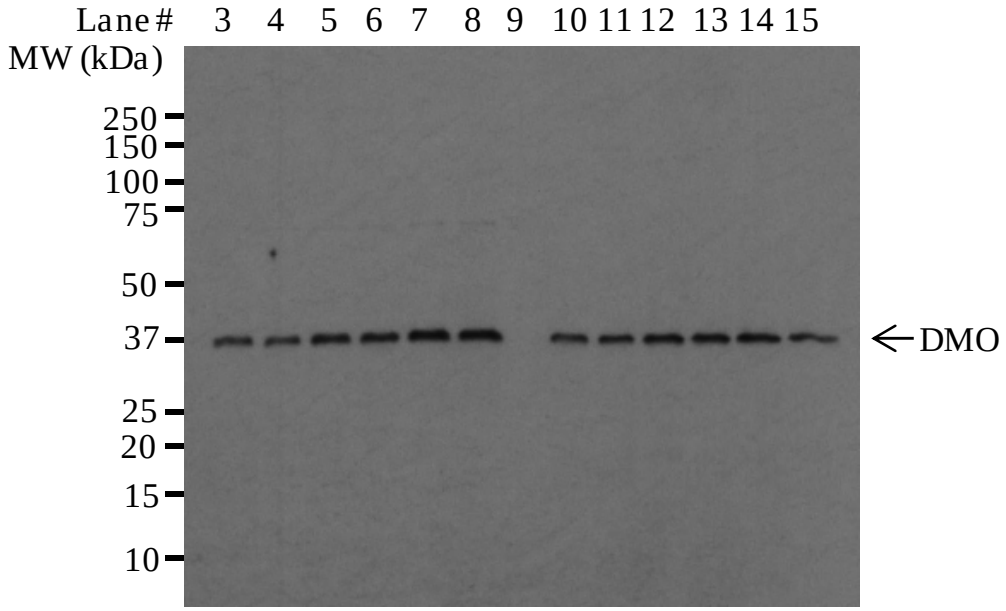


Figure C-3. Western Blot Analysis of MON 87419-produced and *E. coli*-produced MON 87419 DMO Proteins

Aliquots of the MON 87419-produced DMO protein and the *E. coli*-produced MON 87419 DMO protein were subjected to SDS-PAGE and electrotransferred to a PVDF membrane. Proteins were detected using anti-DMO antibodies as the primary antibodies. Immunoreactive bands were visualized using HRP-conjugated secondary antibodies and an ECL system. The approximate MW (kDa) of the standards are shown on the left. Lanes 1 and 2 were cropped from the image. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Precision Plus Protein™ Standards	-
2	Blank	-
3	MON 87419-produced DMO	1
4	MON 87419-produced DMO	1
5	MON 87419-produced DMO	2
6	MON 87419-produced DMO	2
7	MON 87419-produced DMO	3
8	MON 87419-produced DMO	3
9	Blank	-
10	*E. coli*-produced MON 87419 DMO	1
11	*E. coli*-produced MON 87419 DMO	1
12	*E. coli*-produced MON 87419 DMO	2
13	*E. coli*-produced MON 87419 DMO	2
14	*E. coli*-produced MON 87419 DMO	3
15	*E. coli*-produced MON 87419 DMO	3

Table C-3. Comparison of Immunoreactive Signal Between MON 87419-produced and *E. coli*-produced MON 87419 DMO Proteins

Mean Signal Intensity from MON 87419-Produced DMO [1] (OD x mm^2)	Mean Signal Intensity from *E. coli*-Produced MON 87419 DMO [1] (OD x mm^2)	Acceptance Limits[2] (OD x mm^2)
2.51	2.23	1.45-3.01

[1] Value refers to mean calculated based on n = 6. Values are rounded to two decimal places.
[2] The acceptance limits are for the MON 87419-produced DMO protein and are based on the interval between -35% (2.23 x 0.65 = 1.45) and +35 % (2.23 x 1.35 = 3.01) of overall mean of the *E. coli*-produced MON 87419 DMO signal intensity at all loads.

C.2.8. Molecular Weight Estimation using SDS-PAGE

C.2.8.1. Methods

MON 87419-produced and *E. coli*-produced DMO proteins were diluted in 5 × LB and Storage Buffer to obtain a final concentration of 1 × LB and heated to 95-105°C for 3-5 min. The MON 87419-produced DMO protein was loaded in duplicate at ~ 0.5, ~ 1.0, and ~ 1.5 µg based on total protein concentration, onto a Tris-glycine 4-20% polyacrylamide mini-gel (Invitrogen, Carlsbad, CA) in lanes 3, 4, 5, 6, 7 and 8. The *E. coli*-produced DMO protein was loaded at ~ 1.0 µg total protein in lane 2. Broad Range Molecular Weight Standards (Bio-Rad) were prepared and loaded on the gel in parallel. Following electrophoresis at a constant voltage, proteins were briefly fixed in 40% (v/v) methanol, 7% (v/v) acetic acid and stained for 18 ± 2 hr with Brilliant Blue G-Colloidal stain (Sigma). Gels were briefly destained in 10% (v/v) acetic acid, 25% (v/v) methanol followed by 8 ± 2 hr in 25% (v/v) methanol. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer supplied with QuantityOne software. Apparent MW and purity were reported as an average of all 6 lanes containing the MON 87419-produced DMO protein.

C.2.8.2. Results of MON 87419-produced DMO Protein Molecular Weight Equivalence

For apparent MW determination, the MON 87419-produced DMO and the *E. coli*-produced MON 87419 DMO proteins were subjected to SDS-PAGE. Following electrophoresis, the gel was stained with Brilliant Blue G-Colloidal stain and analyzed by densitometry. The intact MON 87419-produced DMO protein migrated to the same position on the gel as the *E coli*-produced MON 87419 DMO protein and the apparent MW was calculated to be 39.8 kDa (Figure C-4, Table C-4). Because the experimentally determined apparent MW of the MON 87419-produced DMO protein was within the acceptance limits for equivalence (Table C-4), the MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins were determined to have equivalent apparent molecular weights.

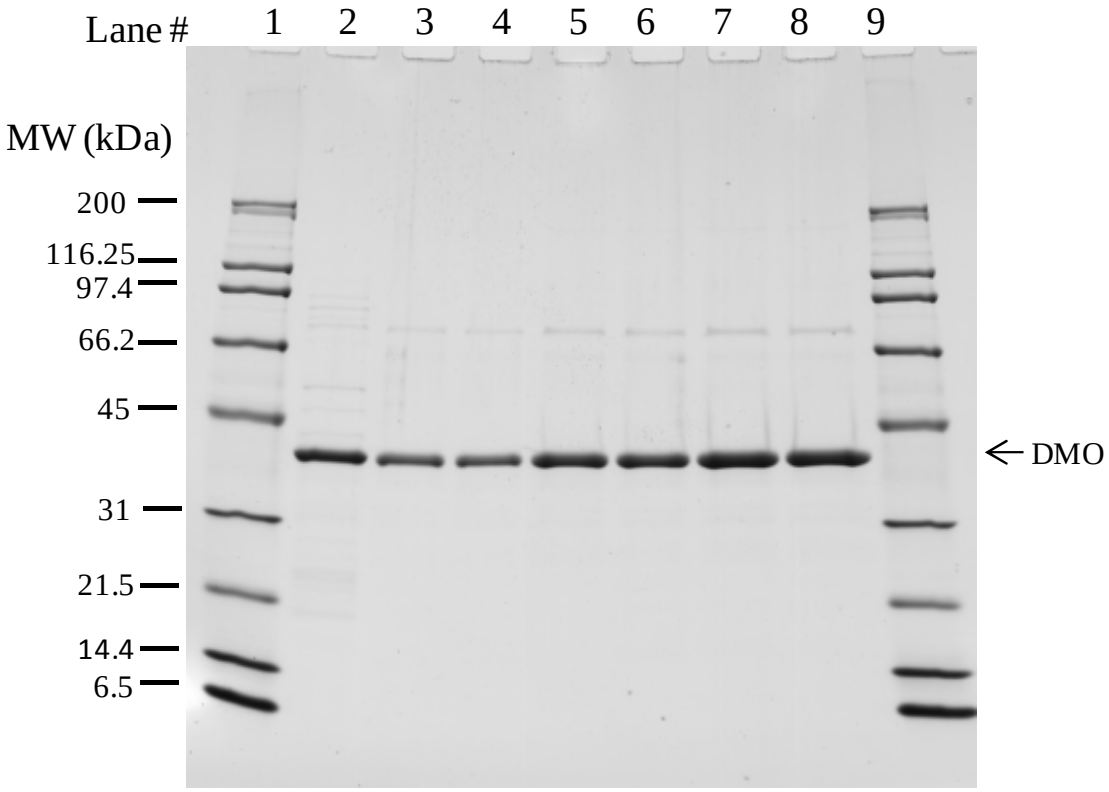


Figure C-4. Molecular Weight Analysis of the MON 87419-produced DMO Protein
Aliquots of the MON 87419-produced DMO and the *E. coli*-produced MON 87419 DMO proteins were subjected to SDS-PAGE and the gel was stained with Brilliant Blue G-Colloidal stain. The MWs (kDa) are shown on the left and correspond to the standards loaded in lanes 1 and 9. Lane 10 was cropped from the image. Lane designations are as follows:

Lane	Sample	Amount (µg)
1	Broad Range MW Standards	4.5
2	*E. coli*-produced MON 87419 DMO	1.0
3	MON 87419-produced DMO	0.5
4	MON 87419-produced DMO	0.5
5	MON 87419-produced DMO	1.0
6	MON 87419-produced DMO	1.0
7	MON 87419-produced DMO	1.5
8	MON 87419-produced DMO	1.5
9	Broad Range MW Standards	4.5
10	Blank	-

Table C-4. Molecular Weight Comparison Between the MON 87419- and *E. coli*-produced DMO Proteins

Apparent MW of MON 87419-Produced DMO Protein (kDa)	Apparent MW of *E. coli*-Produced MON 87419 DMO Protein[1] (kDa)	Acceptance Limits[2] (kDa)
39.8	39.5	38.6 - 40.4

[1] As reported on the Certificate of Analysis for *E. coli*-produced MON 87419 DMO+12 (lot 11383588).

[2] Calculated lower and upper bounds for one future assay based on 95% prediction interval derived from apparent MW determinations for *E. coli*-produced MON 87419 DMO protein (see Appendix C.4).

C.2.9. Glycosylation Analysis

C.2.9.1. Methods

ECL Glycoprotein Detection method (GE Healthcare) was used for glycoprotein detection. The MON 87419-produced DMO protein, the *E. coli*-produced DMO protein, and a positive control, transferrin (Sigma), were diluted in 5 × LB and Storage Buffer to obtain a final concentration of 1 × LB and heated to 95-105°C for 3-5 min. Two amounts (~ 100 and ~ 200 ng) of the intact MON 87419-produced DMO protein (purity corrected) and the *E. coli*-produced DMO protein (purity corrected) were loaded onto a pre-cast Tris-glycine 4-20% polyacrylamide mini-gel (Invitrogen). Three amounts (~ 50, ~ 100, and ~ 200 ng) of the positive control were loaded on the gel. Protein MW standards (Precision Plus Protein™ Standards, Bio-Rad) were also loaded 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 PVDF membrane (Invitrogen).

Glycosylation analysis was performed on the PVDF membrane at room temperature using the ECL Glycoprotein Detection method (GE Healthcare) and following the manufacturer's recommended protocol. Glycosylated proteins were detected using equivalent chemical reagents to the ECL™ reagents (GE Healthcare) and Amersham Hyperfilm (GE Healthcare). The film was developed using a Konica SRX-101A automated film processor (Konica Minolta). An identical gel was run and electrotransferred to a PVDF membrane in parallel. Proteins were stained with Coomassie Brilliant Blue R-250 staining solution (Bio-Rad) and then destained with 1× Coomassie Brilliant Blue R-250 Destaining Solution (Bio-Rad). After washing with water, the blot and gel were scanned using Bio-Rad GS-800 densitometer.

C.2.9.2. Results of Glycosylation Analysis

Eukaryotic proteins can be post-translationally modified with carbohydrate moieties (Rademacher et al., 1988). To test whether DMO protein was glycosylated when expressed in the grain of MON 87419, the MON 87419-produced DMO protein was analyzed using an ECL™ glycoprotein detection method. To assess equivalence of the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins, the *E. coli*-produced MON 87419 DMO protein was also analyzed.

A clear glycosylation signal was observed at the expected molecular weight (~ 80 kDa) in the lanes containing the positive control (transferrin) and the band intensity increased with increasing concentration (Figure C-5A). In contrast, no glycosylation signal was observed in the lanes containing the *E. coli*-produced MON 87419 DMO protein or MON 87419-produced DMO protein (Figure C-5A).

To confirm that MON 87419-produced DMO and *E. coli*-produced MON 87419 DMO proteins were appropriately loaded for glycosylation analysis, a second membrane with identical loadings and transfer time was stained with Coomassie Blue R250 for protein detection. Both the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins were detected (Figure C-5B). These data indicate that the glycosylation status of

MON 87419-produced DMO protein is equivalent to that of the *E. coli*-produced MON 87419 DMO protein and that neither is glycosylated.

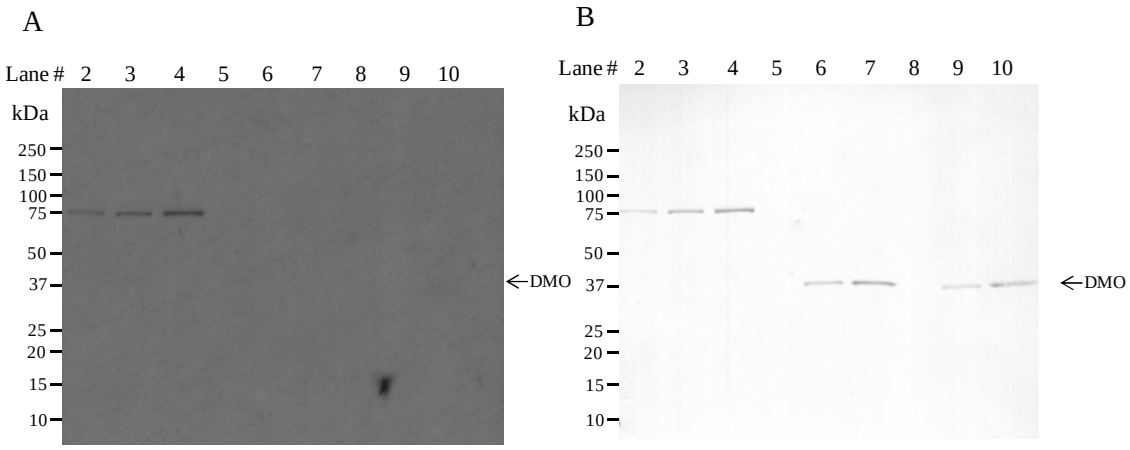


Figure C-5. Glycosylation Analysis of the MON 87419-produced DMO Protein
Aliquots of the transferrin (positive control), *E. coli*-produced MON 87419 DMO and MON 87419-produced DMO were subjected to SDS-PAGE and electrotransferred to a PVDF membrane. The MWs (kDa) correspond to the Precision Plus Protein™ Standards. Lanes loaded with MW standards are cropped. The arrows show the expected migration of the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins. (A) Where present, the labeled carbohydrate moieties were detected by addition of streptavidin conjugated to HRP followed by a luminol-based the detection using ECL reagents and exposure to Hyperfilm®. The 15-minute exposure is shown. (B) An equivalent blot was stained with Coomassie Blue R250 to confirm the presence of proteins. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Precision Plus Protein™ Standards	-
2	Transferrin (positive control)	50
3	Transferrin (positive control)	100
4	Transferrin (positive control)	200
5	Blank	-
6	*E. coli*-produced MON 87419 DMO	100
7	*E. coli*-produced MON 87419 DMO	200
8	Blank	-
9	MON 87419-produced DMO	100
10	MON 87419-produced DMO	200

C.2.10. Functional Activity Analysis

C.2.10.1. Methods

The specific activity of MON 87419-produced DMO protein was assessed and compared to the specific activity of the *E. coli*-produced MON 87419 DMO protein. The specific activity of the DMO proteins was assessed by measuring the amount of dicamba that was converted to DCSA via HPLC (Agilent Technologies 1100 series, Santa Clara, CA) separation and fluorescence detection (Agilent Technologies 1200 series, G1321A). Each assay reaction contained 25 mM potassium phosphate, pH 7.2, 4.0 µg ferredoxin, 15.4 µg reductase, 0.5 mM $FeSO_4$, 10 mM $MgCl_2$, 0.7 mM NADH, 0.3 mM dicamba, 2 µl (42.48 U/ml) of formaldehyde dehydrogenase and either approximately 1 µg MON 87419-produced DMO, *E. coli*-produced MON 87419 DMO, or his-tagged DMO protein as an assay positive control. The reactions were incubated at 30 ± 1°C for 15 ± 1 min. Reactions (200 µl) were initiated by the addition of dicamba and quenched with the addition of 50 µl of 5% H_2SO_4. Reactions were then filtered using Whatman Anotop 10 filters (0.2 µm, GE Healthcare), and 40 µl was transferred to a HPLC sample vial (200 µl, Agilent) for analysis. Twenty five microliters of the filtered reaction was injected onto a Phenomenex® Synergi 4 µm C18/ODS Hydro-RP column (150 × 4.6 mm ID, Torrance, CA). The mobile phase consisted of solvent A (21.5 mM phosphoric acid) and solvent B (100% acetonitrile) running at 1.5 ml/min. DCSA was eluted from the column using a linear gradient from 90% to 40% solvent A for the first 14 min, followed by a step to 10% solvent A for 1 min and then re-equilibration at 90% solvent A for 10 min before the next injection. DCSA was monitored by the detection of fluorescent emission at 424 nm (excitation: 306 nm) and quantified relative to a standard curve of DCSA generated using 0.1, 0.3, 0.6, 0.9, 1.2, 2.4, and 4.8 nmol/250 µl. Chromatographic data were collected using Atlas™ 2003 software (Thermo Fisher Scientific Inc). The specific activity was calculated based on the amount of DMO protein added to the reaction mixture and expressed as nmol of DCSA produced per minute per mg of DMO protein (nmol × $minute^{-1}$ × mg^{-1}).

C.2.10.2. Results of Functional Activity

The functional activity of the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins was determined by measuring the amount of dicamba that was converted to DCSA via HPLC separation and fluorescence detection. In this assay, activity is expressed as specific activity (nmol × $minute^{-1}$ × mg^{-1}).

The specific activity of the MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins were determined to be 232.5 and 240.1 nmol × $minute^{-1}$ × mg^{-1}, respectively (Table C-5). Because the specific activity of MON 87419-produced and *E. coli*-produced MON 87419 DMO proteins were within the acceptance limits (Table C-5), the proteins were determined to have equivalent functional activity.

Table C-5. MON 87419-produced DMO Functional Activity Assay

MON 87419-Produced DMO [1] (nmol × minute^{-1} × mg^{-1})	*E. coli*-Produced MON 87419 DMO [1] (nmol × minute^{-1} × mg^{-1})	Acceptance Limits[2] (nmol × minute^{-1} × mg^{-1})
232.5	240.1	99.3 – 251.5

[1] Value refers to mean calculated based on n = 3. Values are rounded to one decimal place.
[2] Calculated lower and upper bounds for one future assay based on 95% prediction interval derived from functinal activity assays for *E. coli*-produced MON 87419 DMO (see Appendix C.4).

C.3. Substrate Specificity of MON 87419 DMO Protein Produced in MON 87419

C.3.1. Exogenous Specificity Herbicide Tolerance – Greenhouse Analysis

C.3.1.1. Materials

MON 87419 (lot 11356837) and the near isogenic conventional control, NL6169 (lot 11356835) were grown in a greenhouse during 2013. At the 2 to 3 leaf growth stage, MON 87419 and the conventional control, NL6169, were sprayed with a single rate of one of the four herbicides (Table C-6).

The herbicides tested were selected based on a representation of a variety of herbicide classifications (based on mode-of-action) with agronomic importance for controlling grassy weeds. Similar to other grass species, maize has natural tolerance to several of the selective herbicide classes used in agriculture. Pre-study experiments were conducted to determine the rate of herbicide application that would be necessary to result in approximately 40 to 80 percent injury to conventional maize. The eleven herbicides used in the pre-test studies included at least one representative from each of nine major herbicide mechanisms-of-action classes, and each herbicide was applied at a labelled use rate and a rate at least two times the maximum labelled use rate. In these pre-studies, maize was not injured by seven of the herbicides tested (i.e., 2,4-D, 2,4-DB, acetochlor, atrazine, halosulfuron, oxyfluorfen and trifluralin), therefore, no additional testing for these herbicides was conducted. The lack of injury to maize was not unexpected because these herbicides are currently labelled for use in conventional maize crops to target various broadleaf weeds, not grasses. Only dicamba and the three other herbicides that injured maize in the pre-test were selected for use in this study.

C.3.1.2. Exogenous Specificity Herbicide Tolerance Greenhouse Method

MON 87419 and the near isogenic conventional control, NL6169, were planted in pots containing a mixture of one part silt loam soil to one part Redi-earth® potting soil medium. There were 10 replicate pots with one plant in each pot of MON 87419 and the conventional control for each herbicide and rate tested. The pots were randomly placed in a greenhouse and grown under normal agronomic conditions for maize (relative humidity 30 to 80 percent, temperature 22 to 29˚C, 14 hour photoperiod, and watering as needed). When the plants were at the 2 to 3 leaf growth stage, the replicates were

sprayed with a single rate of one of four herbicides (Table C-6). Two different application rates of each herbicide were applied to different replicate sets (Table C-6). Based on the U.S. herbicide labeled rates, the rates for the experiments were chosen and then adjusted for use on maize and for the optimal growing conditions in the greenhouse in order to achieve approximately 40 to 80 percent injury. Fifteen (15) days after herbicide application, all plants were rated for percent injury. Ratings were based on visual assessment of chlorosis, necrosis, malformation, stunting, and biomass reduction with 0 being no visible injury to 100 percent, completely dead. All 10 replicate ratings were averaged.

C.3.1.3. Results of Herbicide Tolerance Greenhouse Trials

MON 87419 demonstrated reduced injury ratings for dicamba, but similar injury ratings and therefore similar levels of susceptibility as the near isogenic conventional control for all other herbicides tested (Table C-6). This result is consistent with previous studies wherein the substrate specificity of DMO for dicamba has been observed (i.e., MON 87708 and MON 88701).

Table C-6. Herbicides and Herbicide Tolerance Injury Ratings

Formulation	Manufacturer	Lot Number	Herbicide[1]	Labeled Rate Range (g/ha)[2]	Rates Applied (g/ha)[2]	Injury ratings (%)[3] Control[4] Average (Range)	Injury ratings (%)[3] MON 87419[5] Average (Range)
Clarity®	BASF	KIH-0702-18134-F	dicamba	128-4488 (a.e.)	2244 (a.e.) 4488 (a.e.)	8.5 (0-15) 30.0 (5-60)	0 (0-0) 0 (0-0)
Gramoxone Max®	Syngenta	GTA-0606-17421	paraquat	232-1566 (a.e.)	561 (a.e.) 1122 (a.e.)	21.5 (15-40) 56.0 (20-90)	27.5 (15-80) 64.0 (20-100)
Roundup WeatherMax®	Monsanto	MUS-0905-19887-F	glyphosate	280-4162 (a.e.)	120 (a.e.) 240 (a.e.)	72.0 (50-90) 89.5 (80-95)	84.0 (60-95) 85.8 (80-98)
Assure II®	DuPont	MPO-0910-20036F	quizalofop	31-462 (a.i.)	20 (a.i.) 40 (a.i.)	95.9 (95-98) 100 (100-100)	94.4 (90-98) 100 (100-100)

[1] All herbicides were applied when the plants were at the V2-V3 growth stage.
[2] a.e. = acid equivalent; a.i. = active ingredient. Each herbicide contains the active ingredient directly or the salt form of the active ingredient. When determining the rate of application, the salt form is calculated back to the acid that is the active ingredient and therefore called acid equivalent. Each labeled rate is for one or more agricultural uses; only dicamba is currently labeled for use in conventional maize. Based on the labeled rates, the rates for the experiments were chosen and then adjusted to induce maize injury under optimal growing conditions in the greenhouse.
[3] Injury ratings were determined by visual inspection of each plant. Ratings were based on visual assessment of chlorosis, necrosis, malformation, stunting, and biomass reduction. 100 percent = completely dead and 0 percent = no visual adverse effects.
[4] Control plants were the near isogenic conventional control, NL6169. Reported average and range of 10 replicate plants.
[5] Reported average and range of 10 replicate plants.

® Gramoxone Max is a registered trademark of Syngenta.
® Assure II is a registered trademark of E.I. du Pont de Nemours and Company.

C.3.2. *In Vitro* Endogenous Specificity Experiments

C.3.2.1. Materials

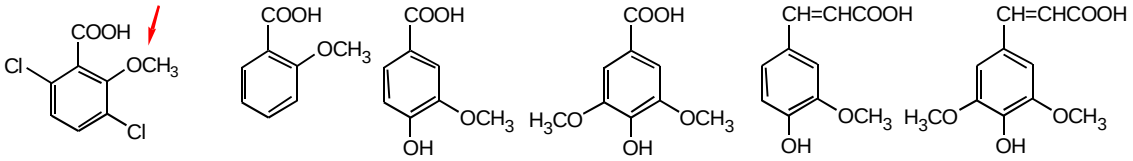
The DMO used in the *in vitro* enzyme assays was generated in *E. coli* with a histidine-tag at the N-terminus and has an identical amino acid sequence to the wild-type DMO found in *S. maltophilia* (Herman et al., 2005), with the exception of the histidine tag at the N-terminus (Figure C-1). The histidine tag was added to aid in the purification of the protein. The *E.coli*-produced MON 87419 DMO was shown to be functionally equivalent to the his-tagged DMO used in these assays (see Appendix C.3.2.5.).

The endogenous compounds tested (Janas et al., 2000) and standard used in the *in vitro* enzyme assays can be found in Table C-7 and Figure C-6. The tested compounds were identified by chemical substructure searching for compounds related to dicamba; followed by a literature search for the presence of these compounds in plants.

The DMO used in the crystallography experiment was generated in *E. coli* with a histidine-tag at the C-terminus and has an identical amino acid sequence to the wild-type DMO found in *S. maltophilia*, with the exception of an alanine added at position 2 and histidine-tag at the C-terminus (D'Ordine et al., 2009; Dumitru et al., 2009). The addition of the alanine was to aid in the cloning of the protein in *E. coli* and the histidine tag at the C-terminus was added to aid in the purification of the protein.

Table C-7. Compounds Used in Specificity *In Vitro* Enzyme Assays

Manufacturer/ Retailer	Compound	Common Name	Lot/Product Number
Compounds Tested:			
Aldrich	2-methoxybenzoic acid	o-anisic acid	A0230443
Chem Service	3,6-dichloro-2-methoxybenzoic acid	Dicamba	341-9143
Fluka	3,5-dimethoxy-4-hydroxybenzoic acid	syringic acid	86230
Fluka	4-hydroxy-3-methoxybenzoic acid	vanillic acid	94770
Fluka	3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	ferulic acid	46278
Sigma	3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid	sinapic acid	D7927-1G
Compounds Used as Standards:			
Monsanto	3,6-dichlorosalicylic acid	DCSA	GLP-0603-16959-T



Dicamba o-Anisic Acid Vanillic Acid Syringic Acid Ferulic Acid Sinapic Acid

Figure C-6. Dicamba and Set of Plant-Based Endogenous Substrates Tested in *In Vitro* Enzyme Assays with DMO
The arrow indicates methyl group removed by DMO.

C.3.2.2. *In Vitro* Specificity Experiments Enzymatic Reaction Mixture Method

The reaction of DMO with different compounds evaluated as potential substrates was carried out using the three enzymes necessary for demethylation: 0.077 µg/µL reductase (lot G825841), 0.0125 µg/µL ferredoxin (lot G828806A), and 0.005 µg/µL DMO in a reaction mixture containing 25 mM KPi, H_2O, 10 mM $MgCl_2$, 0.7 mM NADH, 0.0004 U/µL formaldehyde dehydrogenase, 0.5 mM $FeSO_4$, and the tested compound at 0.2 and 0.012 mM. The final volume for each assay sample was 200 µl. Each assay sample was incubated for 15±2 min at 30°C before quenching the reaction by the addition of 5% H_2SO_4. The concentrations of the compounds tested ensured adequate reaction conditions in terms of the detection of oxidative product formation or disappearance of the tested compound.

C.3.2.3. *In Vitro* Experiments Liquid Chromatography Separation Method

The reaction mixture was separated by UPLC using an ACQUITY UPLC BEH C18 Column containing 1.7 µm Bridged Ethyl Hybrid (BEH) particles and an ACQUITY BEH C18 VanGuard Pre-column. The column was heated to 40°C. The tested compounds and products formed were detected by ACQUITY UPLC photodiode array (PDA) with wavelength range from 200 nm to 320 nm with 1.2 nm resolution (LC-UV). The chromatography was performed at 0.25 ml/min and following the separation the column effluent was then directed to the mass spectrometer. Both mobile phase A (water) and solvent B (acetonitrile) contained 0.1% v/v formic acid. Gradients used were compound specific:

- The gradient for dicamba was run from 40 to 50% solvent B in 3 min, 50 to 100% solvent B in 0.1 min and then kept at 100% solvent B for 1 min before returning to 40% solvent B in 0.1 min. DCSA was used as a standard to determine product retention time.

- The gradients for ferulic acid, *o*-anisic acid, sinapic acid, syringic acid, and vanillic acid were run from 0 to 100% solvent B in 4 min and then held at 100% solvent B for 1 min before returning to 0% solvent B in 0.1 min.

A 5 µl injection of reaction mixture was used for experiments where the tested compound was monitored for disappearance and a 50 µl injection of reaction mixture was used for experiments where the formation of a potential oxidative product was monitored.

C.3.2.4. *In Vitro* Experiments Mass Spectrometry Detection Method

Elution from the UPLC column flowed directly to a Waters Micro Q-TOF mass spectrometer. The parameters used for the mass determination of all compounds were: negative mode, capillary voltage of 2800 V, sample cone voltage of 26 V, extraction cone of 1.5 V, source temperature of 150°C, and the desolvation temperature was 390°C. The desolvation gas flow was 500 L/hour and scan time was 0.76 seconds and inter scan delay was 0.1 sec. The m/z range used was specific to each tested compound and product. The m/z range for dicamba and DCSA was from 160 to 225 from 0 to 4 min. The m/z at 175, which is the fragment ion of dicamba, was used as a detection method for dicamba. This fragment ion of dicamba gave better sensitivity, than the parent ion. The m/z at 205 was used to detect DCSA. The m/z range for all other test compounds and potential oxidative products is from 120 to 250 within 4 min. The m/z at 151 was used to detect *o*-anisic acid. The m/z at 193 was used to detect ferulic acid. The m/z at 223 was used to detect sinapic acid. The m/z at 197 was used to detect syringic acid. The m/z at 167 was used to detect vanillic acid.

C.3.2.5. Results of Endogenous Compound *In Vitro* Reaction Mixture Experiments

The reaction of dicamba with DMO has been well characterized utilizing an *in vitro* enzymatic assay that monitors the formation of DCSA by LC-UV as well as LC-MS, which allows the detection of the product with high sensitivity. The substrate (dicamba) and oxidative product (DCSA) from the reaction of dicamba with DMO can be detected by PDA and LC-MS after separation by UPLC as shown in Figure C-7.

