RECEIVED
By USDA APHIS BRS Document Control Officer at 2:18 pm, Dec 11, 2019

CBI Deleted

MONSANTO

Petition for the Determination of Nonregulated Status for Dicamba, Glufosinate, Quizalofop and 2,4-Dichlorophenoxyacetic Acid Tolerant MON 87429 Maize with Tissue-Specific Glyphosate Tolerance Facilitating the Production of Hybrid Maize Seed

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

June 27, 2019
(Revised December 10, 2019)

OECD Unique Identifier: MON-87429-9

Monsanto Petition number: CR279-19U4

Submitted by:

David Wilson, Ph.D.

Monsanto Company
700 Chesterfield Pkwy West
St. Louis, MO 63017
Email: david.wilson1@bayer.com

Prepared by:

C. Aradhya, Ph.D., T. Butzlaff, B.S., B. Chen, Ph.D., E. Clawson, Ph.D., T. Edrington, Ph.D., G. Frierdich, B.S., D.A.B.T., M. Graneto, B.A., T. Klusmeyer, Ph.D., D. Wilson, Ph.D., and R. Woo, Ph.D.

Contributors and/or Principal Investigators:

A. M. Adio, Ph.D., E. Bell, Ph.D., E. Dickinson, B.S., R. Dobert, Ph.D., D. Kendrick, M.S., C. Kessenich, M.S., A. Jackson-Gheissari, Ph.D., M. Leibman, Ph.D., M. Malven, M.S., J. Petrick, Ph.D., D.A.B.T., K. A. Robinson, Ph.D., A. Schapaugh, Ph.D., A. Silvanovich, Ph.D., and Q. Tian, M.D., Ph.D.

RELEASE OF INFORMATION

Monsanto[1] 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 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.

© 2019 Bayer Group. All Rights Reserved.

This document is protected under national and international copyright law and treaties. This document and any accompanying material are for use only by the regulatory authority to which it has been submitted by Monsanto Company and its affiliates, collectively "Bayer Group", and only in support of actions requested by Bayer Group. Any other use, copying, or transmission, including internet posting, of this document and the materials described in or accompanying this document, without prior consent of Monsanto Company, is strictly prohibited; except that Monsanto Company hereby grants such consent to the regulatory authority where required under applicable law or regulation. The intellectual property, information and materials described in or accompanying this document are owned by Bayer Group, which has filed for or been granted patents on those materials. By submitting this document and any accompanying materials, Monsanto Company and the Bayer Group do not grant any party or entity any right or license to the information, material or intellectual property described or contained in this submission.

[1] Monsanto Company remains a legal entity holding registrations, permits and other approvals, but it is wholly owned by Bayer. We will let you know about any reorganization of legal entities, including transfer of authorizations/registrations from Monsanto and/or address updates. The names of entities holding registrations should not have any bearing on our approvals. These changes are for legal administrative reasons and have no influence on the quality of the end-use products, grain or seeds and they have no consequences for existing safety evaluations.

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.

David Wilson, Ph.D.
Regulatory Affairs Manager

Address:
Monsanto Company
700 Chesterfield Pkwy W, Mail Stop FF4B
St. Louis, MO 63017

Tel: (636)-737-9554

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 87429, any progeny derived from crosses between MON 87429 and conventional maize, and any progeny derived from crosses of MON 87429 with biotechnology-derived maize that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed herbicide tolerant MON 87429 maize, which is tolerant to the herbicides dicamba, glufosinate, aryloxyphenoxypropionate (AOPP) acetyl coenzyme A carboxylase (ACCase) inhibitors (so called "FOPs" herbicides such as quizalofop) and 2,4-dichlorophenoxyacetic acid (2,4-D). In addition, it provides tissue-specific glyphosate tolerance to facilitate the production of hybrid maize seeds. MON 87429 contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, the phosphinothricin-N-acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes* that expresses the PAT protein to confer tolerance to glufosinate herbicide and the *ft_t* gene, a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase *(Rdpa*) gene from *Sphingobium herbicidovorans*, that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that confers tolerance to quizalofop and 2,4-D herbicides. MON 87429 maize also produces the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to confer tolerance to glyphosate for use in hybrid seed production. MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen. Appropriately timed glyphosate applications produce a non-viable pollen phenotype and allow for desirable cross pollinations to be made in maize without using mechanical or manual detasseling methods to control self-pollination in female inbred parents.

Tissue-specific expression of CP4 EPSPS protein in MON 87429, allowing for glyphosate induced non-viable pollen phenotype, is the second generation of Monsanto's Roundup® Hybridization System (RHS) for hybrid seed production. The first-generation RHS event, MON 87427 maize, was deregulated in 2013 (USDA-APHIS Petition #10-281-01p). The second-generation RHS trait in MON 87429 allows inbred MON 87429 lines, treated with glyphosate at the appropriate timings, to serve as a female parent in the production of hybrid

seed. Female inbred MON 87429 lines receive two glyphosate applications at vegetative growth stages ranging from V8 to V13 that correspond to the time when immature male reproductive tissues are forming. Treatment with glyphosate at these stages results in the intended non-viable pollen phenotype in MON 87429 inbred lines due to tissue-specific glyphosate sensitivity in the immature male reproductive tissue. In hybrid maize production systems, treatment of female inbred MON 87429 plants with glyphosate during the time immature male reproductive tissue is developing will inhibit self-pollination. MON 87429 female inbreds instead will be pollinated by a desired male pollen donor inbred grown in close proximity that contains a deregulated glyphosate tolerance trait, such as NK603. This cross-pollination results in hybrid offspring (e.g., MON 87429 × NK603) with full plant tolerance to glyphosate, both in vegetative and reproductive tissues, as well as tolerance to dicamba, glufosinate, quizalofop, and 2,4-D herbicides.

The RHS trait in MON 87429 maize offers the same benefits to hybrid maize seed production as the RHS trait in MON 87427, described in detail in USDA-APHIS Petition #10-281-01p. Briefly, these benefits include enabling hybrid seed producers to discontinue the practice of manually or mechanically detasseling female inbred plants in their production field, which must occur during a critical 3-4 day time period of maize tassel development, which can be influenced by changes in weather (e.g., extreme heat). The ability to treat MON 87429 maize female inbreds with glyphosate (between V8 to V13), in place of detasseling, provides flexibility to hybrid maize seed producers as well as reduces the cost of hybrid seed production by removing the reliance on costly, labor intensive manual/mechanical detasseling. An additional benefit of including the RHS trait in MON 87429 maize, along with dicamba-, glufosinate-, and quizalofop- and 2,4-D-tolerance traits, is the reduction in the number of trait loci that would otherwise need to be managed and combined, via traditional breeding methods.

The MON 87429 event confers glyphosate tolerance in specific plant tissues (i.e., not in tassels) and will be used to facilitate the production of hybrid seed. MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate tolerance (e.g., NK603). MON 87429 maize combined with the glyphosate-tolerant maize system through traditional breeding will provide growers: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system with multiple herbicide sites-of-action for in-crop application in current maize production systems; 3) an opportunity to delay selection for further resistance to glyphosate and other herbicides that are important in crop production; 4) excellent crop tolerance to dicamba, glufosinate, quizalofop, 2,4-D 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.

MON 87429 maize will offer growers multiple choices for effective weed management including tough-to-control and herbicide-resistant broadleaf and grass weeds. The flexibility to use combinations of dicamba, glufosinate, 2,4-D, quizalofop and glyphosate herbicides representing multiple sites-of-action provides an effective weed management system for maize production. Dicamba provides effective control of over 95 annual and biennial broadleaf weed species, approximately 50 perennial broadleaf species and control or suppression of over 50 woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides effective control of

approximately 70 annual broadleaf weed species, over 30 grass weeds and control or suppression of over 30 biennial and perennial grass and broadleaf weed species. Quizalofop, a selective postemergence herbicide, provides effective control of approximately 35 annual and perennial grass weeds including glyphosate-resistant grasses. 2,4-D provides effective control of over 70 annual broadleaf weed species, and approximately 30 perennial broadleaf species. Glyphosate provides control of approximately 100 annual weed species (grass and broadleaf), over 60 perennial weed species (grass and broadleaf) and control or suppression of approximately 65 woody brush, trees and vines. Additionally, dicamba, glufosinate, and 2,4-D individually or in certain combinations 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*).

Data and Information Presented Confirms the Lack of Plant Pest Potential and the Food and Feed Safety of MON 87429 Compared to Conventional Maize

The data and information presented in this petition demonstrate MON 87429 is agronomically and phenotypically comparable to commercially cultivated maize, with the exception of the introduced traits. Moreover, the data and information presented demonstrate MON 87429 is not expected to pose an increased plant pest risk, including weediness, compared to commercially cultivated maize. The food, feed, and environmental safety of MON 87429 was confirmed based on multiple, well-established lines of evidence:

- Maize is a familiar crop that 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 87429.

- 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 FT_T protein and previous assessments of the DMO, PAT and CP4 EPSPS proteins expressed in MON 87429, confirm they are unlikely to be toxins or allergens.

- An extensive evaluation of MON 87429 phenotypic and agronomic characteristics and environmental interactions demonstrates MON 87429 has no increased plant pest risk 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 87429 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 87429, using current cultivation and management practices, leads to the conclusion that deregulation of MON 87429 is not expected to have an adverse effect on maize agronomic practices.

Maize is a Familiar Crop Lacking Weedy Characteristics

Maize is grown extensively throughout the world and is the largest cultivated grain 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 net value. Maize has been studied extensively, and the initial steps in its domestication can be traced back to approximately 9,000 years ago in southern Mexico. Although grown extensively throughout the world, maize is not considered a threat to invade natural or agricultural ecosystems because it does not establish self-sustaining populations outside of cultivation. This lack of weediness may reflect its poor competitive ability, lack of seed dormancy, and barriers to seed dispersal, as maize cobs retain seed and are covered in a husk. Several other characteristics common in weeds, such as rapid flowering following emergence, are lacking in maize. Traits often associated with weediness are typically not selected for during domestication and subsequent breeding and selection, and similarly, the history of maize breeding and production in the U.S. does not indicate there are any changes in the characteristics of maize that would increase the weediness 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. Maize is not sexually compatible with plant species occurring in the U.S. other than teosinte, an introduced wild relative. However, gene introgression from maize into teosinte is unlikely in the U.S. due to barriers to crossing, including morphological and developmental differences and limited geographical distribution of teosinte populations.

Conventional Maize LH244 is an Appropriate Comparator to MON 87429

Conventional control materials developed for use as comparators in safety assessment studies were based on the type of study conducted and the genetic background of the test material. The conventional control materials included the original transformation line (LH244) and LH244 crossed to a conventional line (HCL617) to create F1 starting control materials. LH244 was used as the control in molecular characterization studies. LH244 × HCL617 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 87429

MON 87429 was produced by *Agrobacterium* mediated-transformation of maize tissue using the T-DNA transformation vector PV-ZMHT519224. This plasmid vector contains a single transfer DNA (T-DNA), that is delineated by Right and Left Border regions. The T-DNA contains the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes. Following transformation, traditional breeding, segregation, selection and screening were used to isolate those plants that contain the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes and do not contain any plasmid backbone sequences.

Characterization of the DNA insert in MON 87429 was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatics. The results of this

characterization demonstrate that MON 87429 contains one copy of the intended T-DNA containing the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes 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 87429 by Next Generation Sequencing (NGS) demonstrated that MON 87429 contains a single intended DNA insert. These whole-genome analyses provided a comprehensive assessment of MON 87429 to determine the presence and identity of sequences derived from PV-ZMHT519224 and demonstrated that MON 87429 contains a single T-DNA insert with no detectable plasmid backbone sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87429 was used to determine the complete sequence of the single DNA insert from PV-ZMHT519224, 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-ZMHT519224 T-DNA. Furthermore, the genomic organization at the insertion site in MON 87429 was assessed by comparing the sequences flanking the T-DNA insert in MON 87429 to the sequence of the insertion site in conventional maize. This analysis determined that 54 bases were deleted upon T-DNA integration. There also was a 29 base insertion in the MON 87429 5′ flanking sequence and a 31 base insertion in the MON 87429 3′ flanking sequence.