Compounds structurally similar to dicamba and potentially present in either soybean, cotton, or maize were used as potential substrates to determine if these tested compounds could be catabolized by DMO (Table C-7 and Figure C-6). The compounds tested were sinapic acid, ferulic acid, *o*-anisic acid, syringic acid, and vanillic acid. Mass spectrometry scans were taken 120 m/z to 250 m/z to cover the range of tested compounds and all potential oxidative products formed by the reaction of the compounds tested and DMO. Standard reaction conditions of dicamba with DMO were used as a positive control to demonstrate the functionality of the method (Figure C-8). For the compounds tested (sinapic acid, ferulic acid, *o*-anisic acid, syringic acid, and vanillic acid), no additional peaks that might be associated to the predicted oxidative product were observed in the mass spectrometry scans from 120 m/z to 250 m/z. The results of the reaction mixtures containing the tested compounds, with and without DMO, are shown in Figures C-8 through 10. Each tested compound is shown at their respective detection masses. Based on the known mode-of-action of DMO, a single demethylation was expected and the respective masses for the predicted oxidative products of each tested compound are also shown in Figures C-8 through C-10. These results demonstrate the tested compounds syringic acid, *o*-anisic acid, vanillic acid, ferulic acid, and sinapic acid were not catabolized by DMO.

To assess whether MON 87419 DMO protein has the same specificity as the histidine tagged DMO used in the *in vitro* endogenous specificity experiments, the *E. coli*-produced MON 87419 DMO protein (i.e., lacking a histidine tag), shown to be equivalent to the plant produced MON 87419 DMO protein (Section V.B), was incubated with *o*-anisic acid, the compound that has the greatest structural similarity to dicamba. Again dicamba was used as a positive control to demonstrate the assay system was functional. This analysis demonstrated that *o*-anisic acid was not metabolized by the *E. coli*-produced MON 87419 DMO protein (i.e., lacking a histidine tag), but dicamba was (Figures C-11 and C-12). These results indicate that DMO, including the MON 87419 DMO protein, is specific for dicamba as a substrate.

C.3.2.6. Results of Dicamba and *o*-Anisic Acid *In Vitro* Binding to DMO Protein Crystals

Crystals of DMO were generated by the method detailed in D'Ordine et al. (2009). The DMO protein crystals were soaked in a buffer containing 1.25 mM dicamba for 24 hours. Separately, crystals were soaked in a 5 mM solution of *o*-anisic acid. After soaking, crystals were examined using x-ray diffraction crystallography to determine binding in the catalytic site. All methods for crystallography, data collection, analysis and structure solution, and refinement are described in D'Ordine et al. (2009).

Crystals of DMO were soaked in solutions containing dicamba or *o*-anisic acid to test whether each compound would bind to DMO. *o*-Anisic acid was chosen since it is the most structurally similar to dicamba of the endogenous compounds tested. When DMO crystals were soaked with *o*-anisic acid, at concentrations 4-fold higher than those used for dicamba, no protein crystals were observed that contained *o*-anisic acid in the catalytic site, demonstrating that *o*-anisic acid did not bind to the DMO. It has been previously described that dicamba interacts with amino acids in the catalytic site of DMO through both carboxylate moiety and the chlorine atoms of dicamba, which are primarily involved in orienting the substrate in the catalytic site. The chlorine atoms present in dicamba (and absent in *o*-anisic acid) are required for binding to DMO (D'Ordine et al., 2009; Dumitru et al., 2009).

C.3.2.7. Conclusions

The results presented herein demonstrate that DMO has a high specificity for dicamba as a substrate. Endogenous soybean, cotton, and maize compounds structurally similar to dicamba were tested as substrates, no catabolism of the tested compounds was observed when incubated with DMO, except for dicamba, which was included as a positive control. The soaking crystals of DMO in *o*-anisic acid showed that *o*-anisic acid does not bind to DMO at the catalytic site. Therefore, DMO is specific for its substrate dicamba and did not catabolize other structurally similar compounds tested.

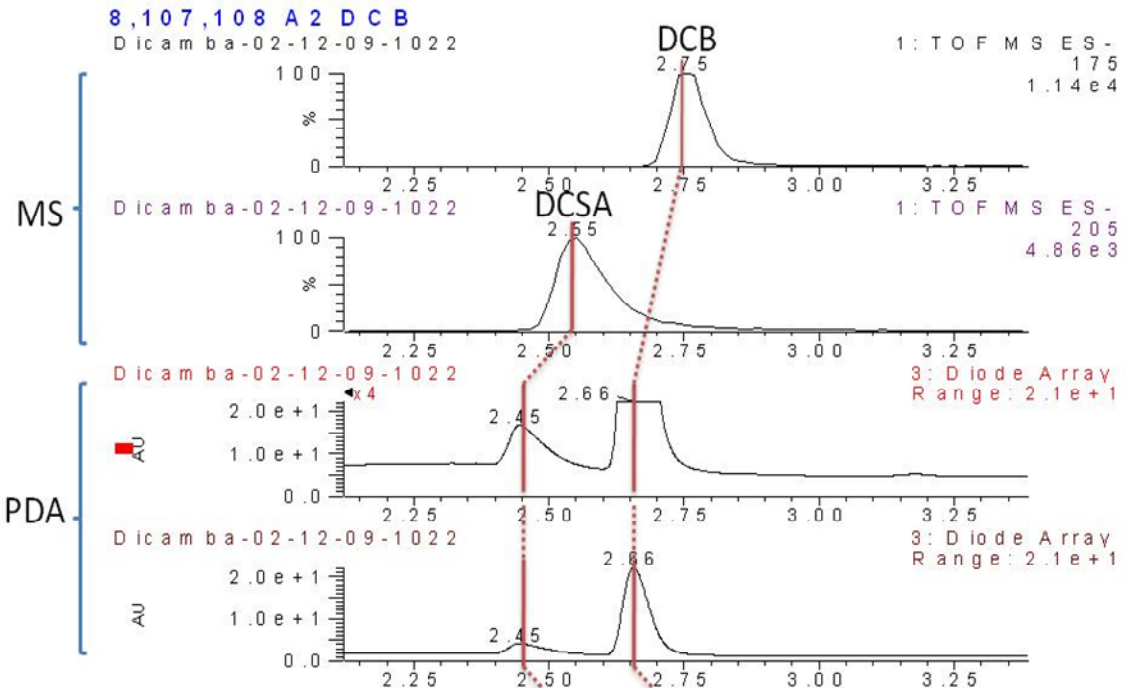


Figure C-7. UPLC Separation of Dicamba (DCB) and DCSA

Dicamba and DCSA were separated by UPLC and detected by UV absorbance using a Photo Diode Array (PDA) and mass spectrometry (MS). Both dicamba and DCSA are shown on the chromatograms using PDA loaded at 1x and 4x; however, due to the different m/z used for optimal detection of dicamba (m/z=175) and DCSA (m/z=205), each compound is shown on different chromatograms for detection by MS. A line connects each compound across all the chromatograms displayed for ease of identification.

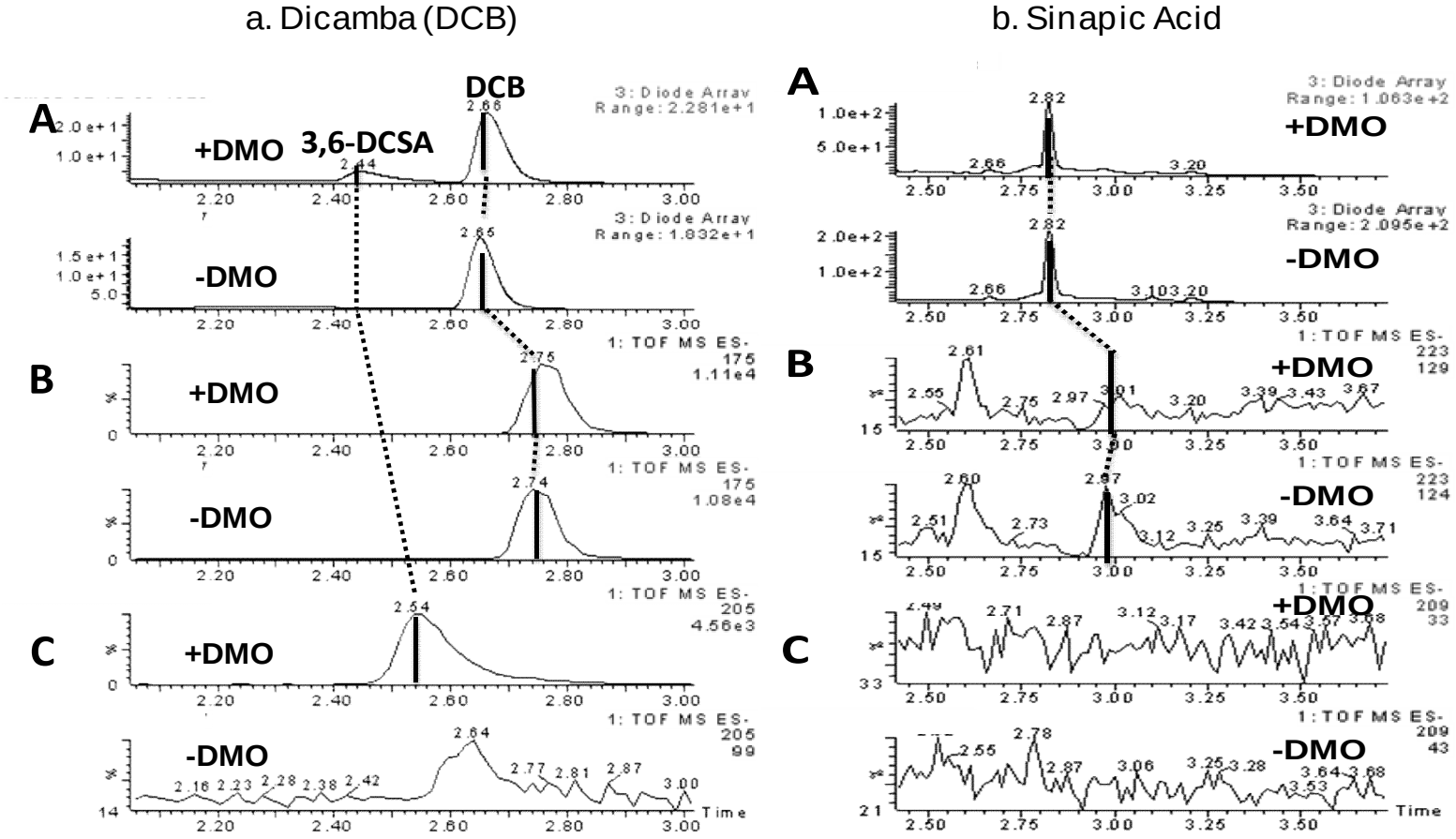


Figure C-8. DMO Conversion of Dicamba and Sinapic Acid

Dicamba and sinapic acid were incubated with the N-terminal histidine-tagged DMO, and the formation of predicted oxidative products and the disappearance of each tested compound was monitored by LC-UV (A chromatograms) and LC-MS (B and C chromatograms). The B chromatograms with a 5 µl injection show the expected m/z for the tested compound, while the C chromatograms with a 50 µl injection show the expected m/z for the predicted oxidative product. Dicamba (a) was used as a positive control. The tested compound was included in a reaction mixture made with (+DMO, upper) and without (-DMO, lower) DMO. The dotted line indicates the migration of the compounds (and DCSA in the case of dicamba) in each chromatogram as a result of the UV and MS detectors being connected in series.

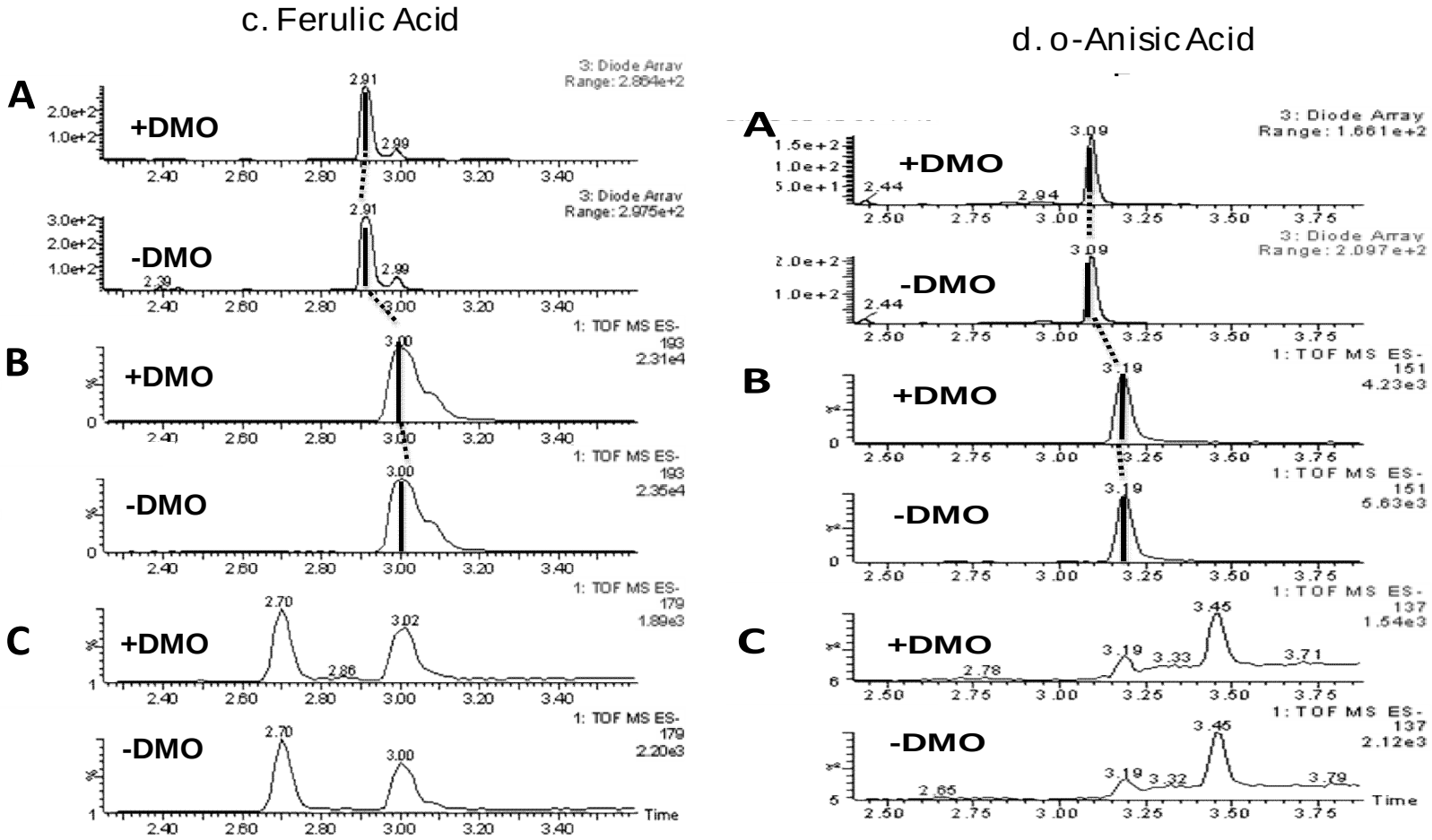


Figure C-9. DMO Conversion of Ferulic Acid and *o*-Anisic Acid

Ferulic acid and *o*-anisic acid were incubated with the N-terminal histidine-tagged DMO, and the formation of predicted oxidative products and the disappearance of each tested compound was monitored by LC-UV (A chromatograms) and LC-MS (B and C chromatograms). The B chromatograms with a 5 µl injection show the expected m/z for the tested compound, while the C chromatograms with a 50 µl injection show the expected m/z for the predicted oxidative product. The tested compound was included in a reaction mixture made with (+DMO, upper) and without (-DMO, lower) DMO. The dotted line indicates the migration of the compounds in each chromatogram as a result of the UV and MS detectors being connected in series.

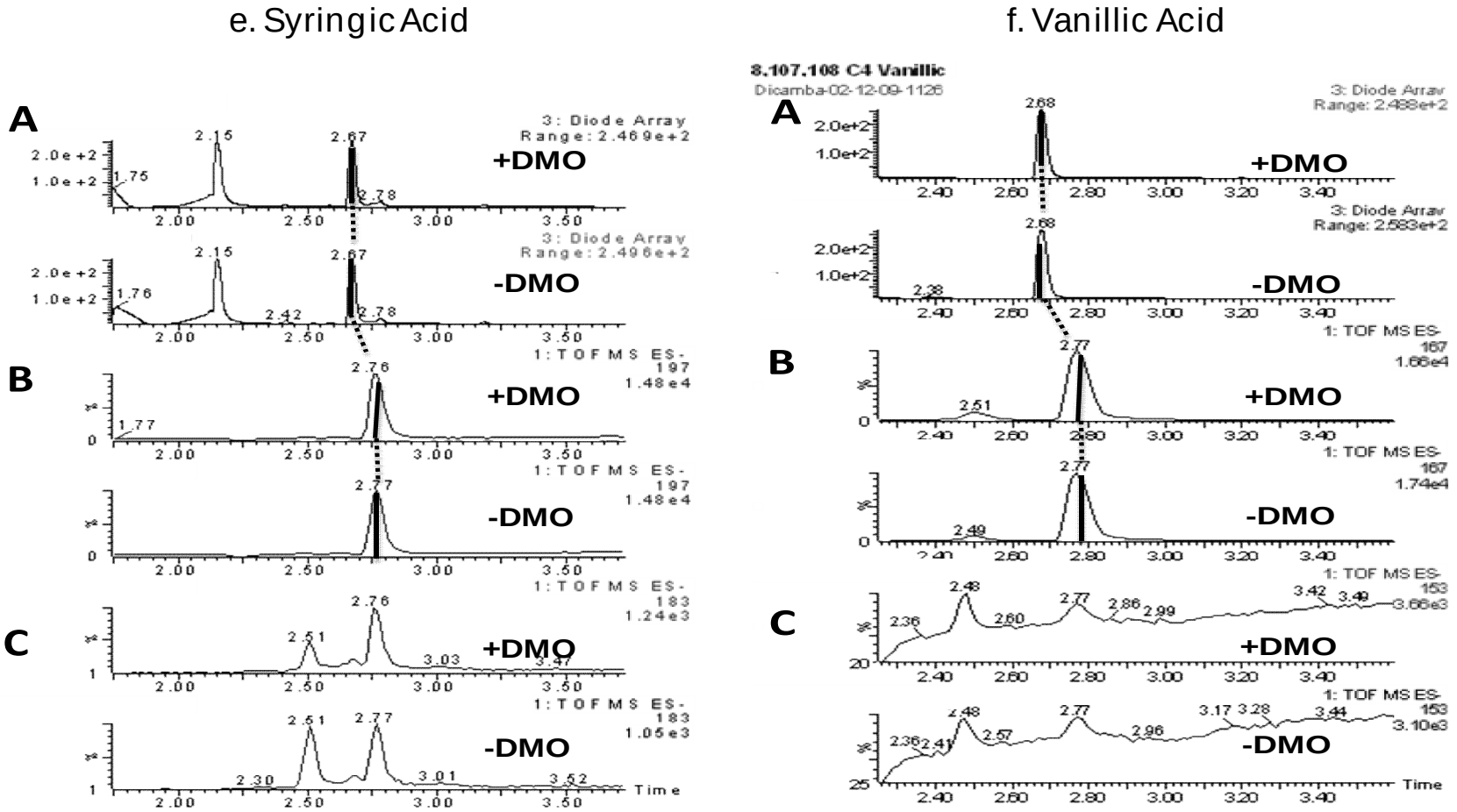


Figure C-10. DMO Conversion of SyringicAcid and Vanillic Acid

Syringic acid and vanillic acid were incubated with the N-terminal histidine-tagged DMO, and the formation of predicted oxidative products and the disappearance of each tested compound was monitored by LC-UV (A chromatograms) and LC-MS (B and C chromatograms). The B chromatograms with a 5 µl injection show the expected m/z for the tested compound, while the C chromatograms with a 50 µl injection show the expected m/z for the predicted oxidative product. The tested compound was included in a reaction mixture made with (+DMO, upper) and without (-DMO, lower) DMO. The dotted line indicates the migration of the compounds in each chromatogram as a result of the UV and MS detectors being connected in series.

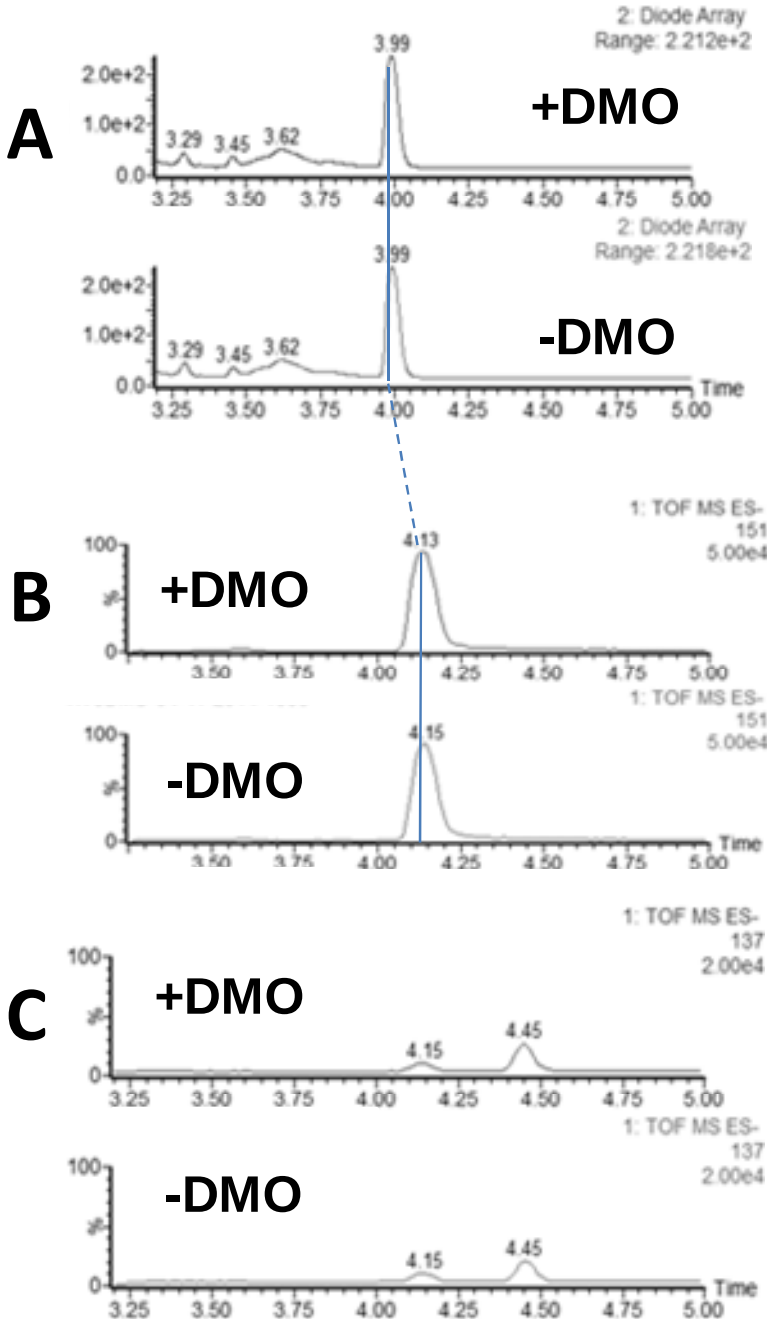


Figure C-11. Assay Results for *o*-Anisic Acid

200 µM of *o*-anisic acid was included in a reaction mixture made with (+DMO, upper) or without (-DMO, lower) MON 87419 DMO, and the presence of the added compound and formation of the predicted demethylated product was monitored by LC-UV (Panel A) and LC-MS (Panels B and C). Panel B chromatograms with a 5 µl injection show the monitored m/z (151) for *o*-anisic acid, while the panel C chromatograms with a 50 µl injection show the monitored m/z (137) for the predicted product. The y-axes in panels B and C were set to 50000 and 20000 counts full scale, respectively.

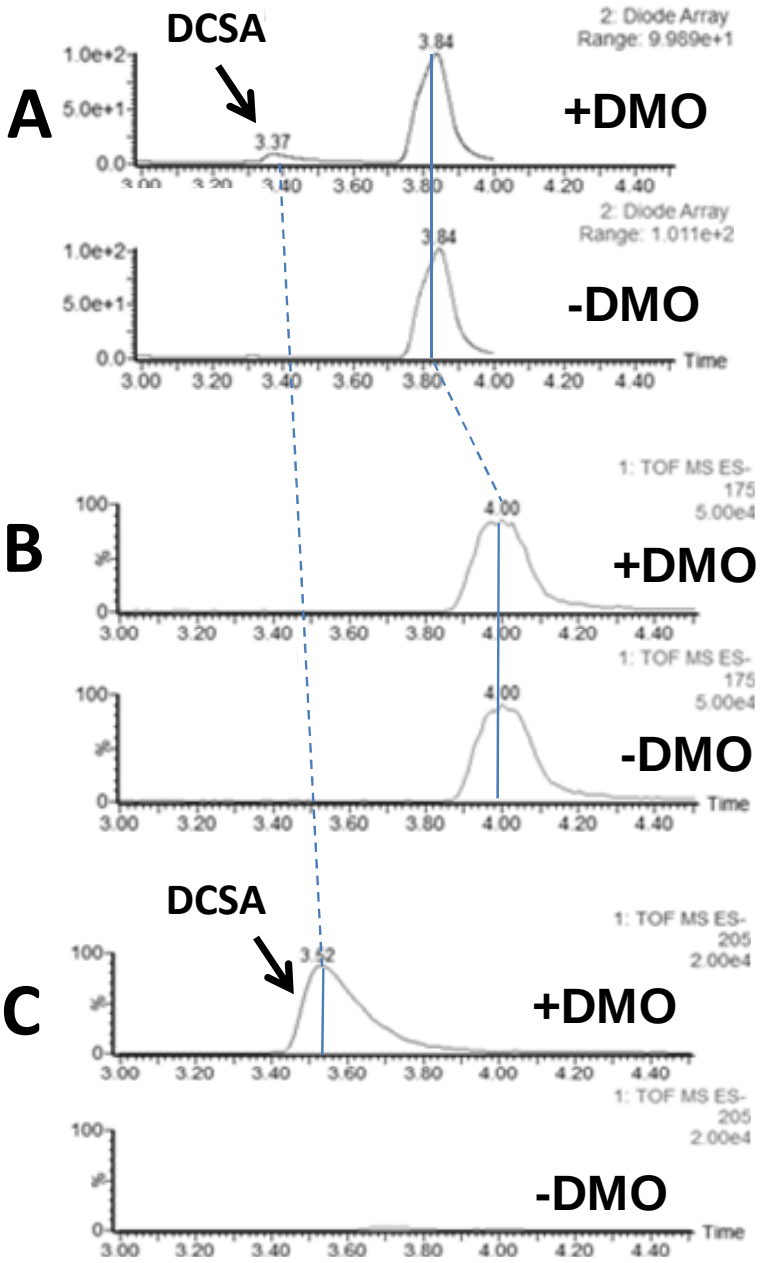


Figure C-12. Assay Results for Dicamba

200 µM of dicamba was included in a reaction mixture made with (+DMO, upper) or without (-DMO, lower) MON 87419 DMO, and the presence of the added compound and formation of the expected demethylated product, DCSA, was monitored by LC-UV (Panel A) and LC-MS (Panels B and C). Panel B chromatograms with a 5 µl injection show the monitored m/z (175) for dicamba, while the panel C chromatograms with a 50 µl injection show the monitored m/z (205) for DCSA. The peak corresponding to DCSA is indicated by arrow in panels A and C. The y-axes in the B and C chromatograms were set to 50000 and 20000 counts full scale, respectively.

C.4. Prediction Intervals as Acceptance criteria

Acceptance criteria (acceptance limits) based on prediction intervals were used to assess the equivalence of the MON 87419-produced and *E. coli*-produced proteins for apparent MW and functional activity. A prediction interval is an estimate of an interval in which a randomly selected future observation from a population will fall, with a certain degree of confidence, given what has already been observed (Hahn and Meeker, 1991a; b); i.e., prediction intervals are generated based on the statistical analysis of the existing data. Data obtained from multiple assays of *E. coli*-produced protein conducted under GLP guidelines were used for this purpose.

To generate the 95% prediction interval (PI), the mean and standard deviation of the data from several assays were calculated. The number of assays used to calculate the mean and the number of future assays (one for equivalence studies) were used in the following formula to generate the PI:

$$\bar{X} \pm r(1-\alpha;\ m,n)\quad (s)$$

$r_{(1-\alpha;\ m,\ n)}$ is estimated using the formula given below:

$$r_{(1-\alpha;m,n)} \cong\ t_{(1-.05/(2m);\ n-1)}\sqrt{1+\frac{1}{n}}$$

Where $\bar{X}$ is mean of the replicate assays; s is standard deviation of the replicates; $1-\alpha$ is the level of confidence; n is the number of assays used to generate the mean; and m is the number of future assays (one for equivalence studies). The t-value is the $100(1-.05/(2m))^{th}$ percentile from Student's t-distribution with n-1 degrees of freedom. With 95% confidence, all m future values of the assay will fall within this interval (Hahn and Meeker, 1991a; b). If the assay means do not appear to have been derived from a normal distribution, but the logarithms of the raw values do follow a normal distribution, then prediction intervals may be applied to the logarithms of the raw values (Hahn and Meeker, 1991a; b).

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Appendix D: Materials and Methods Used for the Analysis of the Levels of DMO and PAT Proteins in MON 87419

D.1. Materials

Leaf, root, forage, and grain tissue samples from MON 87419 were harvested from five field sites in the U.S. during the 2013 growing season from starting seed lot 11356837. *E. coli*-produced DMO (lot 11293429) and PAT protein (lot 11372784) were used as the analytical reference standards.

D.2. Characterization of the Materials

The identity of MON 87419 was confirmed by conducting MON 87419 event-specific polymerase chain reaction (PCR) analyses on the starting seed.

D.3. Field Design and Tissue Collection

Field trials were initiated during the 2013 planting season to generate tissues of MON 87419 at various maize growing locations in the U.S.. Leaf, root, forage, and grain tissue samples from the following field sites were analyzed: Boone County, Iowa (IAPY), Clinton County, Indiana (INKI), Pawnee County, Kansas (KSLA), York County, Nebraska (NEYO), and Lehigh County, Pennsylvania (PAGR). At each site, four replicated plots of plants containing MON 87419 were planted using a randomized complete block field design. Tissue samples were collected from each replicated plot at field sites treated with dicamba and glufosinate. See Table V-1 and Table V-2 for detailed descriptions of when the samples were collected.

D.4. Tissue Processing and Protein Extraction

Tissue samples were shipped to Monsanto Company (St. Louis, Missouri), and were prepared by the Monsanto Sample Management Team. The prepared tissue samples were stored in a -80° C freezer until transferred on dry ice to the analytical facility.

D.4.1. DMO Protein

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

Table D-1. DMO Extraction Methods [1] for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf	1:100	1 × TB[2], pH 7.8
Root	1:100	1 × TB, pH 7.8
Forage	1:100	1 × TB, pH 7.8
Grain	1:100	1 × TB, pH 7.8

[1]DMO protein was extracted from each tissue by adding the appropriate volume of extraction buffer and number of chrome steel beads, and shaking in a Harbil mixer (Harbil Industries Inc.). The extracted samples were clarified using a serum filter.
[2]0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.005 M $MgCl_2$, 0.05% (v/v) Tween-20.

D.4.2. PAT Protein

The PAT protein was extracted from maize tissues as described in Table D-2. The protein extracts were aliquoted and stored frozen in a -80 °C freezer until immunoassay analysis.

Table D-2. PAT Extraction Methods [1] for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf	1:100	1 × PBST + 0.1% (w/v) BSA[2]
Root	1:100	1 × PBST + 0.1% (w/v) BSA
Forage	1:100	1 × PBST + 0.1% (w/v) BSA
Grain	1:100	1 × PBST + 0.1% (w/v) BSA

[1]PAT protein was extracted from each tissue by adding the appropriate volume of extraction buffer and number of chrome steel beads, and shaking in a Harbil mixer (Harbil Industries Inc.). The extracted samples were clarified using a serum filter.
[2]Phosphate buffered saline buffer with Tween-20 and 0.1% bovine serum albumin [0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, and 0.0027 M KCl with 0.05 % (v/v) Tween-20 and 0.1% (w/v) BSA].