- Generational stability analysis by Next Generation Sequencing (NGS) demonstrated that the single PV-ZMHT519224 T-DNA insert in MON 87429 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87429.

- Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA as a single chromosomal locus that shows an expected pattern of inheritance.

Taken together, the characterization of the genetic modification in MON 87429 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 87429.

In addition, MON 87429 maize utilizes an endogenous maize regulatory element (a target sequence for endogenous small interfering RNAs (siRNAs)) to target CP4 EPSPS mRNA for degradation in tassel tissues. A focused study on the MON 87429 siRNA Target Sequence demonstrated the absence of unintended effects on endogenous gene regulation.

Data Confirm DMO, PAT, CP4 EPSPS and FT_T Protein Safety

MON 87429 contains a *dmo* expression cassette that expresses a single MON 87429 DMO precursor protein that is post-translationally processed during the chloroplast targeting process into two forms of the DMO protein; referred to as MON 87429 DMO+1 and MON 87429 DMO+0. MON 87429 DMO+1 is identical to MON 87429 DMO+0 with the exception that it contains an additional amino acid on the N-terminus, a cysteine residue, derived from the alternative processing of the chloroplast transit peptide APG6. Given this degree of similarity, the term MON 87429 DMO protein will be used hereafter to refer to both forms of the protein collectively and distinctions will only be made where necessary. DMO proteins highly similar to those produced in MON 87429 are also present in MON 88701 cotton, MON 87708 soybean and MON 87419 maize which were deregulated by USDA-APHIS in 2015, 2015 and 2016, respectively. MON 88701 cotton, MON 87708 soybean and MON 87419 maize also completed FDA consultation in 2013, 2011 and 2016, respectively, where it was demonstrated that food and feed derived from these crops are not materially different than the respective conventional crops. Data, demonstrating the safety of DMO, were reviewed by U.S. agencies in accordance with the review responsibilities under the Coordinated Framework, resulting in full authorization of these products in the U.S. The safety of DMO protein has been favorably assessed following extensive reviews by regulatory agencies in at least 12 different countries. Although there are minor differences in amino acid sequence, the DMO proteins expressed in MON 87429 are identical to previously reviewed DMO proteins in terms of structure of the catalytic site, function, immunoreactivity, and substrate specificity. Thus, prior safety assessments of DMO proteins are applicable to the DMO protein expressed in MON 87429.

The PAT protein in MON 87429 has the same sequence as PAT protein produced in several other commercially available crops that have been reviewed by USDA and previously deregulated (e.g., T25, DAS-59122-7 and TC1507 maize and A2704-12 and A5547-127 soybean). The safety of PAT proteins has been confirmed following extensive reviews by regulatory agencies in at least 15 different countries for more than 30 biotechnology-derived events in several different crop species (e.g., maize, soybean, cotton, canola and sugar beet). The lack of any documented reports of adverse effects of PAT-containing crops since their commercial introduction further confirms the safety of the PAT protein. The amino acid sequence of the PAT protein expressed in MON 87429 is identical to the wild type PAT protein encoded by *S. viridochromogenes* except for the first methionine, which is removed due to co-translational processing in MON 87429. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins. Thus, prior safety assessments of PAT proteins are applicable to the PAT protein expressed in MON 87429.

The CP4 EPSPS protein in MON 87429 has the same sequence as CP4 EPSPS proteins produced in several other commercially available crops that have been reviewed by USDA and previously deregulated (e.g., 40-3-2 and MON 89788 soybean, NK603, MON 87427 and MON 88017 maize, RT73 canola, H7-1 sugar beet, 1445 and MON 88913 cotton, and J101 and J163 alfalfa). The safety and mode-of-action of CP4 EPSPS proteins is well documented and is the subject of numerous publications. Additionally, in 1996 the U.S. EPA established an exemption from the requirement of a tolerance for residues of the plant pesticide inert ingredient CP4 EPSPS and the genetic material necessary for its production in all plants (40 CFR § 174.523, redesignated from § 180.1174, effective April 25, 2007). The safety of the CP4 EPSPS protein as expressed in

MON 88017 and MON 87427 maize has also been reviewed and approved in at least 12 countries. Thus, prior safety assessments of CP4 EPSPS protein are applicable to the CP4 EPSPS protein expressed in MON 87429.

A multistep approach was used to characterize and assess the safety of the FT_T protein expressed in MON 87429 resulting from the genetic modification. The expression level of the FT_T protein in selected tissues of MON 87429 was determined and potential for exposure to humans and animals was assessed. In addition, the donor species for the FT_T protein coding sequences, *Sphingobium* species, are ubiquitous in the environment and are not commonly known for human or animal pathogenicity or allergenicity. Bioinformatics analysis determined that the FT_T protein lacks structural similarity to known allergens, gliadins, glutenins, or protein toxins. The FT_T protein is rapidly digested in pepsin and pancreatin and demonstrates no acute oral toxicity in mice at a dose level that far exceeds anticipated exposure by humans and animals. Hence, the consumption of the FT_T protein from MON 87429 or its progeny poses no meaningful risk to human and animal health.

MON 87429 is Compositionally Equivalent to Conventional Maize

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process 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. Maize is not known to have any endogenous toxicants or antinutrients associated with overall plant pest potential. For MON 87429, the introduced proteins, DMO, PAT, CP4 EPSPS and FT_T, confer herbicide tolerance and lack catalytic activity that is intended to or expected to affect the plant's metabolism. Given the nature of these introduced traits and the overall lack of meaningful unintended compositional characteristics observed for biotechnology-derived products characterized to date, compositional changes that would affect the levels of nutritional components in MON 87429 maize were not expected.

Monsanto is currently in consultation with the Food and Drug Administration following their policy, "Foods Derived from New Plant Varieties," on the food and feed safety of MON 87429 maize (Submitted 05-Feb-2019). Composition data for 25 components including major nutrients in grain and forage and anti-nutrients in grain was submitted to FDA as part of the voluntary food/feed safety and nutritional assessment for MON 87429 maize. The results of the compositional assessment found that there were no biologically meaningful differences between MON 87429 and conventional control and support the conclusion that MON 87429 maize is compositionally equivalent to the conventional control.

MON 87429 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 interactions assessment of MON 87429 included the genetically similar conventional control as a comparator. This evaluation used a weight of evidence approach and considered statistical differences between MON 87429 and the conventional control with respect to reproducibility, magnitude, and directionality. 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. Assessments included seed germination and dormancy characteristics and pollen characteristics in the laboratory as well as phenotypic and agronomic characteristics and plant responses to abiotic stressors, diseases, and arthropod pests in the field. Results from the phenotypic, agronomic, and environmental interactions assessment indicated that MON 87429 does not possess enhanced weediness characteristics, increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests, or characteristics that would confer an increased plant pest risk compared to conventional maize.

MON 87429 Will Not Negatively Affect Non-Target Organisms (NTOs) Including Those Beneficial to Agriculture

An evaluation of the impacts of MON 87429 on non-target organisms (NTOs) is a component of the plant pest risk assessment. Because MON 87429 does not possess pesticidal activity, all organisms that interact with MON 87429 are considered to be NTOs. Data from the 2017 U.S. phenotypic and agronomic studies, including observational data on plant response to abiotic stressors, diseases, and arthropod pests, were collected for MON 87429 and conventional controls. Results from these studies support conclusions that MON 87429 is unlikely to adversely affect NTOs.

The DMO, PAT, and CP4 EPSPS proteins have been assessed in multiple products by USDA-APHIS and U.S. FDA in the past years. DMO protein is produced in both MON 87708 soybean, MON 88701 cotton and MON 87419 maize that were granted nonregulated status by USDA-APHIS. Starting in 1996 with Bayer's T25 maize, numerous glufosinate tolerant crops (canola, cotton, maize, soybean, sugar beet) containing PAT proteins have been granted nonregulated status by USDA-APHIS. CP4 EPSPS protein is also produced in numerous other glyphosate tolerant crops (soybean, maize, cotton, sugar beet, canola, and alfalfa) that have been granted nonregulated status by USDA-APHIS. Assessment of the FT_T proteins non-toxic mode-of-action (MOA), lack of acute oral toxicity, and lack of impact on plant pest potential supports a conclusion that the FT_T protein will not have adverse impacts to NTOs. In addition, after either extensive testing and/or wide scale commercial cultivation, in no instance have adverse impacts to NTOs been associated with exposure to DMO, PAT, or CP4 EPSPS proteins from these biotechnology-derived crops.

The biochemical information and experimental data for evaluation of MON 87429 included mode of action, molecular characterization, DMO, PAT, CP4 EPSPS and FT_T protein safety assessment, establishment of compositional equivalence to conventional maize, data from the environmental interactions assessment, and demonstration of agronomic and phenotypic equivalence to conventional maize. Taken together, these data support the conclusion that MON 87429 has no plausible mechanism for harm to 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 87429 is Not Expected to Have Effects on Maize Agronomic Practices

An assessment of current maize agronomic practices was conducted to determine whether the cultivation of MON 87429 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 87429 has been developed to offer maize growers multiple choices for effective weed management including tough to control and herbicide resistant broadleaf and grass weeds. The combination of dicamba, glufosinate, quizalofop and 2,4-D tolerance offers multiple herbicide sites-of-actions and provides an effective weed management system for maize production in the U.S. Dicamba, glufosinate, quizalofop, and 2,4-D herbicides are currently labeled for preplant and postemergence applications in maize. In support of Monsanto's third generation herbicide tolerant MON 87419 maize, deregulated by USDA in 2016, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first, without reducing the application rate of dicamba (U.S. EPA, 2019). The combined (pre- and post-emergence) maximum annual application rate of dicamba in MON 87419 maize would be 2.0 lbs. a.e. dicamba per acre per year, increased from the current maximum annual application rate of 0.75 lbs. a.e. in conventional maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. Glufosinate, quizalofop and 2,4-D, are currently labeled for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on herbicide-tolerant hybrids. Use of glufosinate, quizalofop and 2,4-D over the top of MON 87429 will follow the current labeled use patterns of these individual herbicides. The MON 87429 event confers glyphosate tolerance in specific plant tissues (i.e., not in tassels) and will be used to facilitate the production of hybrid seed. MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate tolerance (e.g., NK603). The introduction of MON 87429 stacked with a deregulated glyphosate tolerance trait will follow the current labeled use patterns of glyphosate herbicide. The introduction of MON 87429 maize is not expected to have adverse impacts on current agronomic, cultivation and management practices for maize. No meaningful changes are anticipated in crop rotations, tillage practices, planting practices, fertility management, weed and disease management, and volunteer management from the introduction of MON 87429.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87429 is unlikely to pose a plant pest risk. Therefore, Monsanto Company requests a determination from USDA-APHIS that MON 87429 and any progeny derived from crosses between MON 87429 and conventional maize or deregulated biotechnology-derived maize be granted nonregulated status under 7 CFR Part 340.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND DEFINITIONS[2]

αKG	alpha-ketoglutarate
2,4-D	2,4-Dichlorophenoxyacetic Acid
ACCase	Acetyl Coenzyme A Carboxylase
APHIS	Animal and Plant Health Inspection Service
aroA	5-enolpyruvylshikimate-3-phosphate synthetase gene from *Agrobacterium* sp. strain CP4
CAB	Chlorophyll a/b-Binding
CaMV	Cauliflower Mosaic Virus
CFR	Code of Federal Regulations
CP4 EPSPS	*Agrobacterium tumefaciens* strain CP4, 5-enolpyruvlshikimate-3-phosphate synthase protein
CTP	Chloroplast Transit Peptide
CTAB	Hexadecyltrimethylammonium Bromide
CV	Column Volume
BIO	Biotechnology Industry Organization
BRS	Biotechnology Regulatory Service
DAP	Days After Planting
DCSA	3,6-Dichlorosalycilic Acid
DMO	Dicamba Mono-Oxygenase
DNA	Deoxyribonucleic Acid
dw	Dry Weight
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELN	Electronic Lab Notebook
EPA	Environmental Protection Agency
E-score	Expectation Score
ETS	Excellence Through Stewardship
FDA	Food and Drug Administration (U.S.)
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FOPs	aryloxyphenoxypropionate acetyl coenzyme A carboxylase inhibitor herbicides such as quizalofop
FT_T	FOPs and 2,4-D Dioxygenase Protein
GLP	Good Laboratory Practice
IAC	Immunoaffinity Chromatography
IAM	Iodoacetamide
kb	Kilobase
LOQ	Limit of Quantitation

[2] 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.