D.5. DMO and PAT Antibodies

D.5.1. DMO Antibodies

Goat polyclonal antibodies (lot G-884602) specific for the DMO protein were purified using Protein-G affinity chromatography. The concentration of the purified IgG was determined to be 8.1 mg/ml by spectrophotometric methods. The purified antibodies were stored in phosphate buffer saline (1 × PBS) (0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, and 0.0027 M KCl).

The purified goat polyclonal DMO antibodies were coupled with biotin (Thermo-Fischer Scientific, Inc.) according to the manufacturer's instructions and assigned lot G-884603. The detection reagent was NeutrAvidin (Pierce, Inc.) conjugated to horseradish peroxidise (HRP).

D.5.2. PAT Antibodies

The PAT antibody-coated immunoassay plates and HRP-labeled PAT antibody were commercial reagents purchased from EnviroLogix, Inc. (catalog number AP-014).

D.6. DMO and PAT ELISA Methods

D.6.1. DMO Protein

Goat polyclonal anti-DMO capture antibodies were diluted in a coating buffer (0.015 M Na_2CO_3 and 0.035 M $NaHCO_3$ with 150 mM NaCl) and immobilized onto 96-well microtiter plates at 5 µg/ml. Prior to each step in the assay, plates were washed with 1 × PBS containing 0.05 % (v/v) Tween 20 (1 × PBST). Plates were blocked with the addition of 200 µl per well of blocking buffer (Blocker casein in TBS, Thermo-Fischer Scientific, Inc.) for 60 to 70 minutes at room temperature. DMO protein standard or sample extract was added at 100 µl per well and incubated for 60 to 65 minutes at 37 °C. Biotinylated goat anti-DMO antibodies were added at 100 µl per well and incubated for 60 to 65 minutes at 37 °C. NeutrAvidin-HRP conjugate was added at 100 µl per well and incubated for 30 to 35 minutes at 37 °C. Plates were developed by adding 100 µl per well of horseradish peroxidase substrate, 3,3',5,5'-tetramethyl-benzidine (Kirkegaard & Perry Labs Inc.). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantification of the DMO protein was accomplished by interpolation from a DMO protein standard curve that ranged from 0 – 100 ng/ml.

D.6.2. PAT Protein

The anti-PAT coated immunoassay plates and antibody conjugate were purchased as a kit (Qualiplate Kits for LibertyLink PAT/pat. AP-014.) Prior to each step in the assay, plates were washed with 1 × PBST. The antibody conjugate was added at 50 µl per well. The PAT protein standard or sample extract was added at 50 µl per well and incubated for 60 to 70 minutes at 37 °C. Plates were developed by adding 100 µl per well of HRP

substrate, 3,3',5,5'-tetramethyl-benzidine (Kirkegaard & Perry Labs Inc.). The enzymatic reaction was terminated by the addition of 100 µl per well of 1N HCl. Quantification of the PAT protein was accomplished by interpolation on a PAT protein standard curve that ranged from 0 - 25 ng/ml.

D.7. Moisture Analysis

Tissue moisture content was determined using a Mettler Toledo HR83 Moisture Analyzer System (Mettler-Toledo, Inc.). A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a specific site.

The mean percent moisture for each TSSP was calculated from triplicate analyses. A TSSP dry weight conversion factor (DWCF) was calculated using MoistureDirect software as follows:

$$\text{DWCF} = 1 - \left(\frac{\text{Mean \% TSSP Moisture}}{100} \right)$$

The DWCF was used to convert protein levels assessed on a µg/g fw basis into levels reported on a µg/g dw basis using the following calculation:

$$\text{Protein Level in Dry Weight} = \frac{\text{Protein Level Fresh Weight}}{\text{DWCF}}$$

The protein levels (ng/ml) that were reported to be less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

D.8. Data Analyses

All immunoassay plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices, Inc.) 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 software. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit for DMO and five-parameter logistic curve fit for PAT.

Following the interpolation from the standard curve, for data that were greater than or equal to the LOQ, the protein levels (ng/ml) in the tissues were converted to a µg/g fw value. For each protein, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio. Protein values in "µg/g fw" were also converted to "µg/g dw" by applying the DWCF.

Microsoft Excel 2007 (Microsoft) was used to calculate the protein levels in maize tissues. The sample means, standard deviations (SDs), and ranges were also calculated by Microsoft Excel 2007. All protein expression levels were rounded to two significant figures.

Any test substance extract that resulted in unexpectedly negative results by immunoassay analysis were re-extracted twice for the protein of interest and re-analyzed by immunoassay to confirm the results. Samples with confirmed unexpected results were omitted from all calculations.

Appendix E: Materials and Methods for the Compositional Analysis of MON 87419 Maize Grain and Forage Not Treated With Dicamba and Glufosinate

E.1. Materials

Harvested grain and forage from MON 87419, treated (T) and not treated (NT) with dicamba and glufosinate, and a conventional control that has similar genetic background to that of MON 87419 were compositionally assessed. Treated compositional analyses were discussed in Section VI.

E.2. Characterization of the Materials

The identities of MON 87419 and the conventional control were confirmed prior to use in the compositional assessment.

E.3. Field Production of the Samples

Grain and forage samples from MON 87419, treated (T) and not treated (NT) with dicamba and glufosinate, and the conventional control were collected from five replicated sites in U.S. during the 2013 growing season. The field sites were located in: Boone County, Iowa (IAPY), Clinton County, Indiana (INKI), Pawnee County, Kansas (KSLA), York County, Nebraska (NEYO) and Lehigh County, Pennsylvania (PAGR). Separate entries were included for MON 87419 treated and not treated with dicamba and glufosinate. Starting seeds were planted in a randomized complete block design with four plots for each of MON 87419 and the conventional control. The production was conducted under agronomic field conditions for the different growing regions that are typical areas for maize production in the U.S..

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 and stored in a freezer set to maintain -20°C located at Monsanto Company (Saint Louis, Missouri). Subsamples were shipped on dry ice to EPL Bio Analytical Services (Niantic, Illinois) for compositional analysis.

E.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 [A (β-carotene), 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 EPL Bio Analytical Services (Niantic, Illinois). Methods for analysis were based on internationally-recognized procedures and literature publications. Brief descriptions of the methods utilized for the analyses are provided below.

E.4.1. Acid Detergent Fiber

Subsamples of ground forage and grain were analyzed to determine the percentage of acid detergent fiber (ADF) by digesting with an acid detergent solution and washing with reverse osmosis (RO) water. The remaining residue was dried and weighed to determine ADF content. Samples were analyzed with the Ankom Extraction Apparatus (Ankom Technology, 2010a). The limit of quantitation was 0.01%.

E.4.2. Amino Acid Composition

The following 18 amino acids were analyzed in ground grain:

- 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 amount of tryptophan was determined by reverse phase Ultra Performance Liquid Chromatography (UPLC) with UV detection following hydrolysis with 4M lithium hydroxide and dilution to 50 mL with deionized (DI) water (Rogers and Pesti, 1990).

The amount of cystine and methionine was determined by reverse phase UPLC with UV detection following conversion of the cystine to cysteic acid and methionine to methionine sulfone, after acid oxidation and hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives. The amount of the other 15 amino acids was determined by reverse phase UPLC with UV detection following conversion of the free acids, after acid hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives (AOAC, 2012a; Liu, 1994; Waters Method, 1995).

Reference Standards:

Component	Manufacturer	Lot No.	Purity (%)	LOQ (mg/g)
L-Alanine	Sigma-Aldrich	BCBF7865V	99.8	1.00
L-Arginine Monohydrochloride	Sigma-Aldrich	051M0218V	100	0.828
L-Aspartic Acid	Sigma-Aldrich	060M1511V	100	1.00
L-Cystine	Sigma-Aldrich	081M0123V	100	0.296
L-Cysteic Acid Monohydrate	Sigma-Aldrich	BCBH5081V	100.2	0.296
L-Glutamic Acid	Sigma-Aldrich	060M01711V	100	1.00
Glycine	Sigma-Aldrich	128K0194	100	1.00
L-Histidine Monohydrochloride Monohydrate	Sigma-Aldrich	SLBF0771V	100	0.740
L-Isoleucine	Sigma-Aldrich	040M01172V	100	1.00
L-Leucine	Sigma-Aldrich	045K0387	>99	1.00
L-Lysine Monohydrochloride	Alfa Aesar	10168370	100.6	0.800
L-Methionine	Sigma-Aldrich	90M006934V	100	0.343
L-Methionine Sulfone	Sigma-Aldrich	SLBC5111V	100	0.343
L-Phenylalanine	Sigma-Aldrich	SLBF2036V	100	1.00
L-Proline	Sigma-Aldrich	BCBJ3904V	100.0	1.00
L-Serine	Sigma-Aldrich	SLBC5251V	100.1	1.00
L-Threonine	Sigma-Aldrich	110M00881V	100	1.00
L-Tyrosine	Sigma-Aldrich	075K0015	100	1.00
L-Valine	Sigma-Aldrich	SLBC5601V	100	1.00
L-Tryptophan	Sigma-Aldrich	SLBG1056V	100	0.250

Internal Standards:

- Sigma-Aldrich, L-2-Aminobutyric Acid, 99.5%, Lot Number BCBF3200V

E.4.3. Ash

Subsamples of ground forage and grain were ignited in a muffle furnace for three hours at 650°C. The weight of the ash residue remaining after ignition was determined gravimetrically (AOAC, 2012c). The limit of quantitation was 0.01%.

E.4.4. Carbohydrate

The total carbohydrate level of ground forage and grain was calculated by difference using the fresh weight-derived data and the following equation (USDA-ARS, 1973):

Carbohydrates (%) = 100 – (Moisture (%) + Ash (%) + Fat (%) + Protein (%))

E.4.5. Crude Fat by Acid Hydrolysis

Subsamples of ground forage were dried in an oven for at least 2 hours. The crude fat content was determined gravimetrically after acid hydrolysis and extraction with mixed ethers (AOAC, 2012d). The limit of quantitation was 0.01%.

E.4.6. Crude Fat by ANKOM

Crude fat content in subsamples of ground grain was determined by extraction with petroleum ether using the ANKOM XTl5 extraction system. After extraction, the samples were oven dried and the crude fat content was determined gravimetrically (Ankom Technology, 2010b; AOCS, 2009a). The limit of quantitation was 0.01%.

E.4.7. 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 amount of fatty acids in ground grain was determined by Gas Chromatography with Flame Ionization Detection following fat extraction and derivatization of the fatty acids into methyl esters with boron trifluoride/methanol (AOAC, 2012e; AOCS, 2009b; c).

Reference Standards:

- Nu-Chek Prep, Inc , Fatty Acid Methyl Ester Standard (Major Acids), Lot Number F13-X

  0.5% C12:0 (Limit of quantitation = 0.00187%)
  0.5% C14:0 (Limit of quantitation = 0.00188%)
  9.0% C16:0 (Limit of quantitation = 0.0341%)
  0.5% C16:1 (Limit of quantitation = 0.00190%)
  0.5% C17:0 (Limit of quantitation = 0.00190%)
  0.5% C17:1 (Limit of quantitation = 0.00190%)
  4.0% C18:0 (Limit of quantitation = 0.0152%)
  24.0% C18:1 (Limit of quantitation = 0.0915%)
  48.0% C18:2 (Limit of quantitation = 0.00320%)
  8.5% C18:3 (Limit of quantitation = 0.000567%)

0.5% C20:0 (Limit of quantitation = 0.00191%)
1.0% C20:1 (Limit of quantitation = 0.00191%)
0.5% C20:2 (Limit of quantitation = 0.00191%)
1.0% C22:0 (Limit of quantitation = 0.00384)

- Nu-Chek Prep, Inc , Fatty Acid Methyl Ester Standard (Major Acids), Lot Number N15-P

10.0% C8:0 (Limit of quantitation = 0.00729%)
10.0% C10:0 (Limit of quantitation = 0.00740%)
10.0% C14:1 (Limit of quantitation = 0.00753%)
10.0% C15:0 (Limit of quantitation = 0.00756%)
10.0% C15:1 (Limit of quantitation = 0.00756%,
10.0% C18:2 (Limit of quantitation = 0.00320%)
10.0% gamma C18:3 (Limit of quantitation = 0.00762%)
10.0% C20:3 (Limit of quantitation = 0.00765%)
10.0% C20:4 (Limit of quantitation = 0.00765%)

Internal Standards:

- Nu-Chek Prep, Inc , Tridecanoic Acid (C13:0), >99%, Lot Number N-13A-A20-U
- Nu-Chek Prep, Inc , Methyl Tridecanoate, >99%, Lot Number N-13M-F16-V

E.4.8. Furfural

Subsamples of ground grain were extracted with methanol and analyzed for furfural content by reverse phase high pressure liquid chromatography (HPLC) with ultra-violet (UV) detection (Bredie et al,. 1998; Buttery et al., 1994). The limit of quantitation was 1.00 ppm.

Reference Standards:

- Sigma-Aldrich, Furfural, 99.3%, Lot Number SHBB6776V

E.4.9. Minerals / ICP Emission Spectrometry

The following nine minerals were analyzed:

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

Subsamples of ground forage were digested in a high-pressure microwave unit and diluted with DI water prior to analysis for calcium and phosphorus by inductively

coupled plasma optical emission spectroscopy (AOAC, 2012f; CEM Corporation, 2004). The limit of quantitation for calcium and phosphorus was 62.5 ppm.

Subsamples of ground grain were digested in a high-pressure microwave unit. The resulting digested material was brought to volume with DI water. A dilution was performed and both the diluted and undiluted portions were analyzed for selected elements (magnesium, calcium, sodium, manganese, phosphorus, iron, zinc, copper, and potassium) by inductively coupled plasma optical emission spectroscopy (AOAC, 2012f; CEM Corporation, 2004). The limit of quantitation for calcium, copper, iron, manganese, sodium, and zinc was 0.625 ppm. The limit of quantitation for magnesium, phosphorus, and potassium was 15.6 ppm.

Reference Standards:

- SCP Science, Multi-Element standard for forage analysis containing calcium (5028 µg/ml) and phosphorus (4982 µg/ml), Lot Number S131029009
- SCP Science, Phosphorus standard for forage analysis with a concentration of 1001 µg/ml, Lot Numbers S120706002 and S130611012
- SCP Science, Multi-Element standard for forage and grain analyses containing calcium (1000 µg/ml), copper (1005 µg/ml), iron (1013 µg/ml), potassium (1002 µg/ml), magnesium (1003 µg/ml), manganese (1004 µg/ml), sodium (986 µg/ml), and zinc (987 µg/ml), Lot Number S131002008
- SCP Science, Multi-Element standard for grain analysis containing calcium (90.6 µg/ml), copper (14.96 µg/ml), iron (151.0 µg/ml), manganese (150.1 µg/ml), sodium (150.9 µg/ml), and zinc (149.6 µg/ml), Lot number S130430004
- SCP Science, Multi-Element standard for grain analysis containing potassium (7440 µg/ml), magnesium (3890 µg/ml), and phosphorus (2499 µg/ml), Lot Number S130430005
- SCP Science, Phosphorus standard for grain with a concentration of 7520 µg/ml, Lot Number S130604012

Internal Standards:

- SCP Science, Yttrium standard for forage and grain analyses with a concentration of 998 µg/ml, Lot Number S120917002

E.4.10. Moisture

Moisture content was determined gravimetrically. Subsamples of ground forage were dried to a constant weight in a forced air oven at 135°C for at least 2 hours (AOAC, 2012g). Subsamples of ground grain were dried to a constant weight in a vacuum oven at 100°C and at least 25 inches of mercury pressure for at least 15 hours (AOAC, 2012h). The limit of quantitation was 0.01%.

E.4.11. Neutral Detergent Fiber

Subsamples of ground forage and grain were analyzed to determine the percentage of neutral detergent fiber (NDF) by digesting with a neutral detergent solution, sodium sulfite and alpha amylase. After washing with RO water, the remaining residue was dried and weighed to determine NDF content. Samples were analyzed with the Ankom Extraction Apparatus (Ankom Technology, 2010c). The limit of quantitation was 0.01%.

E.4.12. p-Coumaric Acid and Ferulic Acid

Subsamples of ground grain were hydrolyzed with 2N sodium hydroxide and extracted with ethyl ether after being acidified with hydrochloric acid. The combined ethyl ether layer of two extracts was concentrated and diluted to volume with 1:1 acetonitrile/DI water. The samples were then analyzed for ferulic acid and *p*-coumaric acid content by UPLC with UV detection (Classen et al., 1990; Figueroa-Espinoza et al., 1998; Krygier et al., 1982; Sosulski et al., 1982). The limit of quantitation for the p-coumaric acid and ferulic acid was 33.8 ppm and 300 ppm, respectively.

Reference Standards:

- Sigma-Aldrich, *p*-coumaric acid, 98.6%, Lot Number 110M1259V
- Sigma-Aldrich, ferulic acid, 99.8%, Lot Number STBB8393V

E.4.13. Phytic Acid

Subsamples of ground grain were analyzed to determine the amount of phytic acid by extracting the phytic acid with dilute hydrochloric acid and isolating it using an aminopropyl silica solid phase extraction column. Once isolated and eluted, the phytic acid was analyzed for elemental phosphorus by inductively coupled plasma optical emission spectroscopy. The phytic acid content was calculated from the phosphorus concentration (AOAC, 2012b). The limit of quantitation was 0.355%.

Reference Standards:

- Spectrum, Phytic Acid Sodium Salt, 98%, Lot Number YM0182
- SCP Science, Phosphorus with a concentration of 1001 µg/ml, Lot Numbers S120706002 and S130611012

Internal Standards:

- SCP Science, Yttrium with a concentration of 998 µg/ml, Lot Number S120917002

E.4.14. Protein

Crude protein content was determined using the Foss-Tecator 8400 Kjeltec Analyzer Unit. Subsamples of ground forage and grain were manually digested on a heating block

using sulfuric acid and a catalyst then transferred to the analyzer unit where the digests were distilled and titrated. The crude protein content was calculated by multiplying the amount of nitrogen in the sample by 6.25 (FOSS Analytical AB, 2010). The limit of quantitation was 0.01%.

E.4.15. Raffinose

Subsamples of ground grain were extracted with an ethanol and water mixture. An aliquot of the extract was analyzed for raffinose content by HPLC with refractive index detection (AACC, 2000a; Johansen et al., 1996; Knapp, 1979). The limit of quantitation was 0.0800%.

Reference Standards:

- Sigma-Aldrich, D-(+)-Raffinose pentahydrate, 99.290%, Lot Number 021M1752V

E.4.16. Total Dietary Fiber

Duplicate subsamples of ground grain were gelatinized with heat stable alpha-Amylase, digested with protease and amyloglucosidase to remove starch and protein. Soluble dietary fiber was precipitated with ethanol. The residue was filtered, washed, dried, and quantified gravimetrically. Protein analysis was performed on one of the duplicate samples, while the other duplicate sample was analyzed for ash. The weight of the protein and ash was subtracted from the weight of the residue (Ankom Technology, 2012; AOAC, 2012i). The limit of quantitation was 0.01%.

E.4.17. Vitamin A (Beta Carotene)

Subsamples of ground grain were extracted and filtered. The sample solution was analyzed for beta-carotene content using a UV spectrophotometer. The absorbance was determined by using the straight line equation for the linear regression curve (AOAC, 2012j). The limit of quantitation was 0.250 mg/100g.

Reference Standards:

- Sigma-Aldrich, β-Carotene, 96%, Lot Number SLBG6787V

E.4.18. Vitamin B1 (Thiamine Hydrochloride)

Subsamples of ground grain were analyzed to determine the amount of thiamine by extracting the samples with a 10% acetic acid/4.3% trichloroacetic acid solution. A 50 fold dilution was performed. The samples were analyzed by reverse phase HPLC with tandem mass spectrometric detection (AACC, 2000b). The limit of quantitation was 0.0900 mg/100g.

Reference Standards:

- Sigma-Aldrich, Thiamine hydrochloride, 99.3%, Lot Number 110M0124

E.4.19. Vitamin B2 (Riboflavin)

Subsamples of ground grain were extracted with dilute sulfuric acid. After dilution, filtration, and pH adjustment, riboflavin was assayed microbiologically using the organism *Lactobacillus casei* subspecies *rhamnosus*. The growth of the organism was proportional to the amount of riboflavin in the extract. The turbidity produced by the organism's growth was measured spectrophotometrically (AACC, 2000c). The limit of quantitation was 0.125 µg/g.

Reference Standards:

- Sigma-Aldrich, Riboflavin, 99.5%, Lot Number 011M1785V

E.4.20. Vitamin B3 (Niacin)

Subsamples of ground grain were extracted using DI water to determine the niacin (nicotinic acid) content. After dilution and filtration, niacin was assayed microbiologically using the organism *Lactobacillus plantarum*. The growth of the organism was proportional to the amount of niacin in the extract. The turbidity produced by the organism's growth was measured spectrophotometrically (AACC, 2000d). The limit of quantitation was 6.94 µg/g.

Reference Standards:

- Sigma-Aldrich, Nicotinic acid, 100%, Lot Number SLBC5062V

E.4.21. Vitamin B6 (Pyridoxine Hydrochloride)

Subsamples of ground grain were extracted using dilute sulfuric acid to determine the Vitamin B6 content. After pH adjustment, dilution, and filtration, vitamin B6 was assayed microbiologically using the organism *Saccharomyces cerevisiae*. The growth of the organism was proportional to the amount of vitamin B6 in the extract. The turbidity produced by the organism's growth was measured spectrophotometrically (AACC, 2000e). The limit of quantitation was 0.833 µg/g.

Reference Standards:

- Sigma-Aldrich, Pyridoxine hydrochloride, 100%, Lot Number 041M0042V

E.4.22. Vitamin B9 (Folic Acid)

Subsamples of ground grain were hydrolyzed and digested using protease and amylase enzymes to release the folates from the matrix. A conjugase enzyme was used to convert the naturally occurring folylpolyglutamates to folyldiglutamates. An aliquot of the extracted folates was mixed with a folate and folic acid free microbiological growth

medium. The mixture was inoculated and incubated with *Lactobacillus casei* subspecies *rhamnosus*. The total folate content was determined by measuring the turbidity of the *L. casei* subspecies *rhamnosus* growth response in the sample compared to the turbidity of the growth response in folic acid standards (AACC, 2000f). The limit of quantitation was 0.0156 µg/g.

Reference Standards:

- Sigma-Aldrich, Folic acid, 97%, Lot Number was SLBC2647V

E.4.23. Vitamin E (Alpha-Tocopherol)

Following extraction with hexane, subsamples of ground grain were analyzed to determine the amount of alpha tocopherol by normal phase UPLC with fluorescence detection (Amaral et al., 2005; Weber, 1984). The limit of quantitation was 0.000500 mg/g.

Reference Standards:

- Sigma-Aldrich, (±) α Tocopherol, 97%, Lot Number was 091M1311V

E.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 E-1):

Table E-1. 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
Iron, Manganese, Sodium, 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 15 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, 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, copper, and furfural.

Otherwise, individual results below the LOQ were assigned a value equal to one-half the quantitation limit. Three observations for 22:0 behenic acid and 13 values for sodium were assigned a value equal to one-half of the LOQ (0.00192% fwt and 0.3 ppm fwt, respectively).

The data were assessed for potential outliers using a studentized residuals calculation. A studentized 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 residual range are considered for exclusion, as outliers, from the final analyses. Three results had studentized residual values outside of the ± 6 range.

All three flagged values [lysine from MON 87419 (NT), iron from conventional control and potassium from conventional control] were removed from further analysis as outliers.

The outlier test procedure was reapplied to the remaining lysine, iron and potassium data to detect potential outliers that were masked in the first analysis. No additional values were flagged.

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,

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

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 87419 vs. conventional control were conducted.

E.6. Composition of MON 87419 Not Treated with Dicamba and Glufosinate

E.6.1. Nutrient Levels in Maize Grain Not Treated with Dicamba and Glufosinate

Grain samples were analyzed for levels of nutrients including proximates (protein, fat, ash, moisture), amino acids (18 components), fatty acids (22 components), carbohydrates by calculation, fiber (ADF, NDF, TDF), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [A (β-carotene), B1, B2, B6, E (α tocopherol), niacin, and folic acid]. Moisture was measured for conversion of components from fresh to dry weight, but was not statistically analyzed.

E.6.1.1. Proteins and Amino Acids

Maize grain is typically composed of approximately 10% protein and the levels of protein and associated amino acids can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011; Zhou et al., 2011).

There were no significant differences in protein content between MON 87419 (NT) and the conventional control (11.63 vs. 11.07% dw, respectively; Table E-2). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in protein levels in maize grain and confirmed the similarity of MON 87419 (NT) to the conventional control in levels of this component.

Since total amino acids measured in grain are predominantly derived from hydrolysis of protein, differences in amino acid levels between MON 87419 (NT) and the conventional control were assessed relative to the difference in protein levels. The relative magnitude of the difference in mean protein values for MON 87419 (NT) and the conventional control was 5.07% (Table E-2). Relative magnitudes of difference for the 18 amino acids were 6.33% or less. These differences were significant for five of the amino acids (glycine, histidine, proline, serine, and threonine) (Table E-2), and reflected small relative magnitudes of differences between MON 87419 (NT) and the conventional control, as would be expected based on the small relative magnitude of difference in protein.

The data demonstrated that MON 87419 (NT) was not a major contributor to variation in protein or amino acid levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components. Also, the mean values of these components in MON 87419 (NT) were within the range of values observed in the literature and the ILSI-CCDB (Table E-9). These data confirmed that the significant differences in mean values of glycine, histidine, proline, serine, and threonine were not compositionally meaningful from a food or feed safety perspective.

E.6.1.2. Total Fat and Fatty Acids

Maize grain is typically composed of approximately 4% fat and the levels of total fat and fatty acids can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011; Zhou et al., 2011).

A total of thirteen fatty acids (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, heptadecanoic, heptadecenoic, gamma-linolenic, eicosadienoic, eicosatrienoic, and arachidonic acids) had more than 50% of observations below the assay LOQ, and were excluded from statistical analysis. These fatty acids are present in low amounts in maize grain, if present at all (Harrigan et al., 2009). This study confirmed that this observation can be extended to MON 87419.

There were no significant differences in total fat or fatty acid content between MON 87419 (NT) and the conventional control (Table E-3). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in total fat or fatty acid levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components.

E.6.1.3. Carbohydrates by Calculation and Fiber

In addition to protein and fat, major biomass components assessed in maize grain included carbohydrates by calculation and fiber [acid detergent fiber (ADF), neutral detergent fiber (NDF), and total dietary fiber (TDF)]. Maize grain is typically composed of approximately 85% carbohydrates by calculation, which includes fiber (ADF, NDF, and TDF), and the levels of these components can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011; Zhou et al., 2011).

There were no significant differences in carbohydrates by calculation or fiber content between MON 87419 (NT) and the conventional control (Table E-4). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in carbohydrates by calculation or fiber levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components.

E.6.1.4. Ash and Minerals

Ash and mineral components (calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc) that are constituents of ash were assessed in maize grain. The levels of these components can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011; Zhou et al., 2011).

No statistically significantly differences between MON 87419 (NT) and the conventional control were observed for ash, calcium, iron, magnesium, phosphorus, potassium, sodium, or zinc (Table E-5). A statistically significant difference between MON 87419 (NT) and the conventional control was observed for manganese (Table E-5).

For manganese, the mean value was 6.04 mg/kg dw for MON 87419 (NT) and 5.51 mg/kg dw for the conventional control, a difference of 0.53 mg/kg dw. This difference was evaluated in the context of the conventional control range value, 2.91 mg/kg dw, calculated from the minimum (4.50 mg/kg dw) and maximum (7.41 mg/kg dw) manganese values. The mean difference in manganese values between MON 87419 (NT) and the conventional control was less than the range value of the conventional control, indicating that MON 87419 (NT) does not impact levels of manganese more than natural

variation within the conventional control grown at multiple locations. The MON 87419 (NT) mean manganese value was also within the range of values observed in the literature and the ILSI-CCDB (Table E-9).

The data demonstrated that MON 87419 (NT) was not a major contributor to variation in ash and mineral levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components. Also, the mean values of these components were within the range of values observed in the literature and the ILSI-CCDB. These data confirmed that the significant difference in mean value of manganese was not compositionally meaningful from a food or feed safety perspective.

E.6.1.5. Vitamins

Maize grain contains both water-soluble vitamins (folic acid, niacin, B1, B2, and B6) and fat-soluble vitamins [A (β-carotene) and E]. The levels of these components can vary widely depending on local growing conditions (Egesel et al., 2003; Harrigan et al., 2009; Ridley et al., 2011).

There were no significant differences in vitamin content between MON 87419 (NT) and the conventional control (Table E-6). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in vitamin levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components.

E.6.2. Anti-Nutrient Levels in Maize Grain Not Treated with Dicamba and Glufosinate

The anti-nutrients assessed were phytic acid and raffinose. Phytic acid, the major form of phosphorus in maize grain, is considered an anti-nutrient due to its mineral-chelating properties and the unavailability of phosphorus in phytic acid. Raffinose is a low molecular weight non-digestible carbohydrate that is considered to be an anti-nutrient due to the gas production and resulting flatulence caused by consumption (Liener, 2000). The levels of these components can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011).

No statistically significant differences between MON 87419 (NT) and the conventional control were observed for phytic acid or raffinose (Table E-7). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in phytic acid or raffinose levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components.

E.6.3. Secondary Metabolites Levels in Maize Grain Not Treated with Dicamba and Glufosinate

The secondary metabolites measured in MON 87419 (NT) grain were furfural, ferulic acid, and p-coumaric acid in agreement with the OECD consensus document (OECD, 2002). Ferulic acid and p-coumaric acid are derived from phenylalanine and tyrosine

(Buchanan et al., 2000) and serve as precursors for a large group of phenylpropanoid compounds and fiber. The levels of these secondary metabolites can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011).

Furfural was not detected in the grain of MON 87419 (NT) or the conventional control, and no statistically significant differences between MON 87419 (NT) and the conventional control were observed for ferulic acid and p-coumaric acid (Table E-7). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in ferulic acid and p-coumaric acid levels in maize grain and confirmed the compositional equivalence of MON 87419 (NT) to the control in levels of these components.

E.6.4. Nutrient Levels in Maize Forage Not Treated with Dicamba and Glufosinate

Forage samples were assessed for levels of ash, protein, total fat, carbohydrates by calculation, fiber (ADF and NDF), and minerals (calcium and phosphorus). The levels of these components can vary widely depending on local growing conditions (Harrigan et al., 2009; Ridley et al., 2011).

No statistically significant differences between MON 87419 (NT) and the conventional control were observed for proximates, carbohydrates by calculation, fiber, or minerals in maize forage (Table E-8). The data demonstrated that MON 87419 (NT) was not a major contributor to variation in proximates, carbohydrates by calculation, fiber, or mineral levels in maize forage and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components.

E.6.5. Conclusions

Compositional analysis was conducted on grain and forage of MON 87419 (NT) and a conventional control grown at five sites in the U.S. during 2013. Of the 61 components statistically assessed for MON 87419 (NT) there were no significant differences in 55 components. Only six components (glycine, histidine, proline, serine, threonine, and manganese in grain) showed a significant difference ($p<0.05$) between MON 87419 (NT) and the conventional control. For the amino acids, the differences reflected small relative magnitudes of differences between MON 87419 (NT) and the conventional control, as would be expected based on the small relative magnitude of difference in protein. For manganese, the mean difference in component values between MON 87419 (NT) and the conventional control was less than the range value of the conventional control. The MON 87419 (NT) mean component values were also within the range of values observed in the literature or the ILSI-CCDB.

These results support the overall conclusion that MON 87419 (NT) was not a major contributor to variation in component levels in maize grain and forage and confirmed the compositional equivalence of MON 87419 (NT) to the conventional control in levels of these components. These data indicated that the statistically significant differences observed were not compositionally meaningful from a food and feed safety perspective.

Table E-2. Summary of Maize Grain Protein and Amino Acids for MON 87419 (Not Treated) and Conventional Control

				Difference (Test minus Control)		
Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Mean (S.E.)	p-Value	% Relative[4]
Protein	11.63 (0.55) 9.57 - 14.38	11.07 (0.55) 9.22 - 14.04	4.82	0.56 (0.26)	0.063	5.07
Alanine	0.93 (0.057) 0.72 - 1.24	0.88 (0.057) 0.71 - 1.17	0.46	0.052 (0.026)	0.082	5.93
Arginine	0.46 (0.014) 0.41 - 0.52	0.45 (0.014) 0.39 - 0.52	0.13	0.0087 (0.0076)	0.256	1.95
Aspartic Acid	0.73 (0.036) 0.60 - 0.94	0.70 (0.036) 0.59 - 0.88	0.30	0.032 (0.017)	0.103	4.55
Cystine/Cysteine	0.22 (0.0050) 0.18 - 0.25	0.22 (0.0050) 0.18 - 0.26	0.08	0.0068 (0.0043)	0.118	3.15
Glutamic Acid	2.45 (0.15) 1.91 - 3.23	2.32 (0.15) 1.88 - 3.12	1.24	0.14 (0.070)	0.090	5.84

Table E-2 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87419 (Not Treated) and Conventional Control

				Difference (Test minus Control)		
Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Mean (S.E.)	p-Value	% Relative[4]
Glycine	0.42 (0.011) 0.38 - 0.50	0.40 (0.011) 0.36 - 0.45	0.09	0.013 (0.0060)	0.039	3.14
Histidine	0.33 (0.011) 0.29 - 0.39	0.32 (0.011) 0.28 - 0.37	0.09	0.011 (0.0056)	0.048	3.55
Isoleucine	0.42 (0.024) 0.34 - 0.55	0.40 (0.024) 0.33 - 0.53	0.20	0.020 (0.011)	0.089	5.05
Leucine	1.61 (0.11) 1.23 - 2.17	1.51 (0.11) 1.20 - 2.08	0.89	0.096 (0.048)	0.083	6.33
Lysine	0.28 (0.0062) 0.25 - 0.31	0.28 (0.0061) 0.24 - 0.32	0.07	0.00027 (0.0055)	0.960	0.10
Methionine	0.24 (0.0074) 0.18 - 0.26	0.23 (0.0074) 0.18 - 0.28	0.10	0.0087 (0.0054)	0.113	3.85

Table E-2 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87419 (Not Treated) and Conventional Control

				Difference (Test minus Control)		
Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Mean (S.E.)	p-Value	% Relative[4]
Phenylalanine	0.63 (0.040) 0.51 - 0.83	0.61 (0.040) 0.48 - 0.79	0.31	0.030 (0.018)	0.137	4.93
Proline	1.09 (0.044) 0.93 - 1.33	1.04 (0.044) 0.89 - 1.27	0.37	0.050 (0.021)	0.043	4.80
Serine	0.60 (0.032) 0.49 - 0.83	0.57 (0.032) 0.48 - 0.72	0.25	0.033 (0.014)	0.022	5.81
Threonine	0.43 (0.018) 0.36 - 0.53	0.41 (0.018) 0.35 - 0.50	0.15	0.017 (0.0078)	0.035	4.18
Tryptophan	0.071 (0.0017) 0.062 - 0.081	0.069 (0.0017) 0.055 - 0.083	0.03	0.0016 (0.0018)	0.395	2.30
Tyrosine	0.31 (0.018) 0.25 - 0.40	0.30 (0.018) 0.24 - 0.39	0.15	0.0086 (0.0095)	0.369	2.88

Table E-2 (continued). Summary of Maize Grain Protein and Amino Acids for MON 87419 (Not Treated) and Conventional Control

				Difference (Test minus Control)		
Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Mean (S.E.)	p-Value	% Relative[4]
Valine	0.54 (0.025) 0.45 - 0.67	0.52 (0.025) 0.44 - 0.65	0.22	0.022 (0.011)	0.050	4.31

[1]dw = dry weight.

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

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

[4]The relative magnitude of the difference in mean values between MON 87419 (Not Treated) and the control, expressed as a percent of the control.

Table E-3. Summary of Maize Grain Total Fat and Fatty Acids for MON 87419 (Not Treated) and Conventional Control

Component	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Total Fat (% dw)[1]	3.41 (0.081) 2.96 - 3.87	3.49 (0.081) 2.80 - 3.98	1.18	-0.087 (0.084)	0.327
16:0 Palmitic[4]	14.53 (0.12) 13.90 - 15.45	14.51 (0.12) 13.80 - 15.56	1.77	0.023 (0.14)	0.872
16:1 Palmitoleic	0.12 (0.0040) 0.092 - 0.14	0.12 (0.0040) 0.095 - 0.14	0.05	-0.0012 (0.0022)	0.583
18:0 Stearic	1.62 (0.028) 1.47 - 1.75	1.64 (0.028) 1.46 - 1.84	0.37	-0.019 (0.023)	0.393
18:1 Oleic	22.37 (0.20) 20.60 - 24.16	22.37 (0.20) 20.83 - 24.72	3.89	0.0050 (0.28)	0.985
18:2 Linoleic	59.55 (0.27) 57.39 - 62.12	59.52 (0.27) 57.68 - 61.91	4.23	0.029 (0.38)	0.938

Table E-3 (continued). Summary of Maize Grain Total Fat and Fatty Acids for MON 87419 (Not Treated) and Conventional Control

Component	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
18:3 Linolenic	0.98 (0.027) 0.81 - 1.13	1.02 (0.027) 0.84 - 1.16	0.32	-0.043 (0.024)	0.082
20:0 Arachidic	0.41 (0.0079) 0.37 - 0.44	0.41 (0.0079) 0.37 - 0.45	0.08	-0.00004 (0.0057)	0.994
20:1 Eicosenoic	0.28 (0.0049) 0.25 - 0.33	0.27 (0.0049) 0.25 - 0.33	0.08	0.0065 (0.0063)	0.308
22:0 Behenic	0.15 (0.0070) 0.074 - 0.17	0.15 (0.0070) 0.061 - 0.18	0.11	-0.00046 (0.0074)	0.951

[1]dw = dry weight.

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

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

[4]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, heptadecanoic acid, heptadecenoic acid, gamma linolenic acid, eicosadienoic acid, eicosatrienoic acid, and arachidonic acid.

Table E-4. Summary of Maize Grain Carbohydrates by Calculation and Fiber for MON 87419 (Not Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Carbohydrates by Calculation	83.57 (0.54) 80.90 - 85.39	84.04 (0.54) 81.36 - 85.93	4.57	-0.47 (0.36)	0.230
Acid Detergent Fiber	3.99 (0.12) 3.58 - 4.52	4.04 (0.12) 3.20 - 5.20	2.00	-0.049 (0.13)	0.709
Neutral Detergent Fiber	9.72 (0.11) 9.15 - 10.79	9.42 (0.11) 8.98 - 10.01	1.03	0.30 (0.15)	0.083
Total Dietary Fiber	8.93 (0.23) 7.29 - 10.76	8.97 (0.23) 7.21 - 10.64	3.43	-0.044 (0.31)	0.889

[1]dw = dry weight.

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

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

Table E-5. Summary of Maize Grain Ash and Minerals for MON 87419 (Not Treated) and Conventional Control

Component	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Ash (% dw)[1]	1.39 (0.021) 1.29 - 1.51	1.38 (0.021) 1.30 - 1.51	0.21	0.0058 (0.016)	0.722
Calcium (% dw)	0.0031 (0.00017) 0.0022 - 0.0043	0.0029 (0.00017) 0.0022 - 0.0054	0.003	0.00019 (0.00019)	0.343
Iron (mg/kg dw)	16.65 (0.54) 14.29 - 19.52	16.57 (0.55) 13.39 - 18.71	5.31	0.087 (0.43)	0.840
Magnesium (% dw)	0.13 (0.0019) 0.12 - 0.15	0.12 (0.0019) 0.086 - 0.14	0.05	0.0044 (0.0026)	0.093
Manganese (mg/kg dw)	6.04 (0.45) 4.76 - 8.84	5.51 (0.45) 4.50 - 7.41	2.91	0.53 (0.18)	0.017
Phosphorus (% dw)	0.36 (0.0059) 0.33 - 0.41	0.35 (0.0059) 0.25 - 0.40	0.15	0.013 (0.0077)	0.089

Table E-5 (continued). Summary of Maize Grain Ash and Minerals for MON 87419 (Not Treated) and Conventional Control

Component	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Potassium (% dw)	0.36 (0.0081) 0.33 - 0.41	0.36 (0.0081) 0.33 - 0.40	0.07	0.0019 (0.0048)	0.693
Sodium (mg/kg dw)	6.56 (1.92) 0.36 - 29.15	5.63 (1.92) 0.36 - 35.05	34.69	0.93 (2.65)	0.728
Zinc (mg/kg dw)	22.20 (1.13) 17.74 - 30.03	21.18 (1.13) 16.40 - 26.70	10.30	1.03 (0.67)	0.134

[1]dw = dry weight.

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

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

Table E-6. Summary of Maize Grain Vitamins for MON 87419 (Not Treated) and Conventional Control

Component (mg/kg dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Folic Acid	0.67 (0.035) 0.52 - 0.97	0.66 (0.035) 0.48 - 0.89	0.41	0.011 (0.035)	0.770
Niacin	10.08 (0.41) 8.06 - 12.02	10.20 (0.41) 8.23 - 11.97	3.74	-0.11 (0.46)	0.817
Vitamin A	5.36 (0.45) 4.03 - 11.61	5.47 (0.45) 3.66 - 8.19	4.53	-0.11 (0.48)	0.822
Vitamin B1	2.54 (0.12) 1.75 - 2.90	2.48 (0.12) 1.80 - 3.34	1.54	0.065 (0.095)	0.497
Vitamin B2	2.37 (0.13) 1.46 - 4.79	2.16 (0.13) 1.54 - 3.43	1.89	0.20 (0.18)	0.258
Vitamin B6	5.55 (0.22) 3.35 - 7.49	5.43 (0.22) 2.82 - 7.61	4.79	0.12 (0.32)	0.708

Table E-6 (continued). Summary of Maize Grain Vitamins for MON 87419 (Not Treated) and Conventional Control

Component (mg/kg dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Vitamin E	11.51 (0.43) 9.56 - 13.94	11.07 (0.43) 8.65 - 12.76	4.11	0.44 (0.28)	0.123

[1]dw = dry weight.

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

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

Table E-7. Summary of Maize Grain Anti-nutrients and Secondary Metabolites for MON 87419 (Not Treated) and Conventional Control

Component	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Anti-nutrients (% dw[1])					
Phytic Acid	0.96 (0.031) 0.81 - 1.10	0.93 (0.031) 0.71 - 1.37	0.65	0.033 (0.038)	0.390
Raffinose	0.28 (0.010) 0.22 - 0.34	0.28 (0.010) 0.24 - 0.35	0.11	-0.00063 (0.0070)	0.931
Secondary Metabolites (µg/g dw)					
Ferulic Acid	2341.03 (45.66) 2043.63 - 2730.41	2289.17 (45.66) 1882.22 - 2508.79	626.58	51.86 (37.49)	0.174
p-Coumaric Acid	197.19 (12.40) 149.60 - 251.15	187.70 (12.40) 132.56 - 254.88	122.32	9.48 (6.67)	0.163

[1]dw = dry weight.

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

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

Table E-8. Summary of Maize Forage Proximates, Fiber and Minerals for MON 87419 (Not Treated) and Conventional Control

				Difference (Test minus Control)	
Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Mean (S.E.)	p-Value
Ash	3.78 (0.54) 2.01 - 6.13	3.89 (0.54) 2.27 - 5.70	3.43	-0.11 (0.10)	0.297
Carbohydrates by Calculation	87.14 (0.85) 83.52 - 90.45	87.15 (0.85) 83.47 - 90.85	7.38	-0.0023 (0.29)	0.993
Protein	7.50 (0.36) 6.70 - 9.35	7.27 (0.36) 5.43 - 8.78	3.35	0.23 (0.19)	0.244
Total Fat	1.62 (0.17) 0.55 - 3.45	1.68 (0.17) 0.66 - 3.84	3.18	-0.061 (0.21)	0.771
Acid Detergent Fiber	26.77 (1.15) 20.63 - 33.83	26.72 (1.15) 20.79 - 40.90	20.10	0.054 (1.13)	0.962
Neutral Detergent Fiber	41.49 (1.40) 36.75 - 49.81	41.16 (1.40) 32.32 - 47.52	15.20	0.33 (1.10)	0.765

Table E-8 (continued). Summary of Maize Forage Proximates, Fiber, and Minerals for MON 87419 (Not Treated) and Conventional Control

Component (% dw)[1]	MON 87419 (Not Treated) Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Calcium	0.21 (0.021) 0.12 - 0.29	0.22 (0.021) 0.13 - 0.33	0.20	-0.0067 (0.012)	0.589
Phosphorus	0.20 (0.018) 0.12 - 0.33	0.21 (0.018) 0.13 - 0.32	0.18	-0.012 (0.015)	0.438

[1]dw = dry weight.

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

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

Table E-9. 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 (% dw)		
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 (% dw)		
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 (% dw)		
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
16:1 Palmitoleic	0.059 – 0.23[a]	0.095 – 0.447
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 (% dw)	0.0036 – 0.0068[a]; 0.0035 – 0.0070[b]	0.00127 – 0.02084
Copper (mg/kg dw)	0.85-3.54[c]	0.73 – 18.50
Iron (mg/kg dw)	14.17 – 23.40[a]; 15.90 – 24.66[b]	10.42 – 49.07
Magnesium (% dw)	0.091 – 0.14[a]; 0.10 – 0.14[b]	0.0594 – 0.194

Table E-9 (continued). Literature and ILSI Database Ranges for Components in Maize Forage and Grain

Grain Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Minerals		
Manganese (mg/kg dw)	4.83 – 8.34[a]; 4.78 – 9.35[b]	1.69 – 14.30
Phosphorus (% dw)	0.24 – 0.37[a]; 0.27 – 0.38[b]	0.147 – 0.533
Potassium (% dw)	0.29 – 0.39[a]; 0.36 – 0.43[b]	0.181 – 0.603
Sodium (mg/kg dw)	ND	0.17 – 731.54
Zinc (mg/kg dw)	16.78 – 28.17[a]; 18.25 – 30.44[b]	6.5 – 37.2
Vitamins (mg/kg dw)		
Folic acid	0.19 – 0.35[a]; 0.23 – 0.42[b]	0.147 – 1.464
Vitamin A [β–Carotene]	0.14 – 11.27[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 (% dw)		
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 dw)		
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 (% dw)		
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 (% dw)		
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 (% dw)		
Calcium	0.12 – 0.33[a]; 0.21 – 0.41[b]	0.07139 – 0.57679
Phosphorus	0.090 – 0.26[a]; 0.13 – 0.21[b]	0.09362 – 0.37041

[1]dw=dry weight; FA = fatty acids; ND = not detected.

[2]Literature range references: [a]US and [b]Chile (Harrigan et al., 2009), [c](Ridley et al., 2011) [d](Egesel et al., 2003).

[3]ILSI range is from ILSI Crop Composition Database, 2011 [Accessed 9 May 2014] (ILSI, 2011).

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Appendix F: Materials, Methods, and Individual Site Results for Seed Germination and Dormancy Assessment of MON 87419

F.1. Materials

Seed germination and dormancy characteristics were assessed on seed from MON 87419, the conventional control, and reference hybrids produced in replicated field trials during 2013 at the following sites: Jackson County, Arkansas; York County, Nebraska; and Lehigh County, Pennsylvania (Table F-1).

F.2. Characterization of the Materials

The identities of the MON 87419 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 87419 and the conventional control plots was confirmed by event-specific PCR analyses. Chain-of-custody documentation for all starting seed for this germination and dormancy study was maintained from harvest through shipment to the performing laboratory with the use of packaging labels and plant sample transfer forms.

F.3. Germination Testing Facility and Experimental Methods

Germination and dormancy evaluations were conducted at BioDiagnostics, Inc. in River Falls, Wisconsin. 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, 2013b; a; AOSA/SCST, 2010).

The seed lots (selfed F_2 grain) of MON 87419, the conventional control, and four reference hybrids from each location were tested under seven different temperature regimes. Seven germination chambers were used in this 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 approximately 16 hours and the higher temperature for approximately eight hours. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Approximately 100 seeds each of MON 87419, 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. The germination towels were then rolled up in a wax cover. All rolled germination towels were labeled and placed into an appropriately labeled bucket. Each bucket within a temperature regime represented a replication per site. There were four replications per site for a total of 12 buckets for each temperature regime. Each bucket contained one 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 is 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, 2013b; a). AOSA only provides guidelines for testing seed under a single regime of optimal temperatures (20/30 °C), whereas six 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 alternating 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 radicle 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 101). 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 both 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.

F.4. Statistical Analysis

When appropriate, an analysis of variance (ANOVA) was conducted according to a split-plot design using SAS® (SAS, 2012). When analysis of variance was not appropriate Fisher's Exact test was conducted using SAS® (SAS, 2012). MON 87419 was compared to the conventional control for germination and dormancy 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 three sites. The seed germination and dormancy characteristics analyzed included percent germinated, percent viable hard seed, percent dead, 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 87419 and the conventional control were not statistically compared to the reference hybrids, nor were comparisons made across temperature regimes. The minimum and maximum mean reference values were determined from the reference hybrids across the study sites (i.e., reference range). Results from the combined-site analysis are presented in Table VII-2.

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

In the individual site analyses, no significant differences were detected at site PAGR. Four statistically significant differences were detected between MON 87419 and the conventional control at the ARNE and NEYO sites for the measured characteristics germinated seed and dead seed (Table F-2). Compared to the conventional control, MON 87419 had higher percent germinated seed at the 10 °C temperature regime (97.8 vs. 94.0%) for the seed produced at the ARNE site. Compared to the conventional control, MON 87419 had lower percent dead seed at the 10 °C temperature regime (0.0 vs. 1.5%), higher percent germinated seed at the alternating 10/20 °C temperature regime (100.0 vs. 97.8%), and lower percent dead seed at the alternating 10/20 °C temperature regime (0.0 vs. 2.3%) for the seed produced at the NEYO site.

All statistically significant differences between MON 87419 and the conventional control detected in the individual-site analyses were also detected in combined-site analyses for the corresponding temperature regimes and characteristics (Table VII-2). For the alternating 10/20 °C temperature regime, the combined-site mean values of percent germinated and percent dead seed for MON 87419 were within the respective reference ranges. The differences are therefore unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 3, “no” answer). For the 10 °C temperature regime, the combined-site mean values for percent germinated and percent dead seed were outside of the respective reference ranges in this study. However, they were within the reference ranges at the 10 °C temperature regime from a similar study conducted in support of submission for maize product MON 89034 (USDA Petition Number 06-298-01p, Section VII.A.3.a). Thus the means for MON 87419 were within the known variation for maize and the differences are unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 compared to conventional maize (Figure VII-1, Step 4, “no” answer).

Table F-1. Starting Seed (Selfed F_2 Grain) of MON 87419, the Conventional Control and Reference Hybrids Used in Germination and Dormancy Assessment

Site Code[1]	Material Name	Monsanto Lot Number	Phenotype	T/C/R[2]
ARNE	NL6169[3]	11379912	Conventional	C
	Phillips 717	11379913	Conventional	R
	Stewart S588	11379914	Conventional	R
	Mycogen 2M746	11379915	Conventional	R
	Lewis 7007	11379916	Conventional	R
	MON 87419	11379917	Dicamba and glufosinate-tolerant	T
NEYO	NL6169[3]	11379918	Conventional	C
	Dekalb DKC59-34	11379919	Conventional	R
	Phillips 717	11379920	Conventional	R
	Stine 9724	11379921	Conventional	R
	NC+ 5220	11379922	Conventional	R
	MON 87419	11379923	Dicamba and glufosinate-Tolerant	T
PAGR	NL6169[3]	11379924	Conventional	C
	Dekalb DKC57-73	11379925	Conventional	R
	Dekalb DKC59-34	11379926	Conventional	R
	Phillips 717	11379927	Conventional	R
	Stine 9724	11379928	Conventional	R
	MON 87419	11379929	Dicamba and glufosinate-Tolerant	T

[1] Site code: ARNE = Jackson County, Arkansas; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania.
[2] T/C/R=Test/Control/Reference.
[3] NL6169 = HCL645 × LH244.

Table F-2. Germination and Dormancy Characteristics of MON 87419 and the Conventional Control Seed Produced at each of the Three Field Sites

Temperature (°C)	Characteristic	ARNE[1] Mean % (S.E.)[2]		NEYO[1] Mean % (S.E.)[2]		PAGR[1] Mean % (S.E.)[2]	
		MON 87419	Control	MON 87419	Control	MON 87419	Control
5	Germinated[3]	3.0 (0.71)	1.8 (0.85)	12.0 (1.47)	17.8 (6.33)	12.7 (3.55)	15.0 (5.20)
	Viable Hard[5]	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[4]	0.8 (0.48)	2.3 (0.48)	2.3 (1.31)	0.5 (0.50)	1.5 (0.86)	1.3 (0.48)
	Viable Firm-Swollen[3]	96.3 (0.48)	96.0 (1.08)	85.8 (1.11)	81.8 (5.86)	85.8 (3.25)	83.8 (5.23)
10	Germinated[4]	97.8 (0.63)*	94.0 (1.08)	99.5 (0.50)	98.5 (0.65)	100.0 (0.00)	99.3 (0.48)
	Viable Hard[5]	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[4]	1.0 (0.41)	3.0 (0.71)	0.0 (0.00)*	1.5 (0.65)	0.0 (0.00)	0.8 (0.48)
	Viable Firm-Swollen	1.3 (0.75) [4]	3.0 (0.71)	0.5 (0.50) [4]	0.0 (0.00)	0.0 (0.00) [5]	0.0 (0.00)
20	Germinated[4]	98.5 (0.96)	96.5 (0.87)	99.5 (0.50)	99.3 (0.48)	99.8 (0.25)	99.5 (0.50)
	Viable Hard[5]	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[4]	1.5 (0.96)	3.5 (0.87)	0.5 (0.50)	0.8 (0.48)	0.3 (0.25)	0.5 (0.50)
	Viable Firm-Swollen[5]	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[4]	99.0 (0.58)	97.5 (0.96)	99.8 (0.25)	99.5 (0.29)	100.0 (0.00)	99.3 (0.48)
	Viable Hard[5]	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[4]	1.0 (0.58)	2.5 (0.96)	0.3 (0.25)	0.5 (0.29)	0.0 (0.00)	0.8 (0.48)
	Viable Firm-Swollen[5]	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 F-2 (continued). Germination and Dormancy Characteristics of MON 87419 and the Conventional Control Seed Produced at each of the Three Field Sites

Temperature		ARNE[1] Mean % (S.E.)[2]		NEYO[1] Mean % (S.E.)[2]		PAGR[1] Mean % (S.E.)[2]	
(°C)	Characteristic	MON 87419	Control	MON 87419	Control	MON 87419	Control
10/30	Germinated[4]	99.3 (0.48)	98.8 (0.63)	99.5 (0.29)	98.8 (0.48)	99.5 (0.29)	99.8 (0.25)
	Viable Hard[5]	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[4]	0.8 (0.48)	1.3 (0.63)	0.5 (0.29)	1.3 (0.48)	0.5 (0.29)	0.3 (0.25)
	Viable Firm-Swollen[5]	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
10/20	Germinated[4]	99.3 (0.25)	97.3 (1.49)	100.0 (0.00)*	97.8 (0.75)	99.8 (0.25)	98.8 (0.48)
	Viable Hard[5]	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[4]	0.8 (0.25)	2.8 (1.49)	0.0 (0.00)*	2.3 (0.75)	0.3 (0.25)	1.3 (0.48)
	Viable Firm-Swollen[5]	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	Viable Hard[5]	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
(AOSA)	Dead[4]	0.8 (0.48)	1.0 (0.00)	1.0 (0.41)	1.5 (0.29)	0.3 (0.25)	1.0 (0.41)
	Viable Firm-Swollen[5]	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	Normal Germinated[4]	98.0 (0.71)	98.3 (0.33)	99.0 (0.41)	97.8 (0.25)	99.5 (0.29)	98.5 (0.29)
	Abnormal Germinated[4]	1.3 (0.48)	0.7 (0.33)	0.0 (0.00)	0.8 (0.25)	0.3 (0.25)	0.5 (0.29)

Note: The experimental design was a split-plot with four replications.

*Indicates a statistically significant difference between test and the conventional control (α=0.05).

[1] Site code: ARNE = Jackson County, Arkansas; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania.

[2] MON 87419 and the conventional control values represent means with standard errors (S.E.) in parentheses. n=4 with the following exceptions: n=3 for MON 87419 from site PAGR at the 10 °C temperature regime; n=3 for the control from site ARNE at the 20/30 °C temperature regime. Percentages do not always sum to 100% within a site, material and temperature regime due to numerical rounding of the means.

[3] Indicates statistical comparisons were performed using ANOVA.

[4] Indicates statistical comparisons were performed using Fisher's Exact Test.

[5] No statistical comparisons were made because of lack of variability in the data (all test and control values were 0 or 100).

References for Appendix F

AOSA. 2013a. AOSA Rules for testing seeds. Volume 1: Principles and procedures, Association of Official Seed Analysts, Ithaca, New York.

AOSA. 2013b. AOSA Rules for testing seeds. Volume 4: Seedling evaluation, Association of Official Seed Analysts, Ithaca, New York.

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

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Appendix G: Materials, Methods, and Individual Site Results from Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87419 Not Treated and Treated with Dicamba and Glufosinate under Field Conditions

G.1. Materials

Agronomic, phenotypic, and environmental interaction characteristics were assessed for MON 87419, the conventional control, and the reference hybrids grown under similar agronomic conditions. Four reference hybrids were planted per site (Tables G-1 and G-2). For MON 87419 not treated with dicamba and glufosinate, a total of 17 unique reference hybrids were evaluated among the eight sites. For MON 87419 treated with dicamba and glufosinate, a total of 14 unique reference hybrids were evaluated among the eight sites.

G.2. Characterization of the Materials

The presence or absence of the MON 87419 event in the starting seed of MON 87419 and the conventional control was verified by event-specific polymerase chain reaction (PCR) analyses. No molecular analyses were performed on the reference starting seed.

G.3. Field Sites and Plot Design

Field trials were established at sites that provided a range of environmental and agronomic conditions representative of U.S. maize growing regions. For MON 87419 not treated with dicamba and glufosinate, eight U.S. sites were established in 2013 (Table VII-3). For MON 87419 treated with dicamba and glufosinate, six U.S. sites were established in 2013 and two U.S. sites were established in 2014 (Table VII-4). The Principal Investigator at each site was familiar with the growth, production, and evaluation of maize characteristics.

At all sites, seed of MON 87419, the conventional control, and four reference hybrids were planted in a randomized complete block design with four replications. At all sites except NCBD, PAHM, and WIDL, each replicated plot consisted of six (for 2013 sites) or four (for 2014 sites) rows of maize spaced approximately 0.76 m apart and approximately 6 m long (Tables G-3 and G-4). Phenotypic and qualitative environmental interaction data were collected from rows 4 and 5 in 2013 and rows 2 and 3 in 2014. The remaining rows were used for other purposes.

At the 2013 sites NCBD, PAHM, and WIDL, each replicated plot consisted of 16 rows of maize spaced approximately 0.76 – 0.97 m apart and approximately 6 m long (Table G-3). Phenotypic and qualitative environmental interaction data were collected from rows 2 and 3 (rows 14 and 15 at site NCBD). Rows 6 and 8 (rows 5 and 7 at site NCBD) were used to measure arthropod abundance using sticky traps. Rows 9, 10, 11, and 12 were designated for visual counts of arthropod abundance. Rows 13 and 14 (rows 2 and 3 at site NCBD) were used to assess plant damage caused by corn earworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis*). Suitable rows differing from the above designations were used for most data in two plots at site PAHM. The remaining rows were used for other purposes.

G.4. Planting and Field Operations

Planting information, soil description, and cropping history of the study area are listed in Tables G-3 and G-4. Prior to planting, the Principal Investigator at each site prepared the plot area with a proper seed bed according to local agronomic practices in matters such as 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. General trial maintenance, such as agricultural chemicals, fertilizer, irrigation, and other management practices were applied as necessary throughout the season. Maintenance operations were performed uniformly across all plots.

G.5. Additional Herbicide Applications to MON 87419 Treated with Dicamba and Glufosinate

In addition to the general trial maintenance agrichemical applications, plots of MON 87419 treated with dicamba and glufosinate received a single application of dicamba herbicide followed at least one week later by a single application of glufosinate herbicide. Dicamba was applied at growth stages ranging from V2 to V4 at each site. The dicamba treatment was applied at a common agronomic use rate of 0.56 kg acid equivalent (a.e.) per hectare at all sites except INKI (0.50 kg a.e./ha) and KSLA (0.34 kg a.e./ha). Glufosinate herbicide was applied to the MON 87419 plots at each site at growth stages ranging from V4 to approximately V7. The glufosinate treatment was applied at a common agronomic use rate of 0.45 kg a.i. (active ingredient) per hectare at all sites except INKI (0.40 kg a.i./ha) and KSLA (0.27 kg a.i./ha). Although the application rates were reduced at two sites, they were sufficiently high to allow meaningful assessment of MON 87419 treated with dicamba and glufosinate. Thus, the overall dataset of 8 sites, including 6 sites with intended application rates, provides a robust dataset for assessment of the phenotypic and agronomic characteristics of MON 87419 treated with dicamba and glufosinate compared to a conventional maize control.

Table G-1. Starting Seed for Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87419 Not Treated with Dicamba and Glufosinate

Site Code[1]	Material Name[2]	Monsanto Lot Number	Phenotype	T/C/R/[3]
All	MON 87419	11356837	Dicamba and glufosinate-tolerant	T
	NL6169[4]	11356835	Conventional	C
ARNE	Phillips 717	11300073	Conventional	R
	Stewart S588	11226918	Conventional	R
	Mycogen 2M746	11226705	Conventional	R
	Lewis 7007	11226559	Conventional	R
ILMN	Phillips 713	11300072	Conventional	R
	Gateway 6116	11227211	Conventional	R
	Stewart S602	11226919	Conventional	R
	Dekalb DKC63-43	11354935	Conventional	R
KSLA	Phillips 717	11300073	Conventional	R
	Gateway 6116	11227211	Conventional	R
	Dekalb DKC63-43	11354935	Conventional	R
	Lewis 7007	11226559	Conventional	R
NCBD	Dekalb DKC59-34	11354934	Conventional	R
	Gateway 6116	11227211	Conventional	R
	LG2615CL	11226863	Conventional	R
	Lewis 6442	11226558	Conventional	R
NEYO	Dekalb DKC59-34	11354934	Conventional	R
	Phillips 717	11300073	Conventional	R
	Stine 9724	11298951	Conventional	R
	NC+ 5220	11226701	Conventional	R
PAGR	Dekalb DKC57-73	11354929	Conventional	R
	Dekalb DKC59-34	11354934	Conventional	R
	Phillips 717	11300073	Conventional	R
	Stine 9724	11298951	Conventional	R
PAHM	Jacobsen Seed JS4431	11267096	Conventional	R
	Phillips 717	11300073	Conventional	R
	Midland Phillips 7B15P	11226702	Conventional	R
	LG2597	11226862	Conventional	R
WIDL	Jacobsen Seed JS4431	11267096	Conventional	R
	Dekalb DKC59-34	11354934	Conventional	R
	Phillips 717	11300073	Conventional	R
	LG2597	11226862	Conventional	R

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; KSLA = Pawnee County, Kansas; NCBD = Perquimans County, North Carolina; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.
[2] The trials included additional test entries not relevant to the objectives of this report which were included in the statistical analysis input files. For this analysis, no statistical comparisons were made using these additional test entries.
[3] T/C/R=Test/Control/Reference.
[4] NL6169 = HCL645 × LH244.