ABBREVIATIONS AND DEFINITIONS (continued)

MBu	Million bushels
Mdh	malate dehydrogenase gene from *Arabidopsis thaliana*
MOA	Mode of Action
mRNA	Messenger RNA
mts-siRNA	Male Tissue Specific Small Interfering RNA
MW	Molecular Weight
NGS	Next Generation Sequencing
OECD	Organization for Economic Co-operation and Development
OPA	o-phthalaldehyde
ORF	Open Reading Frame
OSL	Over Season Leaf
PAT	Phosphinothricin N–Acetyltransferase
PCR	Polymerase Chain Reaction
RdpA	R-2,4-Dichlorophenoxypropionate Dioxygenase
RHS	Roundup® Hybridization System
S3P	Shikimate-3phoshate
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
SE	Standard Error
ShkG	5-enolpyruvylshikimate-3-phosphate synthase from *Arabidopsis thaliana*
siRNA	Small Interfering RNA
SOA	Site-of-action
SOP	Standard Operating Procedure
µg/g	Microgram per Gram
USDA	United States Department of Agriculture
WT	Wildtype

I. RATIONALE FOR THE DEVELOPMENT OF MON 87429

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. 106-2a24, 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 87429, any progeny derived from crosses between MON 87429 and conventional maize, and any progeny derived from crosses of MON 87429 with biotechnology-derived maize that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Herbicide Tolerant Maize MON 87429

Maize (*Zea mays* L.) is the largest crop grown in the U.S. in terms of acreage planted and net value. Planted maize acres in the U.S. have ranged between 88 to 94 million acres from 2015 to 2018 (USDA-NASS, 2018a). 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 an herbicide application (USDA-NASS, 2019b).

MON 87429 maize will offer growers multiple choices for effective weed management including tough-to-control and herbicide-resistant broadleaf and grass weeds. The flexibility to use dicamba, glufosinate, quizalofop, 2,4-D and glyphosate and/or combinations of these five herbicides representing multiple sites-of-action will provide an effective weed management system for maize production. Dicamba provides effective control of over 95 annual and biennial broadleaf weed species, approximately 50 perennial broadleaf species and control or suppression of over 50 woody plant species. Glufosinate, a broad-spectrum contact herbicide, provides effective control of approximately 70 annual broadleaf weed species and over 30 grass weed species and control or suppression of over 30 biennial and perennial grass and broadleaf weed

species. Quizalofop, a selective postemergence herbicide, provides effective control of approximately 35 annual and perennial grass weed species including glyphosate-resistant grasses. 2,4-D provides effective control of over 70 annual and biennial broadleaf weed species, and approximately 30 perennial broadleaf weed species. Glyphosate provides control of approximately 100 annual weed species (grass and broadleaf), over 60 perennial weed species (grass and broadleaf) and control or suppression of approximately 65 woody brush, trees and vines. Additionally, dicamba, glufosinate, and 2,4-D individually or in certain combinations 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*), johnson grass (*Sorghum halepense)* and waterhemp (*Amaranthus tuberculatus*).

The MON 87429 event confers glyphosate tolerance in specific plant tissues (i.e., not in tassels) and will be used to facilitate the production of hybrid seed. MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate tolerance (e.g., NK603). MON 87429 maize combined with glyphosate-tolerant maize through traditional breeding will provide: 1) an opportunity for an efficient, effective weed management system for hard-to-control and herbicide-resistant weeds; 2) a flexible system with multiple herbicide sites-of-action for in-crop application in current maize production systems; 3) an opportunity to delay selection for further resistance to glyphosate and other herbicides that are important in crop production; and 4) excellent crop tolerance to dicamba, glufosinate, quizalofop, 2,4-D and glyphosate. These enhanced weed management tools will maintain or improve maize yield and quality to meet the growing needs of the food, feed, and industrial markets.

Maize differs from other major U.S. crops, such as soybean or cotton, in that it is typically planted as a hybrid, and maize hybrids are utilized on nearly all maize production acres currently planted in the U.S. The seed supply used to plant the U.S. maize acreage is produced via hybrid seed production. The volume of seed planted in the U.S., to produce hybrid maize seed, has increased from 22.45 million bushels (MBu) planted in 2000 to 30.90 MBu planted in 2018 (USDA-ERS, 2018a). Hybrid seed production is accomplished through the combining of genetic material from one inbred parent with that of the other inbred parent. Specifically, pollen from the tassel (male flower) of the male parent is used to fertilize the ear (female flower) of the female parent. One challenge inherent to the production of hybrid maize seed is that the female parent produces pollen at the same time as the male parent. Therefore, pollen from the female parent must be removed or eliminated in order to assure uni-directional transfer of genetic material, via pollen, only from the male parent to the female parent. In the past, removal or elimination of the pollen from the female parent required deployment of costly, labor intensive and time-critical methods such as manual or mechanical detasseling, or maintenance of Cytoplasmic Male Sterile lines, to ensure quality hybrid seed was produced. These hybrid seed production methods are further detailed in MON 87427 USDA-APHIS Petition #10-281-01p (p. 128), Monsanto's first-generation

Roundup® Hybridization System (RHS) for hybrid seed production, which was assessed and deregulated by USDA-APHIS in 2013.