Table G-2. Starting Seed for Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87419 Treated with Dicamba and Glufosinate

Site Code[1]	Material Name[2]	Monsanto Lot Number	Phenotype	T/C/R/[3]
	MON 87419 [5]	11356837	Dicamba and glufosinate-tolerant	T
All	NL6169[4]	11356835	Conventional	C
	Phillips 717	11300073	Conventional	R
	Stewart S588	11226918	Conventional	R
	Mycogen 2M746	11226705	Conventional	R
ARNE	Lewis 7007	11226559	Conventional	R
	Phillips 713	11300072	Conventional	R
	Gateway 6116	11227211	Conventional	R
	Stewart S602	11226919	Conventional	R
ILMN	Dekalb DKC63-43	11354935	Conventional	R
	Phillips 713	11300072	Conventional	R
	Dekalb DKC59-34	11354934	Conventional	R
	Stine 9724	11298951	Conventional	R
INKI	H-9180	11226704	Conventional	R
	Phillips 717	11300073	Conventional	R
	Gateway 6116	11227211	Conventional	R
	Dekalb DKC63-43	11354935	Conventional	R
KSLA	Lewis 7007	11226559	Conventional	R
	Dekalb DKC59-34	11354934	Conventional	R
	Phillips 717	11300073	Conventional	R
	Stine 9724	11298951	Conventional	R
NEYO–2013	NC+ 5220	11226701	Conventional	R
	Phillips 713	11300072	Conventional	R
	Stine 9724	11298951	Conventional	R
	Stewart S602	11226919	Conventional	R
NEYO–2014	Dekalb DKC63-43	11354935	Conventional	R
	Dekalb DKC57-73	11354929	Conventional	R
	Phillips 713	11300072	Conventional	R
	LG2597	11226862	Conventional	R
OHTR	Stewart S602	11226919	Conventional	R
	Dekalb DKC57-73	11354929	Conventional	R
	Dekalb DKC59-34	11354934	Conventional	R
	Phillips 717	11300073	Conventional	R
PAGR	Stine 9724	11298951	Conventional	R

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; INKI = Clinton County, Indiana; KSLA = Pawnee County, Kansas; NEYO = York County, Nebraska; OHTR = Miami County, Ohio; PAGR = Lehigh County, Pennsylvania.

[2] The trials included additional test entries not relevant to the objectives of this report which were included in the statistical analysis input files. For this analysis, no statistical comparisons were made using these additional test entries.

[3] T/C/R=Test/Control/Reference. [4] NL6169 = HCL645 × LH244.

[5] Received an application of dicamba herbicide followed by an application of glufosinate herbicide

Table G-3. Field and Planting Information for MON 87419 Not Treated with Dicamba and Glufosinate

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 2012
ARNE	05/14/2013	09/18/2013	7.2	6.1 × 4.6	6	Sandy Loam	1.5	Soybean
ILMN	05/16/2013	09/30/2013	6.6	6.3 × 4.6	6	Silt Loam	4.5	Wheat
KSLA	05/10/2013	10/07/2013	7.2	6.1 × 4.6	6	Sandy Loam	1.7	Soybean
NCBD[4]	05/02/2013	09/11/2013	7.1	6.1 × 15.4	16	Loam	26.7	Soybean
NEYO	05/10/2013	10/21/2013	7.2	6.1 × 4.6	6	Silt Loam	3.0	Soybean
PAGR	05/27/2013	10/29/2013	7.2	6.1 × 4.6	6	Loam	3.2	Soybean
PAHM[4]	05/20/2013	10/29/2013–10/30/2013	7.2	6.1 × 12.2	16	Loam	2.3	Soybean
WIDL[4]	05/17/2013	10/25/2013	7.2	6.1 × 12.2	16	Silt loam	2.4	Maize

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; KSLA = Pawnee County, Kansas; NCBD = Perquimans County, North Carolina; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.
[2] Planting and Harvest Date = mm/dd/yyyy.
[3] % OM = Percent Organic Matter.
[4] Sites with additional rows per plot to permit assessment of quantitative environmental interactions as well as the other characteristics.

Table G-4. Field and Planting Information for MON 87419 Treated with Dicamba and Glufosinate

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 Year Crop
ARNE	05/14/2013	09/18/2013	7.2	6.1 × 4.6	6	Sandy Loam	1.5	Soybean
ILMN	05/16/2013	09/30/2013	6.6	6.3 × 4.6	6	Silt Loam	4.5	Wheat
INKI	05/14/2013	10/28/2013	7.2	6.1 × 4.6	6	Silt Loam	2.0	Maize
KSLA	05/10/2013	10/07/2013	7.2	6.1 × 4.6	6	Sandy Loam	1.7	Soybean
NEYO–2013	05/10/2013	10/21/2013	7.2	6.1 × 4.6	6	Silt Loam	3.0	Soybean
NEYO–2014	06/11/2014	11/10/2014	7.2	6.1 × 3.0	4	Silt Loam	2.2	Soybean
OHTR	05/31/2014	10/29/2014	6.2	6.3 × 3.0	4	Silty Clay Loam	3.8	Soybean
PAGR	05/27/2013	10/29/2013	7.2	6.1 × 4.6	6	Loam	3.2	Soybean

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; INKI = Clinton County, Indiana; KSLA = Pawnee County, Kansas; NEYO = York County, Nebraska; OHTR = Miami County, Ohio; PAGR = Lehigh County, Pennsylvania.
[2] Planting and Harvest Date = mm/dd/yyyy.
[3] % OM = Percent Organic Matter.

G.6. Phenotypic and Agronomic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in Table VII-1.

G.7. Environmental Observations

Environmental interactions (i.e., interactions between the crop plants and their receiving environment) were used to characterize MON 87419 by evaluating plant response to abiotic stressors, disease damage, and arthropod-related damage using qualitative methods described in Section G.7.1 In addition, specific arthropod damage (corn earworm damage and European corn borer damage) and arthropod abundance were evaluated using the quantitative methods described in Section G.7.2.

G.7.1. Plant Response to Abiotic Stress, Disease Damage, and Arthropod-Related Damage

MON 87419, the conventional control, and reference hybrids 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 intervals: V6–V8; V12–VT; R1–R3; and onset of 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

Methods 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 were 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 co-operator 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.

G.7.2. Specific Arthropod Damage and Arthropod Abundance

Specific arthropod (corn earworm and European corn borer) damage and arthropod abundance were assessed quantitatively from observations/collections performed at the NCBD, PAHM, and WIDL sites.

Corn earworm damage was evaluated at R5 to onset of R6 growth stage by examining ears from ten plants (five 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 cell was 0.5 cm^2). Damage (cm^2) per plant was calculated as the total number of grid cells matching the damaged 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 (five consecutive plants per row) in each plot. Damage was assessed by longitudinally splitting the stalk of each plant and counting the number of feeding galleries per plant and measuring the total length of feeding galleries (cm) in each stalk.

Arthropods were collected using yellow sticky traps five times during the growing season at the following approximate intervals: late vegetative – VT, R1, R2, R3, and R4 growth stage. Sticky traps (two per plot) were deployed in the designated rows 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 time, traps were deployed for approximately seven days. Sticky traps were then sent to the University of Arkansas, Fayetteville, Arkansas for arthropod identification and enumeration.

Visual counts for arthropods were conducted during the growing season from five non–systematically selected plants per plot to collect abundance data. Arthropods were enumerated beginning at approximately VT and repeated approximately every five days for a total of five collections. Visual counts were made by examining the stalk, leaf blade, leaf collar, ear tip, silk, and the tassel of each plant.

G.8. 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 ensured that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the study was carefully monitored. Prior to analysis, the datasets were evaluated by the Lead Scientist or Environmental Interactions Scientist for evidence of biologically relevant changes and for possible evidence of unexpected plant responses. Any unexpected observations or issues identified during the study that might impact the study objectives were noted by the Lead Scientist or Environmental Interactions Scientist. Data were then subjected to summarization or statistical analysis as indicated in Section G.9.

G.9. Statistical Analysis/Data Summarization

G.9.1. Agronomic and Phenotypic Data

Plant vigor data were summarized but not subjected to an analysis of variance (ANOVA). MON 87419 was considered different from the conventional control in vigor if the ranges of vigor for MON 87419 did not overlap with the range of vigor for the conventional control across all replications. Any observed differences between MON 87419 and the conventional control were further assessed in the context of the range of the commercial reference hybrids and for consistency at other sites.

For MON 87419 not treated with dicamba and glufosinate, an ANOVA was conducted according to a randomized complete block design using SAS® (SAS, 2012b) to compare MON 87419 to the conventional control for the phenotypic characteristics listed in Table VII-1, with the exception of dropped ears, root lodged plants, and plant vigor. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). Comparisons of MON 87419 to the conventional control were conducted within site (individual-site analysis) and in a combined-site analysis in which the data were pooled across sites. Due to a lack of or insufficient within-material variability, stalk lodged plants at site NCBD and dropped ears and root lodged plants at all eight sites were removed from individual-site comparisons. Nonparametric analysis was used for combined-site analysis of dropped ears and root lodged plants because they lacked sufficient variability to satisfy ANOVA assumptions. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 87419 and the conventional control material were not statistically compared to the commercial reference hybrids. The reference range for each measured phenotypic characteristic was calculated as the minimum and maximum means among the 17 unique conventional commercial references, where each mean was combined over all the sites at which the reference was planted.

For MON 87419 treated with dicamba and glufosinate, an ANOVA was conducted according to a randomized complete block design using SAS® (SAS, 2012b) to compare MON 87419 to 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 87419 to the conventional control were conducted within site (individual-site analysis) and in a combined-site analysis in which the data were pooled across sites. In individual-site analyses, p-values could not be generated for dropped ears at sites ARNE and OHTR and root lodged plants at sites ILMN, KSLA, NEYO–2013, NEYO–2014 and OHTR because of lack of variability in the data. MON 87419 and the conventional control material were not statistically compared to the commercial reference materials. The reference range for each measured phenotypic characteristic was calculated as the minimum and maximum means among the 14 unique conventional commercial references, where each mean was combined over all the sites at which the reference was planted.

Data excluded from the studies and the reasons for their exclusion are listed in Tables G-5 and G-6.

G.9.2. Environmental Interaction Data

The qualitative environmental interaction data consisting of plant response to abiotic stressors, disease damage, and arthropod damage are categorical and were not subjected to ANOVA.

An ANOVA was conducted according to a randomized complete block design using SAS® (SAS, 2012b) 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 87419 was compared to the conventional control for corn earworm damage, European corn borer damage and the arthropod abundance. The reference range for each measured characteristic was determined from the minimum and maximum mean values from the four reference maize hybrids planted at each site.

For the arthropod abundance data, an across-collection analysis within each site was performed with five repeated collections of sticky traps and five repeated visual counts. Statistical analyses and significance testing of differences between MON 87419 and the conventional control material 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. All collection times that met the inclusion criterion for a given arthropod were pooled within the site and subjected to across-collection analysis.

Data excluded from the studies and the reasons for their exclusion are listed in Tables G-5 and G-6.

G.10. Individual Field Site Phenotypic, Agronomic, and Environmental Interactions Results and Discussion for MON 87419.

G.10.1. Phenotypic and Agronomic Characteristics for MON 87419 Not Treated with Dicamba and Glufosinate.

In the individual-site analyses, five statistically significant differences were observed in the 87 statistical comparisons between MON 87419 not treated with dicamba and glufosinate and the conventional control (Table G-7). Compared to the conventional control, MON 87419 had more days to 50% pollen shed at the WIDL site (73.3 vs. 69.0 days), more days to 50% silking at the WIDL site (74.5 vs. 71.5 days), shorter ear height at the PAGR site (106.5 vs. 117.7 cm), fewer stalk lodged plants at the WIDL site (0.5 vs. 3.8 plants/two rows), and lower yield at the ILMN site (14.6 vs. 15.5 Mg/ha). None of the statistically significant differences between MON 87419 and the conventional control detected in the individual-site analyses described above were detected in the combined-site analysis. Thus, the differences detected for these phenotypic characteristics at individual sites do not indicate consistent responses associated with the trait and are unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 not treated with dicamba and glufosinate compared to conventional maize (Figure VII-1, Step 2, “No” answer).

In individual-site assessments of plant vigor, MON 87419 and the conventional control were considered different if their within-site ranges of raw values did not overlap. There were no differences observed between MON 87419 not treated with dicamba and glufosinate and the conventional control in plant vigor at any site (Table G-7).

G.10.2. Phenotypic and Agronomic Characteristics for MON 87419 Treated with Dicamba and Glufosinate.

In the individual-site analyses, eight statistically significant differences were observed in the 97 statistical comparisons between MON 87419 treated with dicamba and glufosinate and the conventional control (Table G-8). Compared to the conventional control, MON 87419 had a higher early stand count at the ILMN site (84 vs. 80 plants/two rows), a lower stay green rating at the PAGR site (5.5 vs. 7.3), lower ear height at the ILMN (102.1 vs. 111.1 cm) and PAGR (107.7 vs. 117.7 cm) sites, shorter plant height at the ILMN site (212.3 vs. 228.4 cm), fewer stalk lodged plants at the NEYO–2013 site (0.0 vs. 1.8 plants/two rows), more stalk lodged plants at the NEYO–2014 site (4.0 vs. 0.3 plants/two rows), and a higher final stand count at the KSLA site (77.5 vs. 74.0 plants/two rows). None of the statistically significant differences between MON 87419 and the conventional control detected in the individual-site analyses described above were detected in the combined-site analysis. Thus, the differences detected for these phenotypic characteristics at individual sites do not indicate consistent responses associated with the trait and are unlikely to be biologically meaningful in terms of pest/weed potential of MON 87419 treated with dicamba and glufosinate compared to conventional maize (Figure VII-1, Step 2, “No” answer).

In individual-site assessments of plant vigor, MON 87419 and the conventional control were considered different if their within-site ranges of raw values did not overlap. There were no differences between MON 87419 treated with dicamba and glufosinate and the conventional control in plant vigor at any site (Table G-8).

G.10.3. Environmental Interactions Assessments for MON 87419

Plant Response to Abiotic Stressor, Disease Damage, and Arthropod-related Damage

In the individual-site assessment, no differences were observed between MON 87419 and the conventional control for any of the 93 comparisons for the assessed abiotic stressors: cold, drought, flooding, hail, heat, nutrient deficiency, soil compaction, and wind (Table G-9).

In the individual-site assessment, no differences were observed between MON 87419 and the conventional control for any of the 107 comparisons for the assessed diseases: anthracnose, bacterial blight, crazy top, ear rot, eyespot, *Fusarium* sp., Goss's bacterial wilt, gray leaf spot, leaf blight, Northern leaf spot, *Pythium* sp., rust, smut, stalk rot, and Stewart's bacterial wilt (Table G-10).

In the individual-site assessment, no differences were observed between MON 87419 and the conventional control for any of the 91 comparisons for the assessed arthropods: aphids, armyworms, billbugs, corn earworms, corn flea beetles, corn rootworm beetles, cutworms, European corn borers, grape colaspis, grasshoppers, Japanese beetles, sap beetles, spider mites, Southwestern corn borers, and stink bugs (Table G-11).

Corn Earworm and European Corn Borer Damage for MON 87419

In the individual-site analysis, one statistically significant difference was detected out of nine comparisons between MON 87419 and the conventional control for corn earworm damage and European corn borer damage among all observations at sites NCBD, PAHM, and WIDL (Table G-12). For the detected difference, MON 87419 had less damage from corn earworm infestation compared to the conventional control at site NCBD (0.0 vs. 0.7 cm^2 damaged area per plant). The mean damage rating for MON 87419 was within the range of the commercial reference hybrids (0.0 – 0.6 cm^2 damaged area per plant) and no differences were detected at other sites. Thus, this difference was not indicative of a consistent plant response associated with the trait and is unlikely to be biologically meaningful in terms of increased pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

Arthropod Abundance for MON 87419

The arthropod taxa included in the statistical analyses and significance testing of differences were those that met the minimum abundance criterion (see Section G.9.2) required for inclusion in the analysis. Corn flea beetles, corn rootworm beetles, ladybird beetles, sap beetles, leafhoppers, planthoppers, minute pirate bugs, parasitic wasps, lacewings, syrphid flies, spider mites, and spiders met the minimum abundance criteria for sticky trap collections. Arthropod taxa that met the minimum abundance criteria for

visual count collections were corn flea beetles, corn rootworm beetles, ladybird beetles, sap beetles, shining flower beetles, minute pirate bugs, and spiders.

In an across-collection analysis of arthropod abundance data from sticky traps, no statistically significant differences were detected between MON 87419 and the conventional control for 21 out of 23 comparisons (Table G-13). The abundance of corn rootworm beetles was lower for MON 87419 than the conventional control (25.9 vs. 37.2 per plot) in sticky traps at the WIDL site, and the value for MON 87419 was slightly lower than the reference range (32.1 – 42.2 per plot). However, a lower value for MON 87419 does not imply increased susceptibility of MON 87419 to the corn rootworm beetle, a pest arthropod species. The abundance of spiders was higher for MON 87419 than the conventional control (2.8 vs. 1.5 per plot) in sticky traps at the NCBD site, and the value for MON 87419 was slightly higher than the reference range (1.7 – 2.3 per plot). The differences in abundance of corn rootworm beetles and spiders were not consistently detected across collection methods (i.e., in visual counts; Table G-14) and/or sites where the taxa occurred. Thus, these differences were not indicative of consistent responses associated with the trait and are not considered biologically meaningful in terms of increased pest potential or adverse environmental impact of MON 87419 compared to conventional maize (Section VII.B.2).

In an across-collection analysis of arthropod abundance data from visual counts, no statistically significant differences were detected between MON 87419 and the conventional control for 10 out of 11 comparisons (Table G-14). The abundance of minute pirate bugs was lower for MON 87419 than the conventional control (3.4 vs. 5.5 per plot) in visual counts at the PAHM site. However, the mean abundance value of minute pirate bugs was within the reference range (3.0 – 4.3 per plot). Additionally, this difference was not consistently detected across collection methods (i.e., in sticky traps; Table G-13) or sites where the taxa occurred. Thus, this difference is unlikely to be biologically meaningful in terms of increased plant pest potential of MON 87419 compared to conventional maize (Section VII.B.2).

Table G-5. Data Missing or Excluded from Analysis of MON 87419 Not Treated with Dicamba and Glufosinate

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
ARNE	Phillips 717 Stewart S588 Phillips 717	Reference	202 304 404	Yield	Stalk lodging that likely impacted yield.
PAGR	Dekalb DKC57-73 MON 87419 Dekalb DKC57-73 Stine 9724 Dekalb DKC57-73 Dekalb DKC59-34	Reference Test Reference Reference Reference Reference	104 108 207 302 308 403	Yield	Low or high stand count in harvested or adjacent row compared to site harvested row average, with potential to impact yield.
PAHM	LG2597	Reference	301	Yield	High stand count compared to site harvested row average, with potential to impact yield.
PAHM	MON 87419 LG2597	Test Reference	101 301	Final stand count	Final stand count was collected from rows that were not thinned.

Table G-5 (continued). Data Missing or Excluded from Analysis of MON 87419 Not Treated with Dicamba and Glufosinate

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
NCBD	LG2615CL Lewis 6442 MON 87419	Reference Reference Test	207 304 306	Sticky traps, Collection #3	Quality of sticky traps were compromised due to dirt
NCBD	LG2615CL	Reference	307	Visual counts, Collection #1 (sap beetle, Grape colaspis)	Data was not collected on all plots as required by the protocol
PAHM	All	All	All	Environmental interaction evaluation #2, #3, and #4 (European corn borer)	Improper selection of stressor: not allowed by protocol for sites with quantitative environmental interaction data collections
PAHM	Dekalb DKC59-34 NL6169 LG2615CL MON 87419 Dekalb DKC59-3	Reference Control Reference Test Reference	103 104 105 301 303	Visual counts, Collection #1 (planthoppers and hoverflies)	Data was not collected on all plots as required by the protocol
PAHM	MON 87419 MON 87419 MON 87419 Dekalb DKC59-34 NL6169	Test Test Test Reference Control	101 204 301 303 407	Visual counts, Collection #1 (unidentified arthropod)	Data was not collected on all plots as required by the protocol
WIDL NEYO	All	All	All	Environmental interaction evaluation #1 (seed corn maggot and white grubs)	Improper data collection: protocol does not allow data collection on below ground arthropods
WIDL	MON 87419	Test	101	Environmental interaction evaluation #3 (Curculio)	Data not collected on all plots as required by the protocol

Table G-5 (continued). Data Missing or Excluded from Analysis of MON 87419 Not Treated with Dicamba and Glufosinate

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
WIDL	Phillips 717	Reference	407	Environmental interaction evaluation #3 (abiotic, disease, and arthropod)	Data was not collected for nine stressors
WIDL	All	All	All	Environmental interaction evaluation #4 (abiotic and disease stressors)	Data are missing for a majority of plots
WIDL	All	All	All	Environmental interaction evaluation #4 (brown stem rot)	Improper selection of stressor; not a disease of maize
ARNE	MON 87419	Test	409	Environmental interaction evaluation #4 (grasshopper)	Data not collected
ILMN	All	All	All	Environmental interaction evaluation #4 (animal damage)	Improper selection of stressor: not allowed by protocol
KSLA	All	All	All	Environmental interaction evaluation #1 (crazy top)	Improper selection of stressor based on the developmental stage of the crop
KSLA	All	All	All	Environmental interaction evaluation #1 (buggy whipping)	Improper selection of stressor based on stressor category
NEYO	All	All	All	Environmental interaction evaluation #3 (grasshoppers)	Duplicate rating for the same stressor

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; KSLA = Pawnee County, Kansas; NCBD = Perquimans County, North Carolina; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.

Table G-6. Data Missing or Excluded from Analysis of MON 87419 Treated with Dicamba and Glufosinate

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
ARNE	Phillips 717	Reference	202	Yield	Stalk lodging with potential to impact yield
	Stewart S588	Reference	304		
	Phillips 717	Reference	404		
INKI	H-9180	Reference	405	Stalk lodging	Data collection error
INKI	MON 87419	Test	402	Final stand count	Data entry error
INKI	Phillips 713	Reference	107	Yield	Low stand count or pre-flowering stalk breakage with potential to impact yield
	H-9180	Reference	405		
NEYO–2014	MON 87419	Test	101	Final stand count	Data collection error
OHTR	Dekalb DKC57-73	Reference	106	Dropped ears, stalk lodged plants, root lodged plants, final stand count, yield	Low stand count with potential to affect count data and yield
PAGR	Dekalb DKC57-73	Reference	104	Yield	Low stand count in harvest or adjacent rows with potential to impact yield
	Dekalb DKC57-73	Reference	207		
	Stine 9724	Reference	302		
	Dekalb DKC57-73	Reference	308		
	Dekalb DKC59-34	Reference	403		

[1] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; INKI = Clinton County, Indiana; KSLA = Pawnee County, Kansas; NEYO = York County, Nebraska; OHTR = Miami County, Ohio; PAGR = Lehigh County, Pennsylvania.

Table G-7. Individual-site Analysis of Phenotypic Characteristics of MON 87419 Not Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant Vigor (1-9 rating)[1]		Early stand count (#/two rows)		Days to 50% pollen shed	
	Range		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	2 – 4	3 – 4	85.8 (1.11)	84.3 (1.18)	55.3 (0.25)	55.0 (0.00)
ILMN	2 – 3	2 – 3	82.3 (1.25)	80.0 (0.71)	64.0 (0.00)	64.0 (0.00)
KSLA	2 – 5	3 – 4	82.5 (0.87)	83.3 (1.11)	61.0 (0.00)	61.3 (0.25)
NCBD	3 – 4	3 – 3	91.5 (2.25)	87.0 (2.80)	62.0 (0.00)	62.0 (0.00)
NEYO	1 – 1	1 – 1	84.0 (1.35)	83.5 (2.18)	66.3 (0.25)	66.0 (0.00)
PAGR	1 – 3	1 – 2	75.8 (2.10)	78.3 (1.55)	61.8 (0.48)	61.5 (0.65)
PAHM	2 – 3	2 – 3	83.5 (1.19)	80.3 (3.33)	65.3 (0.63)	66.5 (1.19)
WIDL	2 – 3	2 – 3	85.8 (0.25)	85.0 (0.41)	73.3 (0.25)*	69.0 (0.41)

Table G-7 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Not Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Days to 50% silking		Stay green rating (1-9 scale)		Ear height (cm)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	56.0 (0.41)	56.0 (0.00)	3.5 (0.29)	4.3 (0.25)	106.6 (3.91)	103.1 (1.48)
ILMN	65.5 (0.87)	66.3 (0.75)	8.3 (0.48)	7.8 (0.63)	105.4 (2.26)	111.1 (1.95)
KSLA	62.5 (0.50)	62.5 (0.65)	4.5 (0.50)	3.8 (0.25)	86.1 (4.45)	91.3 (6.48)
NCBD	61.8 (0.25)	61.8 (0.25)	5.8 (0.25)	5.3 (0.48)	128.3 (2.93)	130.8 (1.70)
NEYO	66.3 (0.25)	66.0 (0.00)	6.3 (0.25)	5.8 (0.25)	104.4 (3.40)	103.5 (2.90)
PAGR	62.3 (0.48)	61.5 (0.50)	7.0 (0.41)	7.3 (0.25)	106.5 (3.26)*	117.7 (1.27)
PAHM	66.0 (0.41)	67.0 (1.00)	4.8 (1.11)	4.5 (1.19)	103.5 (5.64)	107.1 (5.56)
WIDL	74.5 (0.29)*	71.5 (0.87)	9.0 (0.00)	8.5 (0.50)	114.8 (3.42)	120.5 (4.33)

Table G-7 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Not Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant height (cm)		Dropped ears (#/two rows)		Stalk lodged plants (#/two rows)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	230.1 (3.61)	234.8 (1.39)	0.0 (0.00) †	0.0 (0.00)	1.3 (0.75)	1.3 (0.75)
ILMN	224.2 (3.03)	228.4 (3.17)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
KSLA	173.6 (1.64)	177.4 (2.58)	0.0 (0.00) †	0.0 (0.00)	0.3 (0.25)	0.8 (0.48)
NCBD	240.3 (1.31)	240.5 (2.90)	0.0 (0.00) †	0.0 (0.00)	0.0 (0.00) †	0.0 (0.00)
NEYO	201.8 (5.80)	206.1 (7.87)	0.3 (0.25) †	0.0 (0.00)	1.0 (0.71)	1.8 (0.48)
PAGR	225.2 (5.39)	236.4 (3.13)	0.3 (0.25) †	0.3 (0.25)	3.5 (1.76)	3.0 (1.29)
PAHM	223.3 (5.74)	227.5 (0.89)	0.8 (0.75) †	0.0 (0.00)	0.5 (0.50)	0.5 (0.29)
WIDL	210.0 (6.42)	212.3 (8.98)	0.0 (0.00) †	0.0 (0.00)	0.5 (0.50)*	3.8 (0.85)

Table G-7 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Not Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Root lodged plants (#/two rows)		Final stand count (#/two rows)		Grain moisture (%)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	0.0 (0.00) †	0.0 (0.00)	75.5 (0.50)	76.0 (0.00)	15.2 (0.14)	15.2 (0.11)
ILMN	0.0 (0.00) †	0.0 (0.00)	75.8 (0.25)	74.8 (0.75)	20.5 (0.52)	21.4 (0.74)
KSLA	0.0 (0.00) †	0.0 (0.00)	76.0 (1.29)	74.0 (1.22)	8.9 (0.28)	9.0 (0.06)
NCBD	0.0 (0.00) †	0.0 (0.00)	80.0 (0.71)	80.0 (1.08)	19.8 (0.32)	19.7 (0.09)
NEYO	0.0 (0.00) †	0.0 (0.00)	78.0 (0.91)	77.5 (1.04)	14.2 (0.06)	14.1 (0.07)
PAGR	1.0 (0.71) †	0.5 (0.29)	69.5 (2.18)	70.3 (1.93)	21.9 (0.53)	21.3 (0.42)
PAHM	1.0 (1.00) †	0.3 (0.25)	72.3 (0.33)	71.0 (0.71)	19.3 (0.21)	19.4 (0.44)
WIDL	0.0 (0.00) †	0.0 (0.00)	72.8 (1.55)	69.5 (1.55)	22.4 (1.01)	21.5 (0.69)

Table G-7 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Not Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 U.S. Field Trials

	Phenotypic Characteristics (units)			
	Test weight (kg/hl)		Yield (Mg/ha)	
	Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control
ARNE	73.7 (0.41)	73.4 (0.18)	12.4 (0.49)	12.0 (0.17)
ILMN	74.1 (0.48)	73.1 (0.76)	14.6 (0.25)*	15.5 (0.24)
KSLA	75.3 (0.67)	76.4 (0.43)	14.3 (0.34)	13.3 (0.66)
NCBD	71.3 (0.65)	72.2 (0.57)	14.8 (0.15)	13.3 (0.47)
NEYO	78.7 (0.59)	79.0 (0.11)	12.7 (0.73)	13.4 (0.79)
PAGR	68.1 (0.73)	67.6 (0.22)	13.0 (0.75)	14.1 (0.49)
PAHM	71.8 (0.48)	71.3 (0.48)	10.8 (0.93)	10.1 (1.05)
WIDL	73.0 (0.86)	73.3 (0.65)	7.6 (0.50)	7.8 (0.84)

Note: The experimental design was a randomized complete block design with four replications per site.
*Indicates a statistically significant difference between MON 87419 and the conventional control (α =0.05) using ANOVA.
† p-values could not be generated due to a lack of or insufficient within-material variability in the data.
[1] Data were not subjected to statistical analysis. Plant vigor rating ranges are the minimum and maximum raw values. The plant vigor rating ranges across all references are as follows: ARNE: 1 – 3; ILMN: 2 – 3; KSLA: 2 – 5; NCBD: 2 – 4; NEYO: 1 – 1; PAGR: 1 – 3; PAHM: 2 – 3; WIDL: 2 – 3.
[2] MON 87419 and the conventional control values represent means with standard error in parenthesis. N=4 except where noted in Table G-5.
[3] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; KSLA = Pawnee County, Kansas; NCBD = Perquimans County, North Carolina; NEYO = York County, Nebraska; PAGR = Lehigh County, Pennsylvania; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.

Table G-8. Individual-site Analysis of Phenotypic Characteristics of MON 87419 Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 and 2014 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant Vigor (1-9 scale)[1]		Early stand count (#/two rows)		Days to 50% pollen shed	
	Range		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	3 – 4	3 – 4	86.3 (0.75)	84.3 (1.18)	54.8 (0.25)	55.0 (0.00)
ILMN	2 – 2	2 – 3	84.0 (0.58)*	80.0 (0.71)	64.0 (0.00)	64.0 (0.00)
INKI	3 – 3	2 – 3	82.5 (0.29)	82.3 (1.11)	65.3 (0.48)	65.8 (0.48)
KSLA	1 – 4	3 – 4	84.5 (0.96)	83.3 (1.11)	61.3 (0.25)	61.3 (0.25)
NEYO–2013	1 – 1	1 – 1	83.8 (0.25)	83.5 (2.18)	66.0 (0.00)	66.0 (0.00)
NEYO–2014	1 – 1	1 – 1	88.3 (1.31)	85.8 (0.75)	56.0 (0.00)	56.0 (0.00)
OHTR	1 – 2	1 – 3	71.2 (1.01)	72.0 (1.15)	66.8 (1.70)	68.3 (1.49)
PAGR	2 – 3	1 – 2	77.0 (1.41)	78.3 (1.55)	62.3 (0.48)	61.5 (0.65)

Table G-8 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 and 2014 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Days to 50% silking		Stay green rating (1-9 scale)		Ear height (cm)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	55.3 (0.48)	56.0 (0.00)	4.8 (0.25)	4.3 (0.25)	108.0 (2.18)	103.1 (1.48)
ILMN	67.0 (0.00)	66.3 (0.75)	7.5 (0.65)	7.8 (0.63)	102.1 (1.90)*	111.1 (1.95)
INKI	66.0 (0.41)	66.8 (0.75)	8.3 (0.48)	8.5 (0.29)	111.5 (2.43)	111.5 (1.96)
KSLA	62.5 (0.50)	62.5 (0.65)	4.3 (0.48)	3.8 (0.25)	89.0 (4.95)	91.3 (6.48)
NEYO–2013	66.3 (0.25)	66.0 (0.00)	5.5 (0.29)	5.8 (0.25)	104.9 (4.56)	103.5 (2.90)
NEYO–2014	57.0 (0.00)	57.0 (0.00)	9.0 (0.00)	9.0 (0.00)	106.4 (3.91)	106.5 (2.65)
OHTR	66.3 (1.44)	67.0 (1.73)	8.3 (0.48)	7.5 (0.29)	111.1 (3.22)	109.4 (4.63)
PAGR	62.3 (0.25)	61.5 (0.50)	5.5 (0.50)*	7.3 (0.25)	107.7 (1.59)*	117.7 (1.27)

Table G-8 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 and 2014 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Plant height (cm)		Dropped ears (#/two rows)		Stalk lodged plants (#/two rows)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	233.5 (2.78)	234.8 (1.39)	0.0 (0.00) †	0.0 (0.00)	1.5 (0.96)	1.3 (0.75)
ILMN	212.3 (4.55)*	228.4 (3.17)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
INKI	219.3 (3.09)	219.0 (7.24)	0.0 (0.00)	0.0 (0.00)	0.5 (0.50)	0.0 (0.00)
KSLA	176.0 (2.90)	177.4 (2.58)	0.0 (0.00)	0.0 (0.00)	1.3 (0.95)	0.8 (0.48)
NEYO–2013	208.9 (5.72)	206.1 (7.87)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)*	1.8 (0.48)
NEYO–2014	223.1 (4.06)	221.9 (3.13)	1.0 (0.71)	0.0 (0.00)	4.0 (0.41)*	0.3 (0.25)
OHTR	235.3 (4.47)	241.3 (3.64)	0.0 (0.00) †	0.0 (0.00)	0.8 (0.75)	1.3 (0.48)
PAGR	224.5 (6.18)	236.4 (3.13)	0.0 (0.00)	0.3 (0.25)	2.5 (0.50)	3.0 (1.29)

Table G-8 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 and 2014 U.S. Field Trials

	Phenotypic Characteristics (units)					
	Root lodged plants (#/two rows)		Final stand count (#/two rows)		Grain moisture (%)	
	Mean (S.E.)[2]		Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control	MON 87419	Control
ARNE	0.0 (0.00)	0.0 (0.00)	76.0 (0.00)	76.0 (0.00)	15.2 (0.16)	15.2 (0.11)
ILMN	0.0 (0.00) †	0.0 (0.00)	75.8 (0.63)	74.8 (0.75)	22.4 (0.60)	21.4 (0.74)
INKI	2.3 (1.31)	1.8 (1.03)	74.0 (0.00)	72.0 (1.35)	20.3 (0.89)	19.6 (0.31)
KSLA	0.0 (0.00) †	0.0 (0.00)	77.5 (1.19)*	74.0 (1.22)	8.9 (0.17)	9.0 (0.06)
NEYO–2013	0.0 (0.00) †	0.0 (0.00)	77.0 (0.00)	77.5 (1.04)	14.3 (0.07)	14.1 (0.07)
NEYO–2014	0.0 (0.00) †	0.0 (0.00)	78.0 (1.15)	77.3 (0.75)	19.7 (0.37)	19.6 (0.31)
OHTR	0.0 (0.00) †	0.0 (0.00)	66.8 (0.75)	66.8 (0.48)	24.4 (0.97)	24.4 (1.28)
PAGR	0.8 (0.48)	0.5 (0.29)	67.8 (1.49)	70.3 (1.93)	21.9 (0.38)	21.3 (0.42)

Table G-8 (continued). Individual-site Analysis of Phenotypic Characteristics of MON 87419 Treated with Dicamba and Glufosinate Compared to the Conventional Control in 2013 and 2014 U.S. Field Trials

	Phenotypic Characteristics (units)			
	Test weight (kg/hL)		Yield (Mg/ha)	
	Mean (S.E.)[2]		Mean (S.E.)[2]	
Site Code[3]	MON 87419	Control	MON 87419	Control
ARNE	73.9 (0.40)	73.4 (0.18)	12.1 (0.22)	12.0 (0.17)
ILMN	72.8 (0.36)	73.1 (0.76)	15.8 (0.34)	15.5 (0.24)
INKI	70.6 (1.83)	72.1 (0.83)	10.9 (0.78)	10.4 (1.47)
KSLA	77.0 (0.17)	76.4 (0.43)	13.8 (0.75)	13.3 (0.66)
NEYO–2013	79.2 (0.35)	79.0 (0.11)	14.7 (0.28)	13.4 (0.79)
NEYO–2014	72.6 (0.70)	72.0 (0.28)	15.0 (0.18)	15.5 (0.13)
OHTR	71.2 (0.38)	71.2 (0.46)	13.4 (0.32)	13.6 (0.37)
PAGR	67.9 (0.87)	67.6 (0.22)	13.9 (0.65)	14.1 (0.49)

Note: The experimental design was a randomized complete block design with four replicates per site.
*Indicates a statistically significant difference between MON 87419 and the conventional control (α =0.05) using ANOVA.
† p-values could not be generated because of lack of variability in the data.
[1] Data were not subjected to statistical analysis. Plant vigor rating ranges are the minimum and maximum raw values. The plant vigor rating ranges across all references are as follows: ARNE: 1 – 3; ILMN: 2 – 3; INKI: 2 – 4; KSLA: 2 – 5; NEYO–2013: 1 – 1; NEYO–2014: 1 – 1; OHTR: 1 – 4; and PAGR: 1 – 3.
[2] MON 87419 and the conventional control values represent means with standard error in parenthesis. N=4 except where noted in Table G-6.
[3] Site code: ARNE = Jackson County, Arkansas; ILMN = Warren County, Illinois; INKI = Clinton County, Indiana; KSLA = Pawnee County, Kansas; NEYO = York County, Nebraska; OHTR = Miami County, Ohio; PAGR = Lehigh County, Pennsylvania.

Table G-9. Qualitative Assessment: Abiotic Stressor Evaluations Using a Categorical Scale for MON 87419 and the Conventional Control

Abiotic stressor	Number of observations across sites[1]	Number of observations where no differences were observed between MON 87419 and the conventional control
Total	93	93
Cold	1	1
Drought[2]	12	12
Flooding[3]	14	14
Hail	12	12
Heat	16	16
Nutrient deficiency	16	16
Soil compaction	3	3
Wind[4]	19	19

Note: The experimental design was a randomized complete block with four replications.
No differences were observed between MON 87419 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 onset of R6.
[1] Sites included: Jackson County, Arkansas; Warren County, Illinois; Pawnee County, Kansas; Perquimans County, North Carolina; York County, Nebraska; Lehigh County, Pennsylvania; Berks County, Pennsylvania; Walworth County, Wisconsin.
[2] Includes dryness.
[3] Includes wet soil.
[4] Includes storm.

Table G-10. Qualitative Assessment: Disease Damage Evaluations Using a Categorical Scale for MON 87419 and the Conventional Control

Disease	Number of observations across sites[1]	Number of observations where no differences were observed between MON 87419 and the conventional control
Total	107	107
Anthracnose	13	13
Bacterial blight	1	1
Crazy top	1	1
Ear rot[2]	9	9
Eyespot	9	9
***Fusarium* sp.**	2	2
Goss's bacterial wilt	11	11
Gray leaf spot	14	14
Leaf blight[3]	11	11
Northern leaf spot	1	1
***Pythium* sp.**	2	2
Rust[4]	16	16
Smut[5]	7	7
Stalk rot[2]	9	9
Stewart's bacterial wilt	1	1

Note: The experimental design was a randomized complete block with four replications.
No differences were observed between MON 87419 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 onset of R6. Disease data includes additional assessments of ear rot disease and stalk rot disease made at harvest.
[1] Sites included: Jackson County, Arkansas; Warren County, Illinois; Pawnee County, Kansas; Perquimans County, North Carolina; York County, Nebraska; Lehigh County, Pennsylvania; Berks County, Pennsylvania; Walworth County, Wisconsin.
[2] At ILMN, ear rot data were collected both in-season and at harvest. At NEYO, stalk rot data were collected both in-season and at harvest.
[3] Includes northern and southern.
[4] Includes common rust.
[5] Includes common smut.

Table G-11. Qualitative Assessment: Arthropod Damage Evaluations Using a Categorical Scale for MON 87419 and the Conventional Control

Arthropod	Number of observations across sites[1]	Number of observations where no differences were observed between MON 87419 and the conventional control
Total	91	91
Aphids (Aphididae)	1	1
Armyworms (Noctuidae)[2]	6	6
Billbugs (*Sphenophorus parvulus*)	2	2
Corn earworms (*Helicoverpa zea*)	8	8
Corn flea beetles (*Chaetocnema pulicaria*)[3]	10	10
Corn rootworm beetles (*Diabrotica* spp.)[4]	13	13
Cutworms (Noctuidae)[5]	5	5
European corn borers (*Ostrinia nubilalis*)[6]	11	11
Grape colaspis (Chrysomelidae)	1	1
Grasshoppers (*Melanoplus* spp.)	11	11
Japanese beetles (*Popillia japonica*)	9	9
Sap beetles (Nitidulidae)	3	3
Spider mites (*Tetranychus* spp.)	3	3
Southwestern Corn Borers (*Diatraea grandiosella*)	1	1
Stink bugs (Pentatomidae)	7	7

Note: The experimental design was a randomized complete block with four replications.
No differences were observed between MON 87419 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 onset of R6.
[1] Sites included: Jackson County, Arkansas; Warren County, Illinois; Pawnee County, Kansas; Perquimans County, North Carolina; York County, Nebraska; Lehigh County, Pennsylvania; Berks County, Pennsylvania; Walworth County, Wisconsin.
[2] Includes fall armyworms.
[3] Includes flea beetles.
[4] Includes Northern corn rootworms
[5] Includes black cutworms.
[6] European corn borers were assessed at five of eight sites: ARNE, ILMN, KSLA, NEYO, and PAGR.

Table G-12. Quantitative Assessment: Corn Earworm Damage and European Corn Borer Damage to MON 87419 Compared to the Conventional Control

Arthropod pest	Damage Assessment (per plant)	Site[1]	Mean (S.E.)[2]		Reference Range[3]
			MON 87419	Control	
Corn earworm (*H. zea*)[4]	Damage area	NCBD	0.0 (0.00)*	0.7 (0.48)	0.0 – 0.6
		PAHM	0.1 (0.04)	0.1 (0.05)	0.0 – 0.3
		WIDL	0.2 (0.06)	0.1 (0.05)	0.0 – 0.1
European corn borer (*O. nubilalis*)[5]	Number of stalk galleries	NCBD	0.5 (0.09)	0.5 (0.13)	0.2 – 0.4
		PAHM	0.1 (0.03)	0.1 (0.03)	0.0 – 0.1
		WIDL	0.0 (0.03)	0.2 (0.06)	0.0 – 0.2
European corn borer (*O. nubilalis*)	Stalk gallery length (cm)	NCBD	2.5 (0.61)	3.1 (0.99)	0.6 – 1.8
		PAHM	0.5 (0.17)	0.3 (0.19)	0.0 – 0.5
		WIDL	0.1 (0.05)	0.7 (0.40)	0.0 – 0.7

Note: The experimental design was a randomized complete block with four replications.
*Indicates statistically significant difference between MON 87419 and the conventional control ($\alpha = 0.05$) using ANOVA.
[1] Site code: NCBD = Perquimans County, North Carolina; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.
[2] MON 87419 and conventional control values represent means with standard error in parentheses. N = 4.
[3] Reference range is obtained from the minimum and maximum mean reference values among all reference hybrids at each site.
[4] Damage assessments for *H. zea* were conducted at the R5 – onset of R6 growth stage by assessing five consecutive ears from each of two rows. For two plots at PAHM, 10 consecutive ears from a single row were assessed.
[5] Damage assessments for *O. nubilalis* were conducted at R6 growth stage by assessing five consecutive plants from each of two rows.

Table G-13. Quantitative Assessment: Arthropod Abundance from Sticky Traps Collected from MON 87419 Compared to the Conventional Control

Arthropod[1]	Major Role	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 87419	Control	
Corn flea beetles (Chrysomelidae: Coleoptera)	Herbivore	NCBD	15.3 (2.91)	10.9 (2.38)	11.2 – 23.1
		PAHM	3.5 (1.53)	3.2 (1.56)	2.0 – 3.9
Corn rootworm beetles (Chrysomelidae: Coleoptera)	Herbivore	WIDL	25.9 (2.69)*	37.2 (2.54)	32.1 – 42.2
Ladybird beetles (Coccinellidae: Coleoptera)	Predator	NCBD	5.8 (0.78)	5.8 (0.79)	3.6 – 6.6
		PAHM	6.9 (0.36)	8.2 (1.98)	3.9 – 6.7
		WIDL	2.6 (0.40)	2.3 (0.73)	2.7 – 4.5
Sap beetles (Nitidulidae: Coleoptera)	Herbivore	NCBD	0.9 (0.26)	0.5 (0.13)	1.1 – 1.7
Leafhoppers (Cicadellidae: Hemiptera)	Herbivore	NCBD	70.5 (7.68)	66.3 (6.48)	55.3 – 63.7
		PAHM	12.6 (2.49)	12.0 (2.42)	3.8 – 7.2
Planthoppers (Delphacidae: Hemiptera)	Herbivore	NCBD	5.6 (0.85)	5.8 (0.88)	4.8 – 9.0
		PAHM	1.4 (0.33)	1.3 (0.36)	0.3 – 1.5
Minute pirate bugs (Anthocoridae: Hemiptera)	Predator	NCBD	6.9 (1.08)	9.1 (1.24)	6.2 – 12.0
		PAHM	5.1 (1.11)	3.9 (0.42)	3.8 – 5.5
		WIDL	1.8 (0.35)	2.3 (0.46)	5.1 – 10.6

Table G-13 (continued). Quantitative Assessment: Arthropod Abundance from Sticky Traps Collected from MON 87419 Compared to the Conventional Control

Arthropod[1]	Major Role	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 87419	Control	
Parasitic wasps (Hymenoptera)	Parasitoid	NCBD	192.1 (23.87)	198.7 (18.50)	158.5 – 191.6
		PAHM	87.6 (14.12)	93.7 (11.70)	50.3 – 76.3
		WIDL	98.3 (13.74)	106.0 (13.43)	53.2 – 74.4
Lacewings (Chrysopidae: Neuroptera)	Predator	WIDL	1.2 (0.15)	1.5 (0.44)	1.8 – 2.7
Syrphid flies (Syrphidae: Diptera)	Predator	PAHM	1.7 (0.49)	2.2 (0.34)	1.1 – 2.4
		WIDL	1.3 (0.33)	1.6 (0.28)	0.9 – 1.9
Spider mites (Tetranychidae: Acari)	Herbivore	WIDL	0.9 (0.39)	0.9 (0.21)	0.7 – 1.9
Spiders (Araneae)	Predator	NCBD	2.8 (0.36)*	1.5 (0.10)	1.7 – 2.3
		PAHM	1.2 (0.17)	1.6 (0.42)	0.7 – 1.3

*Indicates statistically significant difference between MON 87419 and the conventional control ($\alpha = 0.05$) using ANOVA.
Note: The experimental design was a randomized complete block with four replications.
[1] Arthropods were collected five times for sticky traps at approximately late vegetative–VT, R1, R2, R3, and R4 growth stages.
[2] Site code: NCBD = Perquimans County, North Carolina; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.
[3] MON 87419 and conventional control values represent means with standard error in parentheses. N = 4 except as noted in Table G-5.
[4] Reference range was obtained from the minimum and maximum mean reference values among all reference hybrids at each site.

Table G-14. Quantitative Assessment: Arthropod Abundance from Visual Counts Collected from MON 87419 Compared to the Conventional Control

Arthropod[1]	Major Role	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 87419	Control	
Corn flea beetles (Chrysomelidae: Coleoptera)	Herbivore	PAHM	3.3 (1.14)	2.9 (0.83)	2.1 – 2.7
Corn rootworm beetles (Chrysomelidae: Coleoptera)	Herbivore	WIDL	1.9 (0.44)	2.9 (1.10)	2.4 – 4.9
Ladybird beetles (Coccinellidae: Coleoptera)	Predator	PAHM	1.3 (0.37)	1.3 (0.44)	0.9 – 1.6
Sap beetles (Nitidulidae: Coleoptera)	Herbivore	NCBD	4.3 (1.67)	3.8 (1.03)	2.4 – 3.8
		PAHM	4.8 (1.11)	5.5 (0.41)	3.9 – 6.5
Shining flower beetles (Phalacridae: Coleoptera)	Herbivore	PAHM	3.1 (0.60)	2.4 (0.51)	2.6 – 3.6
Minute pirate bugs (Anthocoridae: Hemiptera)	Predator	NCBD	1.1 (0.13)	1.7 (0.45)	1.0 – 1.3
		PAHM	3.4 (0.88)*	5.5 (0.71)	3.0 – 4.3
		WIDL	3.6 (0.81)	2.3 (0.24)	2.5 – 4.7
Spiders (Araneae)	Predator	NCBD	6.7 (0.79)	6.1 (1.41)	5.1 – 8.2
		PAHM	1.5 (0.40)	1.9 (0.39)	1.8 – 2.3

*Indicates statistically significant difference between MON 87419 and the conventional control ($\alpha = 0.05$) using ANOVA.
Note: The experimental design was a randomized complete block with four replications.
[1] Visual counts were conducted starting at approximately VT and repeating at approximately five-day intervals for a total of five collections.
[2] Site code: NCBD = Perquimans County, North Carolina; PAHM = Berks County, Pennsylvania; WIDL = Walworth County, Wisconsin.
[3] MON 87419 and conventional control values represent means with standard error in parentheses. N = 4 except as noted in Table G-5.
[4] Reference range is obtained from the minimum and maximum mean reference values among all reference hybrids at each site.

References for Appendix G

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Appendix H: Materials and Methods for Pollen Morphology and Viability Assessment

H.1. Plant Production

MON 87419, the conventional control, and reference hybrids were grown under similar agronomic conditions in a 2013 field trial in Clinton County, Indiana (Table H-1). The trial was arranged in a randomized complete block design with four replications. Each plot consisted of six rows approximately 6 m in length.

H.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 uniquely labeled tubes. 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

H.3. Data Collection

Slides were prepared by aliquoting suspended pollen/stain solution onto a slide. Pollen characteristics were assessed under an Olympus© BX53F light microscope equipped with an Olympus© DP72 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows 7® and installed with an Olympus cellSens® (version 1.4.1) software.

H.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 were counted from a random field of view under the microscope. A minimum of 75 pollen grains were counted for each of the three subsamples per plot. Mean pollen viability for each replication was calculated from the subsamples as shown in Table VII-9.

H.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

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diameter for each replication was calculated from the total of 20 diameter measurements as shown in Table VII-9.

H.3.3. General Pollen Morphology

General pollen morphology of MON 87419, the conventional control, and the conventional commercial reference hybrids was observed as shown in Figure H-1.

H.4. Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS® (2012). The level of statistical significance was predetermined to be 5% (α=0.05). MON 87419 was compared to the conventional control material for percent viable pollen and pollen grain diameter. MON 87419 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.

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Table H-1. Starting Seed for Pollen Morphology and Viability Assessment

Material Name[1]	Monsanto Lot Number	Phenotype	T/C/R[2]
MON 87419	11356837	Dicamba and glufosinate-tolerant	T
NL6169[3]	11356835	Conventional	C
Phillips 713	11300072	Conventional	R
Dekalb DKC59-34	11354934	Conventional	R
Stine 9724	11298951	Conventional	R
H-9180	11226704	Conventional	R

[1] The study included an 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.
[2] T/C/R=Test/Control/Reference.
[3] NL6169 = HCL645 x LH244.

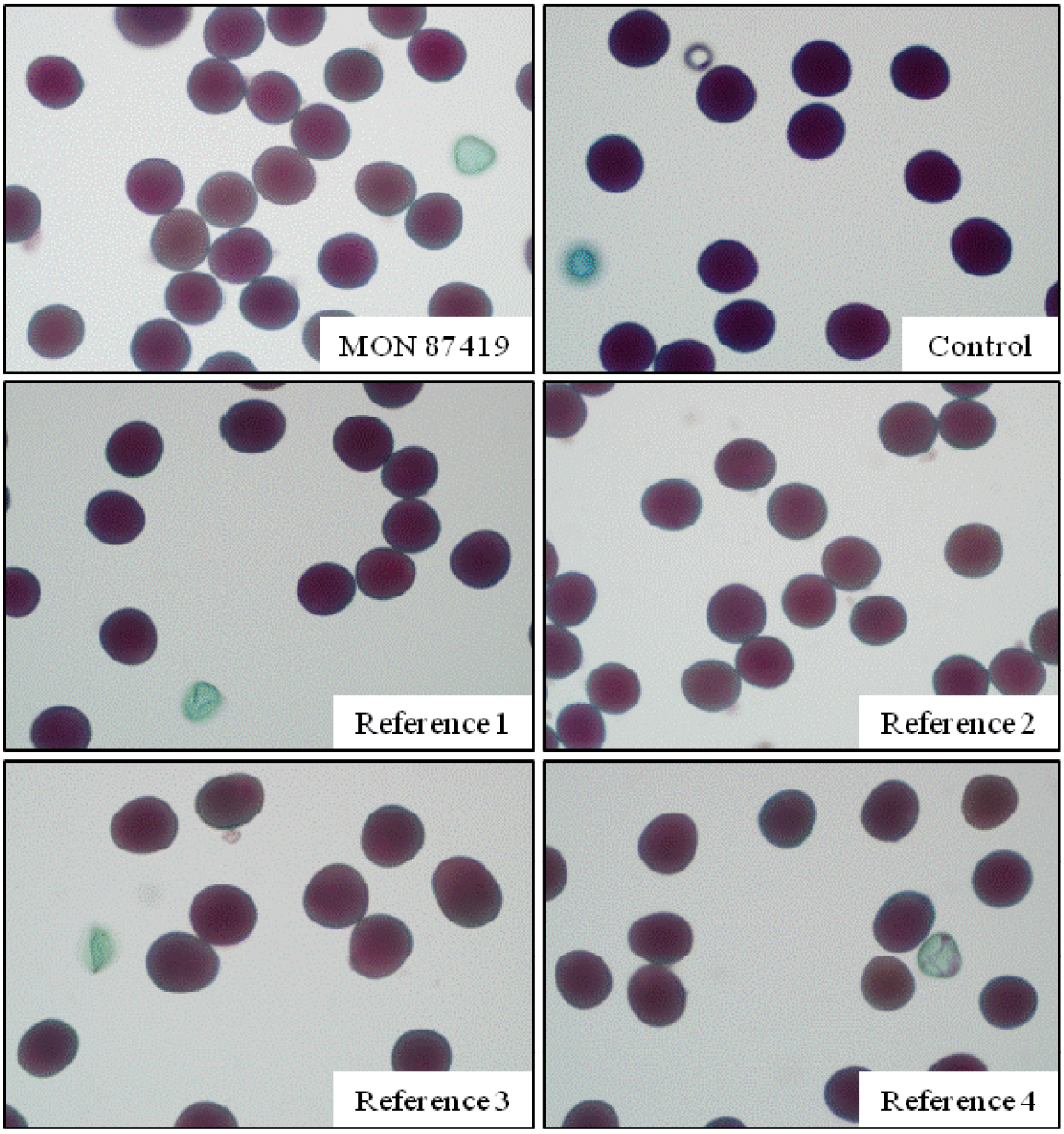

Figure H-1. General Morphology of Pollen from MON 87419, the Conventional Control, and Commercially Available Conventional Reference Hybrids under 200X Magnification

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 (Alexander, 1980).

References for Appendix H

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55:13-18.

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Appendix I: Herbicide Resistance

I.1. Introduction

Based upon theory of natural selection, plant populations can develop resistance to an herbicide due to the selection of individuals that carry variations in genes that can render those individuals unaffected by the typical lethal effects of an herbicide. Rather, over time, those few plant biotypes naturally containing resistant gene(s) become dominant in the population as they survive repeated use of the herbicide in the absence of other control methods (e.g. chemical- other herbicides with different mechanism of actions, mechanical, cultural or biological methods). The development of resistant populations is a possibility for all herbicides. The probability for resistance to develop is a function of: frequency of resistant allele(s)[8], mechanism of resistance, dominance or recessive nature of the resistant allele(s), relative fitness of the resistant biotype, and frequency or duration of herbicide use in the absence of other control methods (Beckie, 2006; Jasieniuk et al.,1996; Sammons et al., 2007). The probability of resistance is not the same for all herbicides, with some herbicides (e.g., ALS and ACCase classes) exhibiting resistance more quickly than other herbicides (e.g. auxin, glyphosate, glufosinate, and acetanilide, classes).

Herbicide resistance can become a limiting factor in crop production if the resistant weed population cannot be controlled with other herbicides, or with other methods of control. In general, this has not been the case for any herbicide. In most crops, there are multiple herbicide options for growers to use. However, good management practices to delay the development of herbicide resistance have been identified and are being actively promoted by the public and private sectors (HRAC, 2015a, b; Norsworthy et al. 2012; WSSA, 2015)[9] and are being implemented by growers.

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of dicamba and glufosinate herbicides to preserve their usefulness for growers is an important aspect of Monsanto's stewardship commitment. Although herbicide resistance may eventually occur in weed species when any herbicide is widely used, resistance can be postponed, contained, and managed through good management practices and associated research and education. These activities are key elements of Monsanto's approach to providing stewardship of dicamba and glufosinate used on MON 87419 that will likely be integrated into the glyphosate tolerant maize cropping systems. Monsanto will invest in research, and grower/retailer education and training programs to provide information on best practices to delay the selection for weed resistance and to manage weeds already selected for resistance to dicamba and glufosinate in maize production. This appendix provides an overview of Monsanto's approach to the development of best management practices to mitigate

[8] An allele is any of several forms of a gene, usually arising through mutations, that are responsible for hereditary variation.

[9] The Herbicide Resistance Action Committee (HRAC) is an international body founded by the agrochemical industry for the purpose of supporting a cooperative approach to the management of herbicide resistance and the establishment of a worldwide herbicide resistance database.

dicamba and glufosinate weed resistance. Monsanto works closely with weed scientists in academia and with other companies to develop best management practices and to consistently communicate such practices to growers. Evidence of this cooperative effort is development and posting of herbicide-resistant training modules on the WSSA website (www.wssa.net) and publication of guidelines by the Herbicide Resistance Action Committee (HRAC) on their website (www.hracglobal.com). The EPA is the U.S. federal regulatory agency that administers federal law governing pesticide sale and use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). EPA encourages pesticide manufacturers to provide growers with information regarding an herbicide's mechanism-of-action to aid growers in planning herbicide use practices and to foster the adoption of effective weed resistance management practices as specified by EPA in Pesticide Registration (PR) Notice 2001-5 (U.S. EPA, 2001). Monsanto incorporates EPA's guidelines for pesticide resistance management labeling on its agricultural herbicide labels, and will continue to do so in the future.

I.2. The Herbicide Dicamba

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is classified as a benzoic acid herbicide belonging to the synthetic auxin group of herbicides (HRAC, 2015a, b). The herbicides in this group act as growth regulators similar to endogenous indole acetic acid (IAA) but are structurally diverse. The synthetic auxin group includes five chemical families (benzoic acid, pyridine-carboxylic acid, quinoline carboxylic acid, phenoxy-carboxylic acid and a separate class which includes one herbicide, benazolin ethyl). The specific site of action among the different synthetic auxin chemical families may be different. In addition to dicamba, other herbicides in the synthetic auxin group include 2,4-D, clopyralid, quinclorac and several other active ingredients. Dicamba and other synthetic auxin herbicides are classified in Herbicide Group 4 by the Weed Science Society of America (HRAC, 2015a, b). Most herbicides in this group are active on broadleaf weeds only, but a few have significant activity on grasses, e.g., quinclorac. Dicamba provides control of over 95 annual and biennial broadleaf weed species and control or suppression of over 100 perennial broadleaf and woody species (BASF, 2008). Dicamba is not active on grass weeds and is often used in combination with other herbicides to provide broad spectrum weed control.

Dicamba herbicide was commercialized in the U.S. for agricultural use in 1967 and is currently labeled for use preemergence and/or postemergence in crops to control emerged weeds in maize, soybean, cotton, sorghum, small grains (wheat, barley and oats), millet, pasture, rangeland, asparagus, sugarcane, turf, grass grown for seed, conservation reserve program land, fallow cropland, and for non-crop uses (U.S. EPA, 2009). Dicamba is sold as standalone formulation which can be tank-mixed with one or more active ingredients depending upon the crop and the weed spectrum. Dicamba is also sold as a premix formulation with other herbicides.

I.3. The Herbicide Glufosinate

Glufosinate [2-amino-4-(hydroxymethylphosphinyl) butanoic acid] is classified as a phosphinic acid herbicide belonging to the glutamine synthetase inhibitor group of herbicides (HRAC, 2015a, b). Bialaphos is the only other herbicide belonging to the

phosphinic acid chemical family. Glufosinate and bialaphos are classified in Herbicide Group 10 by the Weed Science Society of America (HRAC, 2015a, b). Glufosinate provides control of over 90 annual grass and broadleaf weed species and 25 biennial and perennial grass and broadleaf weed species.

Glufosinate was first approved for use in the U.S. in 1994 (U.S. EPA, 2008) and is currently labeled for non-crop uses, preplant burndown to glufosinate-tolerant and non-tolerant crops and/or in-crop postemergence weed control in glufosinate-tolerant canola, maize, cotton, and soybean, (Bayer CropScience, 2011). Glufosinate is sold as a stand-alone formulation which can be tank mixed with other herbicides depending upon the specific herbicide, the crop and the weed spectrum.

I.4. Herbicide-Resistant Weeds and Resistance Management Strategies

The development of herbicide-resistant weeds is not a new phenomenon and resistance is not limited to certain select herbicides. In 1957, the first U.S. herbicide-resistant weed, a spreading dayflower biotype resistant to 2,4-D, was identified in Hawaii (Heap, 2014). See Table VIII-4 for scientific names of weeds mentioned in Appendix I. Through December 2014, there are approximately 146 individual weed species with known herbicide-resistant biotypes to one or more herbicides in the U.S (Heap, 2014). Growers have been managing herbicide-resistant weeds for decades with the use of alternative herbicides and/or other methods of weed control. The occurrence of an herbicide-resistant weed biotype usually does not end the useful lifespan or preclude the effective use of the herbicide as part of an overall diversified weed management system, but may change the way the herbicide is used in the cropping system.

As defined by the Weed Science Society of America, an herbicide resistant weed is one in which there is an inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA, 2015). A herbicide-tolerant weed species is one that is naturally tolerant to a herbicide, for example a grass species is not killed by the application of a broadleaf herbicide (WSSA, 2015). Furthermore, certain weed species, while neither resistant nor tolerant, are inherently difficult to control with a particular herbicide, requiring tank mixing with other herbicides and/or other weed management practices.

Since the first confirmed cases of herbicide resistance, research has been directed at determining which practices are best for managing existing resistance situations and how best to reduce the development of further resistance or multiple herbicide resistance. Resistance management practices most often recommended by University/Cooperative Extension Service (CES) and industry are generally summarized as: a) Understand the biology of the weeds present; b) Use a diversified approach toward weed management focused on preventing weed seed production and reducing the number of weed seed in the soil seedbank; c) Plant into weed-free fields and then keep fields as weed free as possible; d) Plant weed-free crop seed; e) Scout fields routinely; f) Use multiple herbicide mechanisms of action that are effective against the most troublesome weeds or those most prone to herbicide resistance; g) Apply the labeled herbicide rate at recommended weed sizes; h) Emphasize cultural practices that suppress weeds by using crop

competitiveness; i) Use mechanical and biological management practices where appropriate; j) Prevent field-to-field and within-field movement of weed seed or vegetative propagules; k) Manage weed seed at harvest and after harvest to prevent a buildup of the weed seedbank; l) Prevent an influx of weeds into the field by managing field borders (Beckie, 2006; Gressel and Segel, 1990; Norsworthy et al., 2012).