Tissue-specific expression of CP4 EPSPS protein in MON 87429, allowing for glyphosate induced non-viable pollen phenotype, is the second-generation of Monsanto's Roundup® Hybridization System (RHS) for hybrid seed production. The first-generation RHS event, MON 87427 maize, was assessed by USDA-APHIS and deregulated in 2013 (USDA-APHIS Petition #10-281-01p). The second-generation RHS trait in MON 87429 allows inbred MON 87429 lines, treated with glyphosate at the appropriate timings, to serve as a female parent in the production of hybrid seed, similar to previously deregulated MON 87427 maize. Female inbred MON 87429 lines receive two glyphosate applications at vegetative growth stages ranging from V8 to V13, that correspond to the time when immature male tissues are forming. Treatment with glyphosate during this time period, when male tissue is forming, results in the intended non-viable pollen phenotype due to tissue-specific glyphosate sensitivity in the immature male tissue (See Section IV.G).

To produce commercial hybrid maize seed with whole plant glyphosate tolerance using the RHS hybrid seed production system, female inbred MON 87429 plants treated with glyphosate during immature male tissue development will be pollinated by pollen donor plants (male inbred) that contain a deregulated glyphosate tolerance trait (e.g., NK603). The male inbred plants will be cultivated in proximity to the female inbred MON 87429 plants and will not be impacted by the RHS-required glyphosate applications, due to full glyphosate tolerance in all tissues. This cross-pollination results in hybrid offspring (e.g., MON 87429 × NK603) with full tolerance to glyphosate in both vegetative and reproductive tissues, as well as tolerance to dicamba, glufosinate, quizalofop, and 2,4-D herbicides. For weed control in hybrid seed production fields with MON 87429 maize inbred used as female parent, in-crop applications of glyphosate may be made at the same rates and timings as directed on glyphosate agricultural product labels for other glyphosate tolerant maize events.

The RHS trait in MON 87429 maize offers the same benefits to hybrid maize seed production as the RHS trait in MON 87427, described in detail in USDA-APHIS Petition #10-281-01p (p. 5). Briefly, these benefits include enabling hybrid seed producers to discontinue the practice of manually or mechanically detasseling female inbred plants in their production field, which must occur during a critical 3-4 day time period of maize tassel development, which can be influenced by changes in weather (e.g., extreme heat). The ability to treat MON 87429 maize female inbreds with glyphosate (between V8 to V13), in place of detasseling, provides flexibility to hybrid maize seed producers as well as reduces the cost of hybrid seed production by removing the reliance on costly, labor intensive manual/mechanical detasseling. An additional benefit of including the RHS trait in MON 87429 maize, along with dicamba-, glufosinate-, and quizalofop- and 2,4-D-tolerance traits, is the reduction in the number of trait loci that would otherwise need to be managed and combined, via traditional breeding methods.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology (CFR) (USDA-APHIS, 1986), the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of plant incorporated protectants (PIPs), the Environmental Protection Agency (EPA). Deregulation of MON 87429 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87429 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions. As MON 87429 does not contain a PIP, no submission on MON 87429 will be made to the EPA.

I.C.1. Submission to FDA

MON 87429 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 has initiated a consultation with the FDA, identified under BNF No. 000173. A food/feed safety and nutritional assessment summary document was submitted to the FDA in February 2019.

I.C.2. Related Submissions to U.S. EPA

Substances that are pesticides, as defined under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA; 7 U.S.C. §136(u)), are subject to regulation by U.S. EPA. When registering the use of herbicides, U.S. EPA performs a thorough risk assessment including acute, chronic, and aggregate risks for the U.S. population, including setting safe limits for pesticide residues including parent molecules and metabolites. In each case a determination of safety is made that no harm will result to the general population or to infants and children from the aggregate exposure of the herbicide.

MON 87429 provides improved crop tolerance to dicamba, and the ability to extend use of dicamba through the V8 growth stage will provide more effective preemergence and postemergence control of problem weed species compared to currently labeled applications of dicamba in conventional maize hybrids. In support of Monsanto's third generation herbicide tolerant product MON 87419 maize, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first (U.S. EPA, 2019). The combined (pre- and post-emergence) maximum annual application rate of dicamba in MON 87419 maize would be 2.0 lbs. a.e. dicamba per acre per year, increased from the current maximum annual application rate of 0.75 lbs. a.e. in conventional maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419.

Glufosinate use over the top of MON 87429 will not change from current approved use pattern of glufosinate[3]. Glufosinate is labeled for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on glufosinate-tolerant hybrids only.

U.S. EPA has registered the use of 2,4-D on maize[4] up to 1 lb. preemergence and one to two applications of up to 1 lb. between emergence and V8 or 30 inches whichever occurs first. Monsanto, or a partner at our behest, will request the U.S. EPA to register the same already approved use pattern in maize for use on MON 87429 maize.

U. S. EPA has registered the use of quizalofop[5] on maize up to 0.083 lb from V2 to V6 stage of development. Monsanto will request the U.S. EPA to register the same already approve use pattern in maize for use on MON 87429 maize.

Glyphosate use over the top of MON 87429 will follow the current approved use pattern of glyphosate[6]. Glyphosate is labeled for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on glyphosate-tolerant hybrids only.

I.C.3. Submissions to Foreign Government Agencies

Consistent with our commitments to the Biotechnology Industry Organization (BIO) and Excellence Through Stewardship® (ETS)[7], Monsanto will meet applicable regulatory requirements for MON 87429 in the country of intended production and for major import countries, as 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.

[3] Federal Register, 77 FR 59106-59113, Glufosinate Ammonium, Pesticide Tolerances

[4] Federal Register, 82 FR 9523-9529, 2,4-D; Pesticide Tolerances

[5] Federal Register, 83 FR 7111-7115, Quizalofop ethyl; Pesticide Tolerances

[6] Federal Register, 76 FR 27268-27271, Glyphosate; Pesticide Tolerances

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

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

II. THE BIOLOGY OF MAIZE

The biology of maize has been well documented (Anderson and de Vicente, 2010; Farnham et al., 2003; OGTR, 2008). In addition to the wealth of information on maize biology, the Organisation for Economic Co-operation and Development authored a Consensus Document (OECD, 2003) on the biology of maize. This document provides key information regarding:

- 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
- summary of the ecology of maize

Additional information on the biology and uses of maize can also be found on the Australian Government Department of Health and Ageing (Office of the Gene Technology Regulator) web site (OGTR, 2008), and in the USDA-ARS GRIN database (USDA-ARS, 2015). The taxonomic information for maize is available in the USDA's PLANTS Profile (USDA-NRCS, 2019).