Recent research by Beckie and Reboud (2009) indicates that herbicide mixtures offer a better management option than rotating herbicides. Simultaneously using two herbicides with different mechanisms-of-action, each effective on the same weed species, significantly reduce the probability of weeds developing resistance to either or both herbicides (Beckie and Reboud, 2009). The use of multiple methods of weed control including multiple herbicides with different mechanisms of action in a single location is the technical basis for management programs to delay the development of resistance. This general concept has been referred to as applying "diversity" within a crop or across a crop rotation (Beckie, 2006; Powles, 2008).

I.5. Characteristics of Herbicides and Herbicide Use Influencing Resistance

While the incidence of weed resistance is often associated with repeated applications of an herbicide resulting in recurrent selection of the weed in the absence of other herbicides or methods of weed control, the actual probability for the development of resistant populations is related, in part, to the specific herbicide active ingredient, chemical family and herbicide group and especially the resistance mechanism. Some herbicides are more prone to the development of resistance than others (Heap, 2014). The graph in Figure I-1 illustrates the global instances of weed resistance to various herbicide groups. The different slopes of observed resistance are largely due to the factors described above, which relate to the specific herbicide active ingredient as well as to the group and herbicide family and its function.

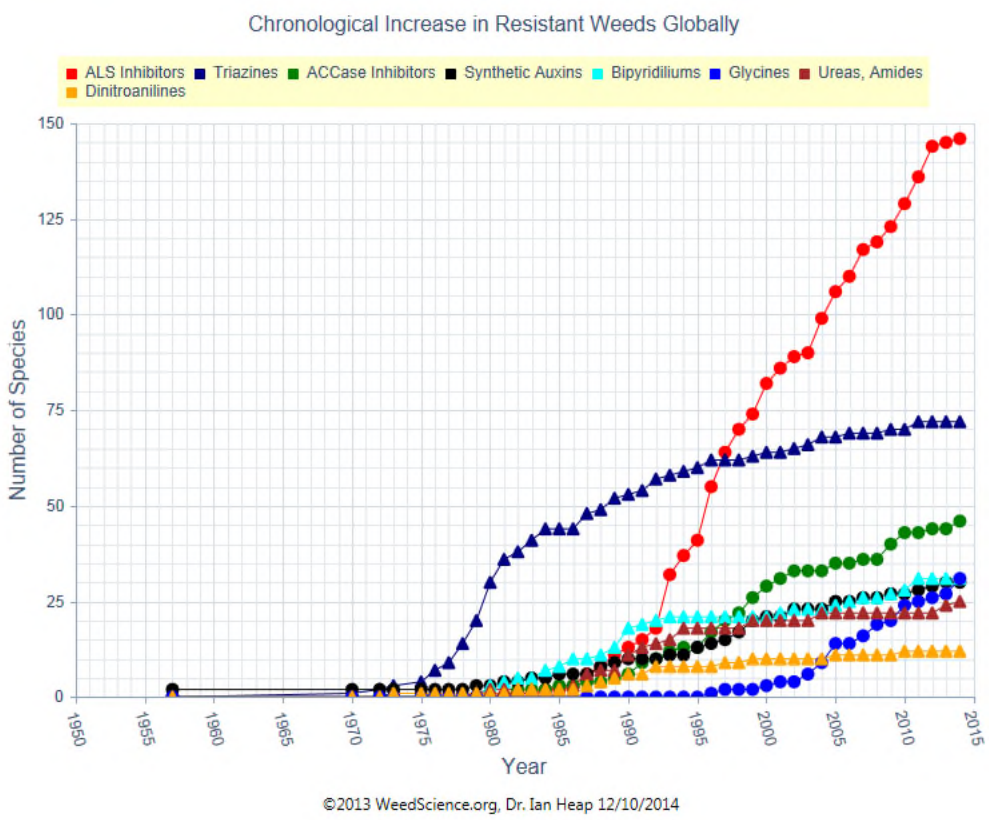

Figure I-1. Weed Resistance to Various Herbicide Families[1]

As can be seen in Figure I-1, weed resistance to the synthetic auxin group of herbicides has been slower to develop than for other herbicide groups even though these were the first synthetic herbicides discovered and used commercially. Possible reasons for this are discussed below.

[1] Global number of resistant biotypes

I.6. Mechanisms of Resistance and Inheritance of Resistance

To date, the three known basic mechanisms by which weed species develop resistance to a herbicide have been identified: 1) target site alteration (point mutations and/or increased expression), 2) enhanced metabolism of the herbicides (metabolism), and 3) reduced herbicide access to the site of action within the plant cell (exclusion) (Sammons et al., 2007).

Herbicide resistance via target site alteration is the most common resistance mechanism among the various herbicide groups and chemical families. It has been found that a target site mechanism is the most common mechanism for ALS inhibitors, ACCase inhibitors, and triazines, but is less common for other herbicide groups, such as glyphosate (Powles and Yu, 2010). The most common type of target site alteration is one where amino acid substitution(s) occur in the protein that is the target of the herbicide such that the alteration prevents herbicide binding to the protein and as a result the activity of the

targeted protein is not altered and the plant grows normally. Recently four species (*Kochia scoparia, Amaranthus palmeri, Amaranthus rudis, Lolium rigidum*) have managed to duplicate the EPSPS gene creating much higher expression levels of EPSPS protein (Sammons and Gaines, 2014).

In the case of synthetic auxin herbicides, resistance has been speculated to be due to mutation(s) in genes encoding an auxin-binding protein causing reduced herbicide binding (Zheng and Hall, 2001; Goss and Dyer, 2003). In several studies, differential herbicide absorption, translocation, and metabolism were ruled out as possible mechanisms of resistance in kochia (Cranston et al., 2001) and in wild mustard (Zheng and Hall, 2001). However, current research has not presented convincing evidence for a single mechanism of resistance and this inability to elucidate the mechanism of resistance may be due to a lack of thorough understanding of the mechanism (mode) of action of auxin herbicides (Jasieniuk et al., 1996). Walsh et al. (2006) identified seven alleles at two distinct genetic loci that conferred significant resistance to picolinate auxins (picloram) in *Arabidopsis*, yet had minimal cross-resistance to 2,4-D and IAA, a naturally occurring plant growth regulator..

Multiple mechanisms for inheritance of dicamba resistance have been reported in the literature. Jasieniuk et al. (1995) reported results indicating that inheritance of dicamba resistance in wild mustard is determined by a single, completely dominant nuclear allele. However, Cranston et al. (2001) reported results indicating that dicamba resistance in kochia is determined by a quantitative trait (two or more genes). The slow development of weed resistance to synthetic auxin herbicides may in part be due to their proposed multiple sites of physiological action in plants (Jasieniuk et al., 1996) and to the possibility that inheritance, at least in some species, is determined by a quantitative trait (Cranston et al., 2001).

Little is known about the resistance mechanisms in glufosinate-resistant biotypes. Avila-Garcia and Mallory-Smith (2011) conducted an initial set of experiments to understand the mechanism of resistance in the ryegrass population that was also resistant to glyphosate. They found that resistance was not due to an insensitive or altered target site and hypothesized that reduced translocation is responsible for the resistance to both glyphosate and glufosinate in these populations.

I.7. Weeds Resistant to Dicamba and Glufosinate

As noted earlier, like other herbicides, the use of dicamba may lead to the development of dicamba-resistant weed species. To date, there are four species with known resistant biotypes to dicamba in the U.S./Canada after over 40 years of use: common hempnettle, kochia, prickly lettuce, and wild mustard (Heap, 2014). Additionally, a population of common lambsquarters has been confirmed to be resistant in New Zealand, and a population of cornflower has been confirmed to be resistant in Poland for a total of six species worldwide with confirmed resistant biotypes to dicamba. For the synthetic auxin group of herbicides there exist a total of 30 species globally with biotypes having confirmed resistance to at least one member of this group, but only eight species in the U.S. and four species in Canada (Heap, 2014). All of the broadleaf populations (except for two (wild carrot in OH and MI, and waterhemp in NE), are found in the western U.S.

or western Canadian provinces. In some weed species, cross-resistance between different herbicides within the auxin group has been confirmed (plant cross-resistance to another herbicide as a result of exposure to a similarly acting herbicide). Therefore, consideration has to be given to the possibility that dicamba resistance could extend to some of the other broadleaf species listed as resistant to other synthetic auxin herbicides (Cranston et al., 2001; Jasieniuk et al., 1995; Miller et al., 2001). However, because of differences in sites of action among the chemistry families within this group (i.e., benzoic acids compared to pyridine-carboxylic acids) cross resistance between the herbicide groups is not a certainty (Monaco et al., 2002).

With the introduction of MON 87419 likely into glyphosate-tolerant maize systems, where dicamba can be applied in combination with glyphosate and glufosinate, it is important to note that kochia is the only broadleaf species with resistant biotypes to either synthetic auxins or glyphosate. However, there are no known kochia biotypes resistant to both of these herbicides or resistant to glufosinate. In addition, the evolution of a dicamba-glyphosate resistant biotype is unlikely because dicamba, glyphosate, and/or glufosinate, each with a distinct mechanism-of-action, will likely be applied in the same season to MON 87419 in the glyphosate-tolerant maize systems. If populations with resistance to both glyphosate and dicamba herbicides were to occur, there are other herbicide options for managing the weed in maize and in its rotational crops (Table I-1). Glyphosate-resistant kochia biotype populations may be found in Montana, North Dakota, South Dakota, Nebraska, Colorado, Kansas and Oklahoma.

To date there are two weed species with confirmed resistance to glufosinate: goosegrass in Malaysia and Italian ryegress in Oregon, U.S. (Heap, 2014). In the case of goosegrass, the resistant populations evolved due to use of glufosinate in a rubber plantation (Seng et al., 2010). In the case of Italian ryegrass, resistance was actually discovered in populations exposed to and evolved resistance to glyphosate and which had not been exposed to glufosinate; exemplifying a case of cross-resistance (Avila-Garcia and Mallory-Smith, 2011). No resistance in a broadleaf species has been found to date.

I.8. Sustainable Use of Dicamba and Glufosinate as a Weed Management Option in Maize

MON 87419 will likely be sold only in maize hybrids that also contain other herbicide-tolerant traits, including glyphosate-tolerance. Maize hybrids containing both MON 87419 and a glyphosate-tolerant system will enable dicamba and glufosinate to be applied with glyphosate and/or other maize herbicides in an integrated weed management program. Dicamba will likely be used in mixtures with either glyphosate or glufosinate or in sequence with glyphosate or glufosinate to control a broad spectrum of grass and broadleaf weed species. Glyphosate and glufosinate will likely not be used in mixtures due to antagonism (i.e., glufosinate damages the leaf tissue before glyphosate gets into the plant and/or can be translocated to growing parts of the plant) and reduced efficacy of glyphosate on susceptible weed species. Dicamba and glufosinate applications on MON 87419 will provide effective control of glyphosate-resistant broadleaf weeds and improve the control of annual and perennial broadleaf weed species, some of which are difficult to control with glyphosate. Dicamba and glufosinate will also help delay development and/or combat existing weed resistance issues that can limit the use of the

PPO- and ALS-inhibiting herbicide groups by providing additional mechanisms-of-action for management of certain broadleaf species known to be prone to resistance to many of the current herbicide options for weed management (i.e., *Amarathus* spp.). Likewise, dicamba will help to mediate potential evolution of resistance to glufosinate in broadleaf species and glufosinate will do the same for the potential evolution of resistant broadleaf species to dicamba.

Upon the likely integration of MON 87419 into glyphosate-tolerant maize systems and pending approval Monsanto's U.S. EPA petition to increase the maximum use rate of dicamba in maize from 0.5 lbs. to 1.0 lbs. a.e. per acre for preemergence applications and up to two applications of 0.5 lbs. a.e. of dicamba per acre for postemergence applications through the V8 growth stage or maize height of 30 inches, whichever comes first. The combined maximum annual application rate of dicamba on MON 87419 would be 2.0 lbs. a.e. dicamba per acre per year. Residual herbicides also will be recommended for use, to provide early season weed control and to supplement dicamba and glufosinate activity on certain hard-to-control and glyphosate-resistant weed biotypes, such as glyphosate-resistant Palmer amaranth where weed populations can be very substantial. See section I.9.1 for specific weed management recommendations.

Dicamba and glufosinate, as complementary herbicides to glyphosate, will provide new weed control options in maize that strengthen the utility and sustainability of glyphosate as a weed control tool in glyphosate-tolerant maize systems. Likewise, glyphosate, as a complementary herbicide to dicamba and glufosinate, would strengthen the utility and sustainability of dicamba and glufosinate as weed control tools for the combined MON 87419 glyphosate-tolerance trait product.

In the event there is known or suspected presence of a dicamba-resistant or glufosinate-resistant weed biotype, other options for managing resistant biotypes are available to the grower. There are multiple preemergence (including soil residuals) and postemergence herbicide options for managing weed populations that are resistant or may potentially develop resistance to dicamba or glufosinate in maize, as well for crops grown in rotation with maize. These options are noted in Table I-1.

Table I-1. Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin-Resistant Weeds

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Maize	Rotational Crops			
			Soybeans	Cotton	Sorghum	Wheat
Kochia	dicamba, fluroxpyr (populations also resistant to glyphosate)	Glyphosate[a] Atrazine[a] Isoxaflutole[a] Saflufenacil[a] Mesotrione[a]	Glyphosate[a] Clomazone[a] Flumioxazin[a] Saflufenacil[a] Paraquat[a]	Glyphosate[a] Clomazone[a] Flumioxazin[a] Saflufenacil[a]	Glyphosate[a] Atrazine[a] Acetochlor[a] Saflufenacil[a]	Glyphosate[a] Saflufenacil[a] Triasulfuron[a]
Lambsquarters (confirmed only in New Zealand)	Dicamba	Atrazine[a] Mesotrione[a] Isoxaflutole[a] Saflufenacil[a] Bromoxynil[a]	Glyphosate[b] Cloransulam[b] Acetochlor[a] Flumioxazin[b] Metribuzin[b]	Glyphosate[c] Trifluralin[c] Fluometuron[c] Diuron[c]	Glyphosate[a] Paraquat[a] Atrazine[a] Saflufenacil[a] Acetochlor[a]	Glyphosate[a] Chlorsulfuron/ metsulfuron[a] Bromoxynil[a] Saflufenacil[a] Metsulfuron[a]

Table I-1 (continued). Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin- Resistant Weeds

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Maize	Rotational Crops			
			Soybeans	Cotton	Sorghum	Wheat
Prickly Lettuce	Dicamba, 2,4 D, MCPA	Saflufenacil[a]	Glyphosate[a]	Glyphosate[c]	Glyphosate[a]	Glyphosate[a]
		Atrazine[a]	Chlorimuron/ metribuzin[a]	Glyphosate + Flumioxazin[c]	Atrazine[a]	Triasulfuron[a]
		Isoxaflutole[a]	Saflufenacil[a]	Saflufenacil[c]	Saflufenacil[a]	Metsulfuron + thifensulfuron[a]
			Saflufenacil/ imazethapyr[a]		Paraquat[a]	Saflufenacil[a]
Spreading Dayflower	2,4 D	Spreading dayflower infestations are present in rice. Bentazon, halosulfuron, penoxsulam, and bispyribac are recommended in rice.[c]				
Tall Waterhemp	2,4-D	Atrazine[a]	Metribuzin[b]	Fomesafen[d]	Atrazine[a]	Triasulfuron[a]
		Saflufenacil[a]	Acetochlor[b]	Trifluralin[d]	Saflufenacil[a]	Chlorsulfuron/ metsulfuron[a]
		Isoxaflutole[a]	Flumioxazin[b]	Fluometuron[d]	Acetochlor[a]	Saflufenacil[a]
		Mesotrione[a]	Fomesafen[b]	Diuron[d]	Carfentrazone[a]	Metsulfuron[a]
		Pyroxasulfone[a]	Lactofen[b]			

Table I-1 (continued). Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin- Resistant Weeds

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Maize	Rotational Crops			
			Soybeans	Cotton	Sorghum	Wheat
Wild Carrot	Dicamba, 2,4-D, MCPA, picloram, dichlorprop, mecoprop	Glyphosate[e] Atrazine[e] Primisulfuron[e] Nicosulfuron[e] Halosulfuron[e]	Glyphosate[e] Chlorimuron[e] Chlorimuron/ Metribuzin[e]	Glyphosate[e]	Glyphosate[e] Atrazine[e]	Glyphosate[e]
Wild mustard (confirmed only in Canada & Turkey)	Dicamba , 2,4 D,MCPA, picloram, dichlorprop, mecoprop	Glyphosate[f] Atrazine[f] Flumetsulam[f] Mesotrione[f] Halosulfuron[f]	Glyphosate[f] Chlorimuron[f] Bentazon[f] Fomesafen[f] Cloransulam[f]	Glyphosate[c] Paraquat[c] Flumioxazin[c] Fomesafen[c]	Glyphosate[f] Atrazine[f] Bentazon[f] Bromoxynil[f]	Pyraxlfotole[f] Bromoxynil[f] Thifensufuron/ Tribenuron[f] Prosulfuron[f] Pyroxsulam[f]
Yellow Starthistle	Picloram	Yellow starthistle infestations are present in pastures and roadsides. Chlorsulfuron is recommended for control in pastures.[g]				

Table I-1 (continued). Management Recommendations for Control of Dicamba-, Glufosinate- and Other Selected Synthetic Auxin- Resistant Weeds

Weed Species[1]	Herbicide Resistant Biotypes	Primary Crop Maize	Rotational Crops			
			Soybeans	Cotton	Sorghum	Wheat
Goosegrass (confirmed only in Malaysia)	Glufosinate	Glyphosate[d] Pendimethalin[d] Pyroxasulfone[d]	Clethodim[d] Glyphosate[d] Pendimethalin[d] Metolachlor[d]	Clethodim[d] Glyphosate[d] Pendimethalin[d] Diuron[d]	Glyphosate[d] Metolachlor[d] Paraquat[d]	Glyphosate[d]
Italian ryegrass	Glufosinate (populations also resistant to glyphosate)	Paraquat[d] Metolachlor/ atrazine[d] Glyphosate[d] Pyroxasulfone[d]	Parquat[d] Clethodim[d] Glyphosate + oxyfluorfen[d] Pyroxasulfone[d]	Paraquat[d] Clethodim[d] Glyphosate + flumioxazin[d] Pyroxasulfone[d]	Paraquat[d] Metolachlor/ atrazine[d] Glyphosate[d]	Glyphosate[d] Pyroxasulfone[d] Pinoxaden[d] Flufenacet/ Metribuzin[d]

[1] Scientific names for each weed species can be found in Table VIII-4.
[a] Knezevic 2014.
[b] Loux et al. 2014.
[c] Scott and Smith 2012.
[d] MSU 2015.
[e] MSU 2014.
[f] Sprague 2014.
[g] Peachey 2014.

I.9. Stewardship of Dicamba and Glufosinate Use on MON 87419

In order to steward the use of agricultural herbicides and herbicide-tolerant cropping systems such as the likely combined trait MON 87419 and glyphosate-tolerant maize product, Monsanto has conducted investigations and worked extensively with academics and other herbicide manufacturers to understand and recommend best practices to manage herbicide resistance. These investigations have demonstrated that one of the major factors contributing to the development of resistant weed biotypes has been poor weed control management practices. The primary reasons for lack of adequate management includes: 1) application of herbicides at rates below those indicated on the product label for the weed species, and 2) sole reliance on a particular herbicide for weed control without the use of other herbicides or cultural control methods (Beckie, 2006; Peterson et al., 2007).

I.9.1. Weed Control Recommendations

The proposed label for dicamba use on MON 87419 is based on the maximum allowable use rates and patterns. Prior to launch of MON 87419 likely in glyphosate-tolerant maize systems, Monsanto, in cooperation with academics, will conduct trials to confirm the optimum rate and timing for dicamba, glufosinate and glyphosate, alone and in combination with each other, and with other herbicides. Recommendations to growers will be developed from this information and will be provided in herbicide product labels, Monsanto's Technology Use Guide (TUG), and in other education and training materials to be broadly distributed. Specifically, current research conducted by Monsanto to define the optimum weed management systems support use recommendations that include the use of products that provide soil residual activity and the application of dicamba and glyphosate prior to maize emergence on conservation tillage acres and early postemergence in-crop applications. In some situations, a second in-crop application of either dicamba tank-mixed with glyphosate or glufosinate, with or without a soil residual will be recommended (see Section VIII.F.1. for additional details).

These recommendations will encourage the use of more than one mechanism of action against the targeted species, which is a fundamental component of a good weed resistance management program. These management systems, which include the use of multiple effective herbicide mechanisms-of-action, will reduce the potential for further resistance development to glyphosate, dicamba, and glufosinate, as well as other critical maize herbicides.

I.9.2. Dispersal of Technical and Stewardship Information

Monsanto will use multiple methods to distribute technical and stewardship information to growers, academics and grower advisors. Monsanto's TUG will set forth the requirements and best practices for cultivation of MON 87419 including recommendations on weed resistance management practices. Growers who purchase hybrids containing MON 87419 will be required to enter into a limited use license with Monsanto and must sign and comply with the Monsanto Technology Stewardship Agreement (MTSA), which requires the grower to follow the TUG.

The weed resistance management practices that will be articulated in the TUG will also be broadly communicated to growers and retailers in order to minimize the potential for development of resistant weed populations. These practices will be communicated through a variety of means, including direct mailings to each grower purchasing a maize hybrid containing MON 87419, a public website[10], and reports in farm media publications. The overall weed resistance management program will be reinforced through collaborations with U.S. academics, who will provide their recommendations for appropriate stewardship of dicamba and glufosinate in maize production, as well as by collaboration with crop commodity groups who have launched web-based weed resistance educational modules. Finally, Monsanto will urge growers to report any incidence of repeated non-performance of dicamba or glufosinate on weeds in fields planted with MON 87419 to the manufacturer of the herbicide. Appropriate investigations of unsatisfactory weed control will be conducted.

The EPA is the U.S. federal regulatory agency that administers federal law governing pesticide sale and use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). EPA encourages pesticide manufacturers to provide growers with information regarding an herbicide's mechanism-of-action to aid growers in planning herbicide use practices and to foster the adoption of effective weed resistance management practices as specified by EPA in Pesticide Registration (PR) Notice 2001-5 (U.S. EPA, 2001). In that document EPA states that "this approach to resistance management is sound and would be highly beneficial to pesticide manufacturers and pesticide users." The EPA approves all pesticide label use instructions based on its evaluation of supporting data supplied by the pesticide registrant or manufacturer. By approving a label, EPA has concluded that the product will not cause unreasonable adverse effects to the environment when used in accordance with the label's directions. After EPA approves a pesticide label, it is a violation of federal law to use the pesticide for a use or in a manner not in accordance with the label directions. Monsanto incorporates EPA's guidelines for pesticide resistance management labeling on its agricultural herbicide labels, and will continue to do so in the future. Monsanto will adopt a similar approach to pesticide resistance management guidance on its dicamba product labels.

In summary, Monsanto will require weed resistance management practices through the MTSA and TUG for its biotechnology-derived herbicide-tolerant products, including MON 87419. Upon deregulation, MON 87419 will be integrated with other maize traits systems, and Monsanto will promote these resistance management practices through product labeling and educational outreach efforts as an effective means to manage weed resistance development for dicamba, glufosinate, and glyphosate.

I.9.3. Weed Resistance Management Practices

Monsanto will provide information to growers and grower advisors on best management practices to delay development of resistance to dicamba and glufosinate. Weed resistance management recommendations for the use of dicamba and glufosinate in conjunction with maize hybrids containing MON 87419 will be consistent with the

[10] http://www.monsanto.com/weedmanagement/Pages/default.aspx

Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2015a, b). These guidelines recommend an integrated approach to weed resistance management, including cultural, mechanical and chemical methods that include the use of multiple herbicide mechanisms-of-action to manage a weed population.

In cases where resistance is confirmed for dicamba or glufosinate in maize producing areas, Monsanto and University/Cooperative Extension Service (CES) personnel will provide recommendations for alternative herbicide control methods to growers. These recommendations will be made available through Monsanto supplemental labels, Monsanto and university publications, and internet sites to growers, consultants, retailers and distributors. For all existing cases of dicamba-resistant and glufosinate-resistant weeds in the U.S. and globally today, alternative herbicides and cultural methods are available to growers to effectively control these biotypes. Examples of recommended alternative herbicides from University/CES personnel that are applicable to weed species known to be resistant to glufosinate, dicamba and other synthetic auxin herbicides are found in Table I-1. However, these examples in Table I-1 are only a subset of product combinations of available maize herbicides.

I.10. Monsanto Weed Performance Evaluation and Weed Resistance Management Plan

An important part of a weed resistance management plan is the timely acquisition of information regarding product performance. Monsanto has an extensive technical, sales and marketing presence in maize markets where MON 87419 will be grown. Through our relationships with farm advisors, key University/CES personnel, and growers using our seeds and traits products, Monsanto will acquire important and timely information regarding product performance. This will allow the timely recognition of performance issues that could arise related to weed resistance or other means. Field employees and hired consultants are trained and provided processes for responding to product performance inquiries. Individual performance issues that could be related to potential resistance are promptly handled. In addition, performance inquiries are periodically reviewed by Monsanto for trends that could indicate the need for follow up action on a broad scale.

If dicamba or glufosinate resistance is confirmed, the scientific and grower communities will be notified and a weed resistance management plan will be implemented by Monsanto in cooperation with the University/CES and/or the appropriate herbicide producer. The management plan will be designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The plan considers what is technically appropriate for a particular weed and incorporates practical management strategies.

After a management plan is developed, Monsanto communicates the plan to the grower community through various means, that may include informational fact sheets, retailer training programs, agriculture media and/or other means, as appropriate.

I.11. Summary

Development of weed resistance is a complex process that can be difficult to accurately predict. Multiple methods for managing weed resistance are available and no single option is best for all farming situations. No single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis, tailored for the particular herbicide and weed species, and utilize an integrated system approach to meet grower needs. Using good weed management principles, built upon achieving high levels of control through proper application rate, choice of cultural and mechanical practices, and appropriate companion weed control products will allow dicamba and glufosinate herbicides to continue to be used effectively. In cases where weed populations have evolved or developed resistance to dicamba and/or glufosinate, effective management options are available and experience has shown that growers will continue to find value in using dicamba and glufosinate in their weed control programs.

The key principles for effective stewardship of dicamba and glufosinate use, including the likely integration of MON 87419 in the glyphosate-tolerant maize systems, comprise: 1) basing weed management and weed resistance management practices on local needs and using the tools necessary to optimize crop yield, 2) using proper rate and timing of application, 3) not relying solely on one herbicide weed control option across a cropping system, 4) responding rapidly to instances of unsatisfactory weed control, and 5) providing up-to-date weed management and weed resistance management training.

Overall, there is a low potential for dicamba-resistant broadleaf weed populations to arise from the use of dicamba applied to MON 87419 integrated into glyphosate-tolerant maize systems. The reasons are as follows:

- Dicamba will be used in combination with glyphosate and/or glufosinate in a majority of cropping situations and weed recommendations will also include the concurrent use of residual herbicides for complementary weed control and additional mechanisms-of-action. These use patterns mean that there will be multiple mechanisms-of-action against the major broadleaf species present in maize production. This is a primary way to delay the development of resistance.
- The development of resistance to auxin herbicides has been found to be relatively slow. This observation is hypothesized to be due to multiple sites of action within plants and evidence suggesting that resistance is determined by multiple genes (quantitative traits), at least in some species.
- Only four broadleaf weed species have been confirmed to be resistant to dicamba in the U.S., and relatively low numbers of broadleaf species have been confirmed to be resistant to synthetic auxin herbicides even though dicamba has been widely in use for over 40 years.
- Known resistant broadleaf weed populations to dicamba and other auxin herbicides are primarily found in the western U.S. and, thus, are not present in the

major maize geographies. In addition, the known dicamba-resistant biotypes are not major weed species present in U.S. maize cropping systems.

Likewise, the probability for weed species to evolve resistance to glufosinate as a result of glufosinate use in the MON 87419 system is considered to be low because:

- Only two species have been confirmed to be resistant to glufosinate worldwide and one (ryegrass) in the US. This suggests that the frequency for resistant alleles in native weed populations is fairly low.
- Known resistant populations to glufosinate herbicide within the U.S. are only found in Oregon, and thus, are not present in the major maize geographies.

In the MON 87419 system, glufosinate will likely be used in combination with dicamba and in sequence with glyphosate. Residual herbicides will also be recommended and likely used in this cropping system. As noted above, these use patterns mean that there will be multiple mechanisms-of-action against the major broadleaf species present in maize production. This is a primary way to delay development of resistant weed populations.

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MONSANTO

Petitioner's Supplemental Data for Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate Tolerant MON 87419 Maize

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Submitted by:

Mark E. Groth
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
Phone: (314) 694-3369
Fax: (314) 694-3080
E-mail: mark.e.groth@monsanto.com

Prepared by:

Mark E. Groth

RELEASE OF INFORMATION

Monsanto is submitting the supplemental information included herein 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 submission, Monsanto expects that, in advance of the release of the document(s), USDA will 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 Environmental Assessment 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.

Crop Rotation Practices in Field 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). While there are tangible benefits from crop rotations, many other factors such as 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. Approximately 30% of the U.S. maize acres are rotated back to maize and 57% are rotated to soybean the following year. Wheat and cotton are other significant rotational crops with approximately 5% and 2%. Table 1 provides an assessment of the dicamba, glufosinate and glyphosate herbicide use in each of the rotational crops following maize at the U.S. country level. For the purpose of this assessment, a 50% adoption rate in U.S. maize production was assumed for MON 87419 and all these acres would receive an application of dicamba. The adoption rate for glyphosate-tolerant alfalfa was assumed to be 50% also since it has only been available a short time. Since MON 87708 soybean and MON 87701 cotton also contain the dicamba-tolerance trait and received a determination of nonregulated status (USDA-APHIS Petitions #10-188-01p and #12-185-01p), the 50% adoption rate was assumed for these products also and all these acres would receive an application of dicamba. With these adoption rate assumptions and the current useage of dicamba in other rotational crops, the usage of dicamba in rotational crop acres following maize is approximately 45% for the U.S. In comparison the usage of glyphosate in rotational crop acres is approximately 83%.

Introduction of MON 87419 is not, however, expected to impact crop rotation practices any more so than current biotechnology-derived herbicide tolerant products available to growers.

Rotation practices for maize vary from region to region in the U.S. However, there are similarities among states within certain growing regions. Tables 2 through 6 provide a detailed description and quantitative assessment of the rotational cropping practices immediately following maize production, by region and state. This assessment is based on current agronomic practices following maize production and accounts for essentionally all of the U.S. maize acreage. These data on rotational patterns are presented in the table below. A common rotation in the Midwest is the maize-soybean rotation (Singer and Bauer, 2009). In the Great Plains states, where water is the most limiting factor for crop production, small grains are dominate in the rotation because of their lower water requirement compared to other annual crops. In the humid southeastern states, peanuts, cotton and maize are included in a three- or four- year crop rotation to help manage weeds, diseases and nematodes while maximimizing profits.

In addition to the on rotational crop patterns, these tables provide an assessment of the dicamba, glufosinate and glyphosate herbicide use in each of the rotational crops following corn at the state, regional and U.S. level.

Table 1. Rotational Practices in the U.S. Following Maize Production

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
United States 95,365	Corn	28291	29.7	50	14146	1	302	82	23310			
	Soybean	54.451	57.1	50	27226	2	1349	95	51586			
	Wheat	4,527	4.7	8	355	NL		17	751			
	Cotton	1,870	2.0	50	935	21	397	85	1597			
	Alfalfa[8]	1,303	1.4	NL		NL		50	652			
	Other Hay	1,118	1.2	NL		NL		NA	0			
	Sorghum	799	0.8	10	82	NL		42	336			
	Oats	469	0.5	0	0	NL		0	0			
	Sugar Beets	455	0.5	NL		0	0	100	455			
	Sunflower	453	0.5	11	52	NL		85	386			
	Barley	320	0.3	3	8	NL		12	39			
	Peanut	281	0.3	NL		NL		28	78			
	Vegetables[9]	283	0.3	NL		NL		NA	0			
	Dry Beans	273	0.3	NL		NL		25	69			
	Potatoes	213	0.2	NL		NL		12	25			
	Tobacco	140	0.1	NL		NL		5	7			
	Millet	99	0.1	NL		NL		0	0			
	Rice	16	0.02	NL		NL		50	8			
	Safflower	6	0.01	NL		NL		0	0			
		Total[10]: 95,365			**Total: 42804**		**Total: 2048**		**Total: 79214**	**44.9**	**2.1**	**83.1**

This table was developed by compiling the data from all four regional summaries (Tables 2 through 6). All acreages are expressed as 1000s of acres.
NL indicates not labeled for use. NA indicates not available

1 Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

2 Column C is obtained by compiling the data from the four regional summaries.

3 Column D is obtained by dividing Column C by Column A.

4 Column E is obtained by dividing Column F by Column C; Column F is obtained by compiling the data from all five regional summaries.

5 Column G is obtained by dividing Column H by Column C; Column H is obtained by compiling the data from all five regional summaries.

6 Column I is obtained by dividing Column J by Column C; Column J is obtained by compiling the data from all five regional summaries

7 Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total; Column M is obtained by dividing Column J Total by Column C Total.

[8] Newly seeded alfalfa.
[9] Vegetables: chili peppers, cantaloupe, watermelon, tomatoes, onions, snap beans, sweet corn, cabbage, lima beans cucumbers, bell peppers, squash, green peas, carrots.
[10] Totals may not be exact due to rounding.

Table 2. Rotational Practices Following Maize Production in the Midwest Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region	Corn	13261	23.8	50	6631	1	161	79	10411			
55,680	Soybean	38785	69.7	50	19392	3	1193	95	36721			
	Wheat	1076	1.9	1	10	NL		7	77			
	Sorghum	12	0	0	0	NL		9	1			
	Barley	21	0	0	0	NL		0	0			
	Oats	229	0.4	0	0	NL		0	0			
	Dry Beans	151	0.3	NL		NL		16	25			
	Sugar Beets	216	0.4	NL		0	0	90	215			
	Potatoes	73	0.1	NL		NL		5	4			
	Alfalfa[8]	908	1.6	NL		NL		50	454			
	Other Hay	735	1.3	NL		NL		NA				
	Vegetables[9]	213	0.4	NL		NL		NA				
		Total[10]: 55,680			**Total: 26033**		**Total: 1354**		**Total: 47909**	**46.8**	**2.4**	**86.0**
IL	Corn	2640	22.0	50	1320	0	0	80	2112			
12,000	Soybean	9060	75.5	50	4530	4.0	362	94	8516			
	Wheat	168	1.4	0	0	NL		1	2			
	Sorghum	12	0.1	0	0	NL		9	1			
	Alfalfa	48	0.4	NL		NL		50	24			
	Other Hay	48	0.4	NL	0	NL		NA				
	Oats	24	0.2	0	0	NL		0	0			
		Total: 12000			**Total: 5850**		**Total: 362**		**Total: 10655**	**48.8**	**3.0**	**88.8**

Table 2 (cont.). Rotational Practices Following Maize Production in the Midwest Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
IN	Corn	1080	18.0	50	540	0	0	74	799			
6,000	Soybean	4800	80.0	50	2400	3.0	144	97	4656			
	Wheat	60	1.0	0	0	NL		11	7			
	Alfalfa	18	0.3	NL		NL		50	9			
	Other Hay	18	0.3	NL		NL		NA				
	Vegetables	24	0.4	NL		NL		NA				
		Total: 6,000			**Total: 2940**		**Total: 144**		**Total: 5471**	**49.0**	**2.4**	**91.2**
IA	Corn	4080	30.0	50	2040	3	122	79	3223			
13,600	Soybean	9112	67.0	50	4556	2	182	95	8656			
	Alfalfa Hay	136	1.0	NL		NL		50	68			
	Other Hay	272	2.0	NL		NL		NA				
		Total: 13,600			**Total: 6596**		**Total: 305**		**Total: 11948**	**48.5**	**2.2**	**87.9**
KY	Corn	520	34.0	50	260	0	0	94	489			
1,530	Soybean	581	38.0	50	291	2.0	12	98	570			
	Wheat	428	28.0	0	0	NL		9	39			
		Total: 1,530			**Total: 551**		**Total: 12**		**Total: 1097**	**36.0**	**0.8**	**71.7**
MI	Corn	676	26.0	50	338	3	20	68	460			
2,600	Soybean	1534	59.0	50	767	1	15	95	1457			
	Dry Beans	104	4.0	NL		NL		13	14			
	Sugar Beets	130	5.0			0	0	99	129			
	Alfalfa Hay	91	3.5	NL		NL		50	46			
	Other Hay	65	2.5	NL		NL		NA				
		Total: 2,600			**Total: 1105**		**Total: 36**		**Total: 2105**	**42.5**	**1.4**	**81.0**

Table 2 (cont.). Rotational Practices Following Maize Production in the Midwest Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
MN	Corn	1634	19.0	50	817	0	0	88	1438			
8,600	Soybean	6321	73.5	50	3161	1	63	96	6068			
	Dry Beans	43	0.5	NL		NL		26	11			
	Sugar Beets	86	1.0	NL		0	0	100	86			
	Alfalfa	224	2.6	NL		NL		50	112			
	Other Hay	120	1.4	NL		NL		NA				
	Potatoes	43	0.5	NL		NL		0	0			
	Vegetables	129	1.5	NL		NL		NA				
		Total: 8,600			**Total: 3978**		**Total: 63**		**Total: 7716**	**46.3**	**0.7**	**89.7**
MO	Corn	402	12.0	50	201	2	8	66	265			
3,350	Soybean	2781	83.0	50	1390	11	306	88	2447			
	Wheat	168	5.0	3	5	NL		8	13			
		Total: 3,350			**Total: 1596**		**Total: 314**		**Total: 2726**	**47.7**	**9.4**	**81.4**
OH	Corn	507	13.0	50	254	2	10	59	299			
3,900	Soybean	3120	80.0	50	1560	3	94	94	2933			
	Wheat	121	3.1	4	5	NL		1	1			
	Alfalfa Hay	39	1.0	NL		NL		50	20			
	Other Hay	113	2.9	NL		NL		NA				
		Total: 3,900			**Total: 1818**		**Total: 104**		**Total: 3253**	**46.6**	**2.7**	**83.4**

Table 2 (cont.). Rotational Practices Following Maize Production in the Midwest Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
	Corn	1722	42.0	50	861	0	0	77	1326			
WI	Soybean	1476	36.0	50	738	1	15	96	1417			
4,100	Wheat	131	3.2	0	0	NL		12	16			
	Barley	21	0.5	0	0	NL		0	0			
	Oats	205	5.0	0	0	NL		0	0			
	Dry Beans	4	0.1	NL		NL		0	0			
	Potatoes	30	0.7	NL		NL		12	4			
	Alfalfa	353	8.6	NL		NL		50	176			
	Other Hay	98	2.4	NL		NL		NA				
	Vegetables	60	1.5	NL		NL		NA				
		Total: 8,600			**Total: 1599**		**Total: 15**		**Total: 2939**	**39.0**	**0.4**	**71.7**

The Midwest region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

1 Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

2 Column C is obtained by multiplying Column A by Column D.

3 The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist, Extension Weed Control Specialist, and Monsanto technical personnel in maize (Personal Communications, November, 2003).

4 Dicamba usage data in Column E except for cotton and soybean is based on 2013 data (Monsanto Company, 2014). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

5 Glufosinate usage data in Column G is based on 2013 data (Monsanto Company, 2014).

6 Glyphosate usage data in Column I except for alfalfa is based on 2013 data (Monsanto Company, 2014). Glyphosate useage in alfalfa (50%) is a future market adoption estimate.

7 Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

8 Newly seeded alfalfa.

9 Vegetables: Sweet corn, tomatoes, snap beans, cantoloupe, watermelon, cucumbers, green peas, carrots, and cabbage.

10 Totals may not be exact due to rounding.

Table 3. Rotational Practices Following Maize Production in the Northeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region	Corn	2204	52.8	50	1102	0	0	63	1385			
4,175	Soybean	836	20.0	50	418	0.1	1	84	702			
	Wheat	417	10.0	0.4	2	NL		1	5			
	Barley	109	2.6	0.1	0	NL		1	2			
	Oats	156	3.7	0	0	NL		0	0			
	Alfalfa[8]	144	3.4	NL		NL		50	72			
	Other Hay	272	6.5	NL		NL		NA				
	Vegetables[9]	37	0.9	NL		NL		NA				
		Total[10]: 4,175			**Total: 1522**		**Total: 1**		**Total: 2165**	**36.5**	**0.03**	**51.9**
DE	Corn	68	38.0	50	34	0	0	45	31			
180	Soybean	61	34.0	50	31	2	1	97	59			
	Wheat	36	20.0	0	0	NL		0	0			
	Barley	13	7.0	0	0	NL		0	0			
	Vegetables	2	1.0	NL		NL		NA				
		Total: 180			**Total: 65**		**Total: 1**		**Total: 90**	**36.0**	**0.7**	**50.1**
MD	Corn	182	38.0	50	91	0	0	90	164			
480	Soybean	168	35.0	50	84	0	0	99	166			
	Wheat	96	20.0	0	0	NL		0	0			
	Barley	34	7.0	0	0	NL		0	0			
		Total: 480			**Total: 175**		**Total: 0**		**Total: 330**	**36.5**	**0.0**	**68.9**
NJ	Corn	52	58.0	50	26	0	0	0	0			
90	Soybean	29	32.0	50	14	0	0	0	0			
	Wheat	3	3.0	0	0	0	0	0	0			
	Vegetables	6	7.0	NL			0	NA				
		Total: 90			**Total: 41**		**Total: 0**		**Total: 0**	**45.0**	**0.0**	**0.0**

Table 3 (cont.). Rotational Practices Following Maize Production in the Northeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
NY	Corn	744	62.0	50	372	0	0	56	417			
1,200	Soybean	156	13.0	50	78	0	0	46	72			
	Wheat	60	5.0	0	0	NL		0	0			
	Oats	60	5.0	0	0	NL		0	0			
	Alfalfa Hay	59	4.9	NL		NL		50	29			
	Other Hay	97	8.1	NL		NL		NA				
	Vegetables	24	2.0	NL		NL		NA				
		Total: 1,200			**Total: 450**		**Total: 0**		**Total: 518**	**37.5**	**0.0**	**43.2**
PA	Corn	784	53.0	50	392	0	0	77	604			
1,480	Soybean	192	13.0	50	96	0	0	99	190			
	Wheat	145	9.8	0	0	NL		0	0			
	Oats	89	6.0	0	0	NL		0	0			
	Barley	50	3.4	0	0	NL		3	2			
	Alfalfa	78	5.3	NL		NL		50	39			
	Other Hay	136	9.2	NL		NL		NA				
	Vegetables	4	0.3	NL		NL		NA				
		Total: 1,480			**Total: 488**		**Total: 0**		**Total: 835**	**33.0**	**0.0**	**56.4**
VA	Corn	209	41.0	50	105	0	0	81	169			
510	Soybean	214	42.0	50	107	0	0	100	214			
	Wheat	77	15.0	2	2	NL		6	5			
	Barley	10	2.0	1	0	NL		1	0			
		Total: 510			**Total: 213**		**Total: 0**		**Total: 388**	**41.8**	**0.0**	**76.1**

Table 3 (cont.). Rotational Practices Following Maize Production in the Northeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
WV	Corn	37	69.0	50	18	0	0	0	0			
53	Soybean	15	29.0	50	8	0	0	0	0			
	Wheat	1	2.0	0	0	NL		0	0			
		Total: 53			**Total: 26**		**Total: 0**		**Total: 0**	**49.0**	**0.0**	**0.0**
New England 182	Corn	127	70.0	50	64	0	0	0	0			
	Barley	2	1.0	0	0	NL		0	0			
	Oats	7	4.0	0	0	NL		0	0			
	Alfalfa Hay	6	3.5	NL		NL		50	3			
	Other Hay	39	21.5	NL		NL		NA				
		Total: 182			**Total: 64**		**Total: 0**		**Total: 3**	**35.0**	**0.0**	**1.8**

The Northeast region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

1 Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

2 Column C is obtained by multiplying Column A by Column D.

3 The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist, Extension Weed Control Specialist, and Monsanto technical personnel in maize (Personal Communications, November, 2003).

4 Dicamba usage data in Column E except for cotton and soybean is based on 2013 data (Monsanto Company, 2014). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

5 Glufosinate usage data in Column G is based on 2013 data (Monsanto Company, 2014).

6 Glyphosate usage data in Column I except for alfalfa is based on 2013 data (Monsanto Company, 2014). Glyphosate useage in alfalfa (50%) is a future market adoption estimate.

7 Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

8 Newly seeded alfalfa.

9 Vegetables: Sweet corn, snap beans, and cabbage.

10 Totals may not be exact due to rounding.

Table 4. Rotational Practices Following Maize Production in the Southeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region	Corn	1753	31.7	50	876	2	37	90	1579			
5,535	Soybean	1513	27.3	50	757	5	69	93	1412			
	Wheat	476	8.6	9	44	NL		24	112			
	Cotton	1331	24.1	50	666	29	381	88	1172			
	Sorghum	20	0.4	34	7	NL		82	17			
	Peanuts	281	5.1	NL		NL		28	78			
	Tobacco	140	2.5	NL		NL		5	7			
	Rice	16	0.3	NL		NL		54	8			
	Vegetables[8]	5	0.1	NL		NL		NA				
		Total[9]: 5535			**Total: 2350**		**Total: 486**		**Total: 4386**	**42.5**	**8.8**	**79.2**
AL	Corn	163	51.0	50	82	0	0	96	157			
320	Soybean	6	2.0	50	3	2	0.1	96	6			
	Wheat	6	2.0	0	0	NL		0	0			
	Cotton	96	30.0	50	48	4	4	99	95			
	Peanuts	48	15.0	NL		NL		66	32			
		Total: 320			**Total: 133**		**Total: 4**		**Total: 290**	**41.5**	**1.2**	**90.5**
AR	Corn	202	23.0	50	101	0	0	95	192			
880	Soybean	273	31.0	50	136	14	38	83	226			
	Wheat	44	5.0	1	44	NL		6	3			
	Cotton	352	40.0	50	176	34	120	84	296			
	Rice	9	1.0	NL		NL		56	5			
		Total: 90			**Total: 458**		**Total: 158**		**Total: 722**	**52.0**	**17.9**	**82.0**

Table 4 (cont.) Rotational Practices Following Maize Production in the Southeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
FL	Corn	63	55.0	50	32	0	0	0	0			
115	Soybean	1	1.0	50	1	0	0	0	0			
	Wheat	1	1.0	0	0	NL		0	0			
	Cotton	17	15.0	50	9	13	2	87	15			
	Peanuts	32	28.0	NL		NL		21	7			
		Total: 115			**Total: 41**		**Total: 2**		**Total: 22**	**35.5**	**2.0**	**18.9**
GA	Corn	224	44.0	50	112	6	13	85	191			
510	Soybean	5	1.0	50	3	7	0.4	81	4			
	Wheat	10	2.0	0	0	NL		55	6			
	Cotton	153	30.0	50	77	46	70	76	116			
	Peanuts	112	22.0	NL		NL		13	15			
	Vegetables	5	1.0	NL		NL		NA				
		Total: 510			**Total: 191**		**Total: 84**		**Total: 331**	**37.5**	**16.5**	**65.0**
LA	Corn	245	36.0	50	122	2	5	96	235			
680	Soybean	272	40.0	50	136	0	0	99	269			
	Wheat	14	2.0	0	0	NL		0	0			
	Cotton	122	18.0	50	61	1	1	100	122			
	Sorghum	20	3.0	34	7	NL		82	17			
	Rice	7	1.0	NL		NL		51	3			
		Total: 680			**Total: 327**		**Total: 6**		**Total: 647**	**48.0**	**0.9**	**95.1**
MS	Corn	258	30.0	50	129	0	0	97	250			
860	Soybean	344	40.0	50	172	0	0	97	334			
	Cotton	258	30.0	50	129	22	57	92	237			
		Total: 860			**Total: 430**		**Total: 57**		**Total: 821**	**50.0**	**6.6**	**95.5**

Table 4 (cont.). Rotational Practices Following Maize Production in the Southeast Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
NC	Corn	233	25.0	50	116	6	14	93	216			
930	Soybean	279	30.0	50	140	6	17	94	262			
	Cotton	205	22.0	50	102	30	61	97	198			
	Peanuts	74	8.0	NL		NL		30	22			
	Tobacco	140	15.0	NL		NL		5	7			
		Total: 930			**Total: 358**		**Total: 92**		**Total: 706**	**38.5**	**9.9**	**75.9**
SC	Corn	133	38.0	50	67	0	0	87	116			
350	Soybean	151	43.0	50	75	4	6	86	129			
	Cotton	53	15.0	50	26	58	30	74	39			
	Peanuts	14	4.0	NL		NL		19	3			
		Total: 350			**Total: 168**		**Total: 36**		**Total: 287**	**48.0**	**10.4**	**81.9**
TN	Corn	231	26.0	50	116	2	5	96	222			
890	Soybean	182	20.5	50	91	4	7	99	181			
	Wheat	401	45.0	0	0	NL		26	104			
	Cotton	76	8.5	50	38	46	35	70	53			
		Total: 890			**Total: 245**		**Total: 47**		**Total: 560**	**27.5**	**5.3**	**62.9**

The Southeast region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

1 Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

2 Column C is obtained by multiplying Column A by Column D.

3 The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist, Extension Weed Control Specialist, and Monsanto technical personnel in maize (Personal Communications, November, 2003).

4 Dicamba usage data in Column E except for cotton and soybean is based on 2013 data (Monsanto Company, 2014). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

5 Glufosinate usage data in Column G is based on 2013 data (Monsanto Company, 2014).

[6] Glyphosate usage data in Column I is based on 2013 data (Monsanto Company, 2014).
[7] Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.
[8]Vegetables: Sweet corn, tomatoes, snap beans, cantoloupe, watermelon, cucumbers, cabbage, bell peppers, and onions.
[9] Totals may not be exact due to rounding.

Table 5. Rotational Practices Following Maize Production in the Plains Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region	Corn	10337	36.2	50	5169	1	104	91	9382			
28,580	Soybean	13317	46.6	50	6659	1	86	96	12751			
	Wheat	2352	8.2	12	279	NL		23	545			
	Barley	128	0.4	6	7	NL		26	34			
	Oats	62	0.2	0	0	NL		0	0			
	Cotton	521	1.8	50	260	3	16	78	408			
	Sorghum	766	2.7	10	75	NL		42	318			
	Sunflower	453	1.6	11	52	NL		67	302			
	Sugar Beets	239	0.8	NL		0	0	100	239			
	Millet	99	0.3	0	0	NL		0	0			
	Potatoes	77	0.3	NL		NL		9	7			
	Dry Beans	93	0.3	NL		NL		33	31			
	Alfalfa[8]	83	0.3	NL		NL		50	42			
	Other Hay	54	0.2	NL		NL		NA				
	Vegetables[9]	1	0.002	NL		NL		NA				
		Total[10]: 28,580			**Total: 12501**		**Total: 206**		**Total: 24058**	**43.7**	**0.7**	**84.2**
CO	Corn	842	69.0	50	421	0	0	89	749			
1,220	Wheat	207	17.0	20	41	NL		26	54			
	Sorghum	73	6.0	47	34	NL		52	38			
	Sunflower	61	5.0	44	27	NL		48	29			
	Millet	37	3.0	0	0	NL		0	0			
		Total: 1,220			**Total: 524**		**Total: 0**		**Total: 870**	**42.9**	**0.0**	**71.4**

Table 5 (cont.). Rotational Practices Following Maize Production in the Plains Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
KS	Corn	1806	42.0	50	903	0	0	93	1680			
4,300	Soybean	2064	48.0	50	1032	1	21	95	1961			
	Wheat	301	7.0	17	51	NL		10	30			
	Sorghum	86	2.0	35	30	NL		74	64			
	Sunflower	43	1.0	39	17	NL		65	28			
		Total: 4,300			**Total: 2033**		**Total: 21**		**Total: 3762**	**47.3**	**0.5**	**87.5**
MT	Corn	12	10.0	50	6	0	0	0	0			
120	Wheat	12	10.0	8	1	NL	0	48	6			
	Barley	12	10.0	3	0	NL		40	5			
	Sugar Beets	60	50.0	NL	0	0	0	100	60			
	Dry Beans	18	15.0	NL		NL		59	11			
	Alfalfa Hay	4	3.1	NL		NL		50	2			
	Other Hay	2	1.9	NL		NL		NA				
		Total: 120			**Total: 7**		**Total: 0**		**Total: 83**	**6.1**	**0.0**	**69.2**
NE	Corn	3881	39.0	50	1940	1	39	88	3415			
9,950	Soybean	4975	50.0	50	2488	1	50	98	4876			
	Wheat	498	5.0	17	85	NL		24	119			
	Sorghum	498	5.0	1	5	NL		37	184			
	Sugar beets	25	0.3	NL		0	0	100	25			
	Dry Beans	75	0.8	NL		NL		27	20			
		Total: 90			**Total: 4517**		**Total: 89**		**Total: 8639**	**45.4**	**0.9**	**86.8**

Table 5 (cont.). Rotational Practices Following Maize Production in the Plains Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
NM	Corn	97	81.0	50	49	0	0	95	92			
120	Wheat	11	9.0	1	0.1	NL		8	1			
	Cotton	4	3.0	50	2	0	0	0	0			
	Sorghum	1	0.5	30	0.2	NL		6	0.04			
	Alfalfa	5	4.1	NL		NL		50	2			
	Other Hay	2	1.9	NL		NL		NA				
	Vegetables	1	0.5	NL		NL		NA				
		Total: 120			**Total: 51**		**Total: 0**		**Total: 96**	**42.2**	**0.0**	**79.8**
ND	Corn	1463	38.0	50	732	0	0	96	1404			
3,850	Soybean	1425	37.0	50	712	1	14	98	1396			
	Wheat	578	15.0	13	75	NL		38	219			
	Barley	116	3.0	6	7	NL		25	29			
	Sugar Beets	154	4.0	NL		0	0	100	154			
	Sunflower	39	1.0	5	2	NL		87	33			
	Potatoes	77	2.0	NL		NL		9	7			
		Total: 3,850			**Total: 1528**		**Total: 14**		**Total: 3243**	**39.7**	**0.4**	**84.2**
OK	Corn	174	47.0	50	87	0	**0**	96	167			
370	Soybean	93	25.0	50	46	2	**2**	93	86			
	Wheat	104	28.0	8	8	NL		17	18			
		Total: 370			**Total: 141**		**Total: 2**		**Total: 271**	**38.2**	**0.5**	**73.1**

Table 5 (cont.). Rotational Practices Following Maize Production in the Plains Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
SD	Corn	930	15.0	50	465	0	0	95	884			
6,200	Soybean	4526	73.0	50	2263	0	0	94	4254			
	Wheat	124	2.0	3	4	NL		18	22			
	Oats	62	1.0	0	0	NL		0	0			
	Sorghum	62	1.0	2	1	NL		28	17			
	Sunflower	310	5.0	2	6	NL		68	211			
	Millet	62	1.0	0	0	NL		0	0			
	Alfalfa	74	1.2	NL		NL		50	37			
	Other Hay	50	0.8	NL		NL		NA				
		Total: 6,200			**Total: 2739**		**Total: 0**		**Total: 5426**	**44.2**	**0.0**	**7.5**
TX	Corn	1081	46.0	50	541	6	65	87	940			
2,350	Soybean	235	10.0	50	118	0	0	76	179			
	Wheat	470	20.0	3	14	NL		16	75			
	Cotton	517	22.0	50	259	3	16	79	408			
	Sorghum	47	2.0	8	4	NL		32	15			
		Total: 2,350			**Total: 934**		**Total: 80**		**Total: 1618**	**39.8**	**3.4**	**68.8**
WY	Corn	52	52.0	50	26	0	0	98	51			
100	Wheat	48	48.0	0	0	NL		0	0			
		Total: 100			**Total: 26**		**Total: 0**		**Total: 51**	**26.0**	**0.0**	**51.0**

The Plains region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

[1] Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

[2] Column C is obtained by multiplying Column A by Column D.

[3] The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist, Extension Weed Control Specialist, and Monsanto technical personnel in maize (Personal Communications, November, 2003).

[4] Dicamba usage data in Column E except for cotton and soybean is based on 2013 data (Monsanto Company, 2014). Dicamba usage in cotton (50%) and soybean (50%) are future market adoption estimates.

[5] Glufosinate usage data in Column G is based on 2013 data (Monsanto Company, 2014).

[6] Glyphosate usage data in Column I except for alfalfa is based on 2013 data (Monsanto Company, 2014). Glyphosate useage in alfalfa (50%) is a future market adoption estimate.

[7] Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8] Newly seed alfalfa

[9] Vegetables: Chili peppers.

[10] Totals may not be exact due to rounding.

Table 6. Rotational Practices Following Maize Production in the West Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
Region	Corn	735	52.7	50	368	0	0	75	553			
1,395	Cotton	18	1.3	50	9	0	0	93	17			
	Wheat	206	14.8	10	20	NL		6	12			
	Oats	22	1.5	0	0	NL		0	0			
	Barley	64	4.6	2	1	NL		4	3			
	Dry Beans	29	2.1	NL		NL		44	13			
	Potatoes	64	4.6	NL		NL		21	14			
	Alfalfa[8]	168	12.0	NL		NL		50	84			
	Other Hay	56	4.0	NL		NL		NA				
	Safflower	6	0.4	NL		NL		0	0			
	Vegetables[9]	27	1.9	NL		NL		NA				
		Total[10]: 1,395			**Total: 398**		**Total: 0**		**Total: 695**	**28.5**	**0.0**	**49.8**
AZ	Corn	30	35.0	50	15	0	0	0	0			
85	Alfalfa Hay	23	27.0	NL		NL	0	50	11			
	Other Hay	4	5.0	0	0	NL	0	0	0			
	Barley	22	26.0	0	0	NL	0	0	0			
	Vegetables	6	7.0	NL		NL	**0**	NA				
		Total: 85			**Total: 15**		**Total: 0**		**Total: 113**	**17.5**	**0.0**	**13.5**
CA	Corn	286	48.0	50	144	0	0	90	259			
600	Wheat	156	26.0	12	19	NL		7	11			
	Alfalfa Hay	67	11.2	NL		NL		50	34			
	Other Hay	29	4.8	NL		NL		NA				
	Cotton	18	3.0	50	9	0	0	93	17			
	Oats	18	3.0	0	0	NL		0	0			
	Safflower	6	1.0	NL		NL		0	0			
	Vegetables	18	3.0	NL		NL		NA				
					Total: 172		**Total: 0**		**Total:320**	**28.6**	**0.0**	**53.4**

Table 6 (cont.). Rotational Practices Following Maize Production in the West Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
ID	Corn	193	55.0	50	96	0	0	97	187			
350	Wheat	18	5.0	1	0.2	NL		1	0			
	Alfalfa	70	20.0	NL		NL		50	35			
	Other Hay	18	5.0	NL		NL		NA				
	Oats	4	1.0	0	0	NL		0	0			
	Barley	18	5.0	2	0.4	NL		6	1			
	Dry Beans	18	5.0	NL		NL		37	6			
	Potatoes	14	4.0	NL		NL		4	1			
		Total: 350			**Total: 97**		**Total: 0**		**Total: 230**	**27.7**	**0.0**	**65.7**
NV	Corn	4	52.0	50	2	0	0	0	0			
7	Wheat	2	24.0	0	0	NL		0	0			
	Barley	2	24.0	0	0	NL		0	0			
		Total: 7			**Total: 2**		**Total: 0**		**Total: 0**	**26.0**	**0.0**	**0.0**
OR	Corn	53	66.0	50	26	0	0	0	0			
80	Wheat	3	4.0	12	0.4	NL		11	0.4			
	Alfalfa Hay	2	2.2	NL		NL		50	1			
	Other Hay	2	2.8	NL		NL		NA				
	Barley	1	1.0	0	0	NL		26	0.2			
	Vegetables	1	1.0	NL		NL		NA				
	Dry Beans	2	3.0	NL		NL		0	0			
	Potatoes	16	20.0	NL		NL		19	3			
		Total: 80			**Total: 27**		**Total: 0**		**Total: 4**	**33.5**	**0.0**	**5.6**

Table 6 (cont.). Rotational Practices Following Maize Production in the West Region.

A	B	C	D	E	F	G	H	I	J	K	L	M
State/ Total Maize Acres[1]	Rotational Crops Following Maize	Rotational Crop Acres[2]	% Rotational Crop of Total Maize[3]	Dicamba Usage in Rotational Crop[4]		Glufosinate Usage in Rotational Crop[5]		Glyphosate Usage in Rotational Crop[6]		% Usage in Total Rotational Crop Acres[7]		
				%	Acres	%	Acres	%	Acres	Dicamba	Glufosinate	Glyphosate
UT	Corn	43	52.0	50	22	0	0	0	0			
83	Wheat	20	24.0	0	0	NL		0	0			
	Barley	20	24.0	5	1	NL		5	1			
		Total: 83			**Total: 23**		**Total: 0**		**Total:1**	**27.2**	**0.0**	**1.2**
WA	Corn	125	66.0	50	63	0	0	85	107			
190	Wheat	8	4.0	6	0.5	NL		13	1			
	Alfalfa Hay	6	3.1	NL		NL		50	3			
	Other Hay	4	1.9	NL		NL		NA				
	Barley	2	1.0	0	0	NL		25	0.5			
	Vegetables	2	1.0	NL		NL		NA				
	Dry Beans	10	5.0	NL		NL		69	7			
	Potatoes	34	18.0	NL		NL		29	10			
		Total: 190			**Total: 63**		**Total: 0**		**Total: 127**	**33.2**	**0.0**	**67.1**

The West region summary was developed by compiling the data from all the states within the region. Column C, Column F, Column H, and Column J are obtained by compiling data from all the states within this region; Column D is obtained by dividing Column C by Column A, Column E is obtained by dividing Column F by Column C, Column G is obtained by dividing Column H by Column C, Column I is obtained by dividing Column J by Column C. All acreages are expressed as 1000s of acres. NL indicates not labeled for use.

1 Maize acreage based on 2013 planting data (USDA-NASS, 2014b).

2 Column C is obtained by multiplying Column A by Column D.

3 The rotational crop percentages in Column D are based on estimates from individual state Extension Crop Production Specialist, Extension Weed Control Specialist, Monsanto technical personnel in maize (Personal Communications, November, 2003).

4 Dicamba usage data in Column E except for cotton is based on 2013 data (Monsanto Company, 2014). Dicamba usage in cotton (50%) is a future market adoption estimate.

5 Glufosinate usage data in Column G is based on 2013 dat (Monsanto Company, 2014).

6 Glyphosate usage data in Column I except for alfalfa is based on 2013 data (Monsanto Company, 2014). Glyphosate useage in alfalfa (50%) is a future market adoption estimate.

7 Column K is obtained by dividing Column F Total by Column C Total; Column L is obtained by dividing Column H Total by Column C Total, and Column M is obtained by dividing Column J Total by Column C Total.

[8] Newly seed alfalfa
[9] Vegetables: Tomatoes, cantaloupe, watermelons, onions, and chili peppers.
[10] Totals may not be exact due to rounding.

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