To support the evaluation of the plant pest potential of MON 87429 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 widely grown in nearly all areas of the world and is the largest grain crop in the world, ahead of both wheat (*Triticum sp.*) and rice (*Oryza sativa* L.), in total metric ton production (FAOSTAT, 2019). In the 2016/2017 marketing year, world maize area was approximately 194 million hectares (ha) with a total grain production of approximately 1,122 million metric tons (MMT) (USDA-FAS, 2018). The top five production regions were: USA (385 MMT), China (264 MMT), Brazil (99 MMT), the European Union (62 MMT), and Argentina (41 MMT) (USDA-FAS, 2018). In the U.S., maize is grown in most states and in 2017, its production value of over $49 billion was the highest of any crop (USDA-NASS, 2019a).

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 familiar plant that has been rigorously studied due to its use as a staple food/feed and the economic opportunity it brings to growers. Archeological and genetic evidence suggests that maize domestication began in southern Mexico approximately 9,000 years ago, and that it was derived from Balsas teosinte, *Zea mays* subsp. *parviglumis* (Kistler et al., 2018; Matsuoka et al., 2002; Piperno et al., 2009). Although grown extensively throughout the world, maize is not considered a threat to invade natural or agricultural ecosystems. Maize does not establish self-sustaining populations outside of cultivation (Crawley et al., 2001; OECD, 2003; Raybould et al., 2012). This lack of weediness may reflect its poor competitive ability (Olson and Sander, 1988), lack of seed dormancy, and barriers to seed dispersal, as maize cobs retain seed and are covered in a husk (Wilkes, 1972). A number of other characteristics common in weeds, such as rapid flowering following emergence, are lacking in maize (Keeler, 1989). Today, the majority of U.S. maize acreage is planted to hybrids, a practice that started in the 1920s (Wych, 1988). Maize hybrids have advantages in yield 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 obtained by intra- and inter-specific crossing, mutation breeding or utilization of other traditional breeding methodologies. However, there is a limit to the 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 traits that are highly desirable to growers and downstream crop users.

II.B. Characteristics of the Recipient Plant

The MON 87429 transformation 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 of Stiff Stalk background that is best adapted to the central regions of the U.S. corn belt.

Following transformation of immature LH244 embryos, a single transformed plant was selected and self-pollinated to increase seed supplies. An inbred line homozygous for MON 87429 was selected and then used to produce other MON 87429 materials for product testing, safety assessment studies, and commercial hybrid development. The non-transformed LH244 was used to produce conventional maize comparators (hereafter referred to as conventional controls) in the safety assessment of MON 87429.

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

In studies utilizing hybrid maize, the test is a hybrid of LH244 (MON 87429 expressing DMO, PAT, FT_T and CP4 EPSPS proteins) × HCL617 unless otherwise noted (Figure IV-4). LH244 × HCL617 was used as near isogenic, conventional control for this submission (hereafter referred to as conventional control). LH244 was used as the conventional control in molecular characterization studies. LH244 × HCL617 was used as the conventional control in compositional analysis and in phenotypic, agronomic and environmental interactions assessments. Where appropriate conventional commercial maize hybrids (hereafter referred to as reference hybrids) were used to establish ranges of natural variability or responses 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.

To conduct the studies reported in this petition, appropriate MON 87429 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 R3 generation (Figure IV-4). Protein characterization and expression analysis, composition analysis, and phenotypic, agronomic and environmental interactions assessment were conducted with MON 87429 breeding generation R3F1.

III. DESCRIPTION OF THE GENETIC MODIFICATION

This section provides a description of the transformation process and plasmid vector used in the development of MON 87429 (Figure III-1 and Figure III-2). Molecular analyses are an integral part of the characterization of maize products with new traits introduced by methods of biotechnology. Vectors and methods are selected for transformation to achieve high probability of obtaining the trait of interest and integration of the introduced DNA into a single locus in the plant genome. This helps ensure that only the intended DNA encoding the desired trait(s) is integrated into the plant genome and facilitates the molecular characterization of the product. Information provided here allows for the identification of the genetic material present in the transformation vector delivered to the host plant and for an analysis of the data supporting the characterization of the DNA inserted in the plant found in Section IV.

III.A. Description of Transformation Plasmid PV-ZMHT519224

Plasmid vector PV-ZMHT519224 was used in the transformation of maize to produce MON 87429 and its plasmid map is shown in Figure III-2. A detailed description of the genetic elements and their prefixes (e.g., B, P, L, I, TS, CS, T, and OR) in PVZMHT519224 is provided in Table III-1. Plasmid vector PV-ZMHT519224 is approximately 17.8 kb in length and contains a single T-DNA that is delineated by Right and Left Border regions. The T-DNA contains the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes. During transformation, the T-DNA was inserted into the maize genome. Following transformation, traditional breeding, segregation, selection and screening were used to isolate those plants that contained the *pat*, *dmo*, *ft_t*, and *cp4 epsps* expression cassettes and did not contain the backbone sequences.

The *pat* expression cassette contains the following genetic elements: promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Erianthus ravennae* (plume grass), the *pat* gene from *Streptomyces viridochromogenes,* and the 3' UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet). The *dmo* expression cassette contains the following genetic elements: Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Coix lacryma-jobi* (adlay millet), chloroplast-targeting sequence of the *Albino and pale green 6* (*Apg6)* gene from *Arabidopsis thaliana*, the *dmo* gene from *Stenotrophomonas maltophilia,* and the 3' UTR sequence of the *OsMt* gene from *Oryza sativa* (rice). The *ft_t* expression cassette contains the following genetic elements: promoter, 5' UTR, and intron sequences for a *ubiquitin* gene (*Ubq*) from *Arundo donax* (giant reed), chloroplast-targeting sequence malate dehydrogenase (*Mdh)* gene from *Arabidopsis thaliana*, the *ft_t* gene from *Sphingobium herbicidovorans,* and the 3' UTR sequence from the gene coding for a no apical meristem (*Nam*) protein domain from *Oryza sativa* (rice). The *cp4 epsps* expression cassette contains the following genetic elements: promoter and leader sequence from the 35S RNA of cauliflower mosaic virus (CaMV), 5' UTR leader sequence from the gene coding for chlorophyll a/b-binding (*CAB*) protein of *Triticum aestivum* (wheat), intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice), chloroplast-targeting sequence of the 5-enolpyruvylshikimate-3-

phosphate synthase (*ShkG*) gene from *Arabidopsis thaliana*, the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4, and 3' UTR sequence of *Zea mays* cDNA (Genbank Accession: EU974548) that contains male tissue specific siRNA target sequence, and 3' UTR sequence of the glycine-rich RNA binding protein (*Grp3*) gene from *Oryza sativa* (rice).

The backbone region of PV-ZMHT519224 contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori V, ori pBR322*), and a bacterial selectable marker gene (*aadA*).

III.B. Description of the Transformation System

MON 87429 was developed through *Agrobacterium tumefaciens* mediated transformation of immature maize embryos based on the method described by (Sidorov and Duncan, 2009) utilizing PV-ZMHT519224. 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 (R0) 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 87429.

The R0 plants generated through the transformation process described above had already been exposed to glyphosate in the selection medium and demonstrated glyphosate tolerance. The R0 plants were self-pollinated to produce R1 seed. Subsequently, the R1 population was screened for the presence of T-DNA and absence of vector backbone sequences by construct-level PCR assay and Southern blot analysis. Only plants that were homozygous positive for T-DNA and negative for vector backbone were selected for further development and their progenies were subjected to further molecular and phenotypic assessments. As is typical of a commercial event production and selection process, hundreds of different transformation events (regenerants) were generated in the laboratory using PV-ZMHT519224. After careful selection and evaluation of these events in the laboratory, greenhouse and field, MON 87429 was selected as the lead event based on superior trait efficacy, agronomic, phenotypic, and molecular characteristics (Prado et al., 2014). Studies on MON 87429 were initiated to further characterize the genetic insertion and the expressed product, and to establish the food, feed, and environmental safety relative to conventional maize. The major steps involved in the development of MON 87429 are depicted in Figure III-1. The result of this process was the production of MON 87429 maize with the *pat, dmo, ft_t* and *cp4 epsps* expression cassettes.

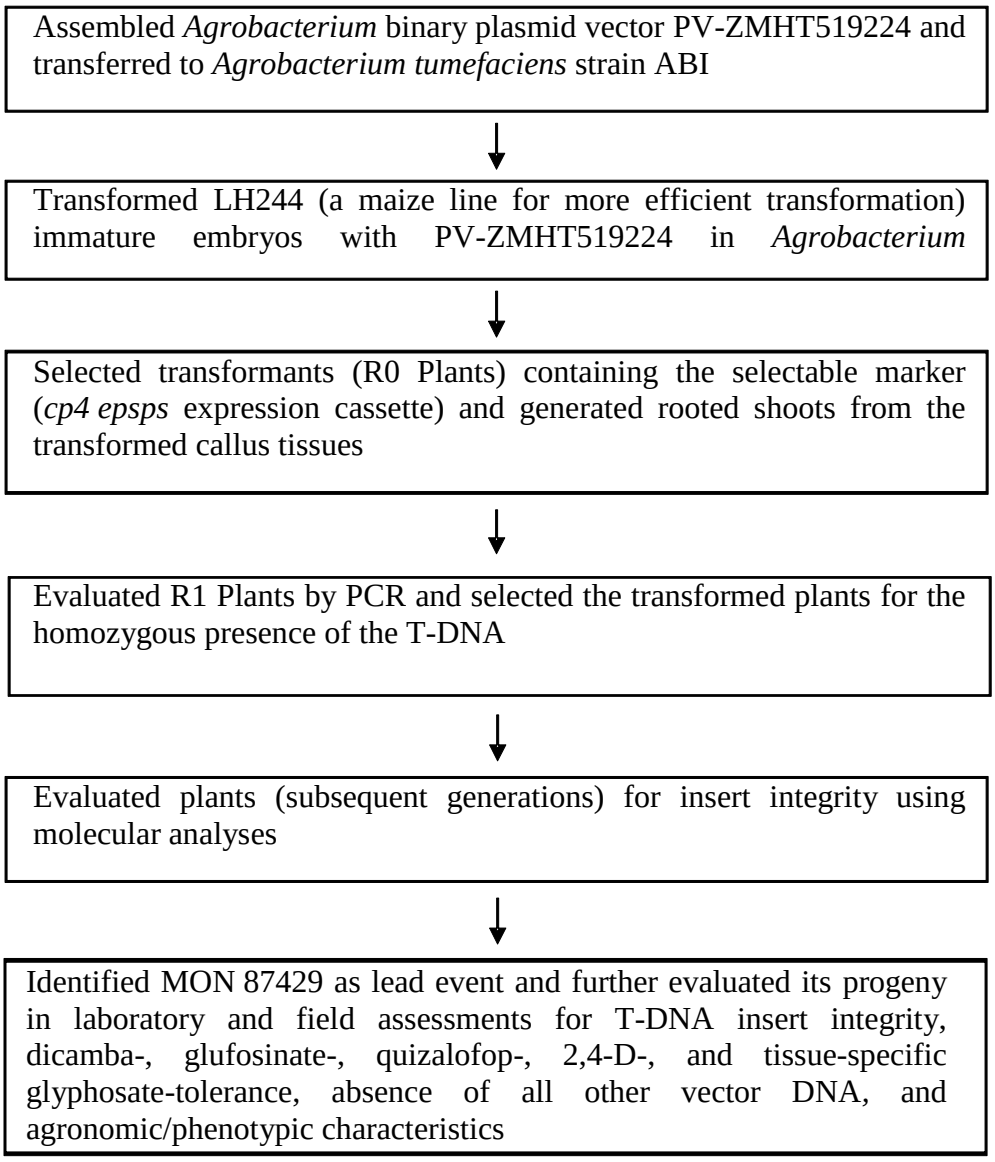


Figure III-1. Schematic of the Development of MON 87429

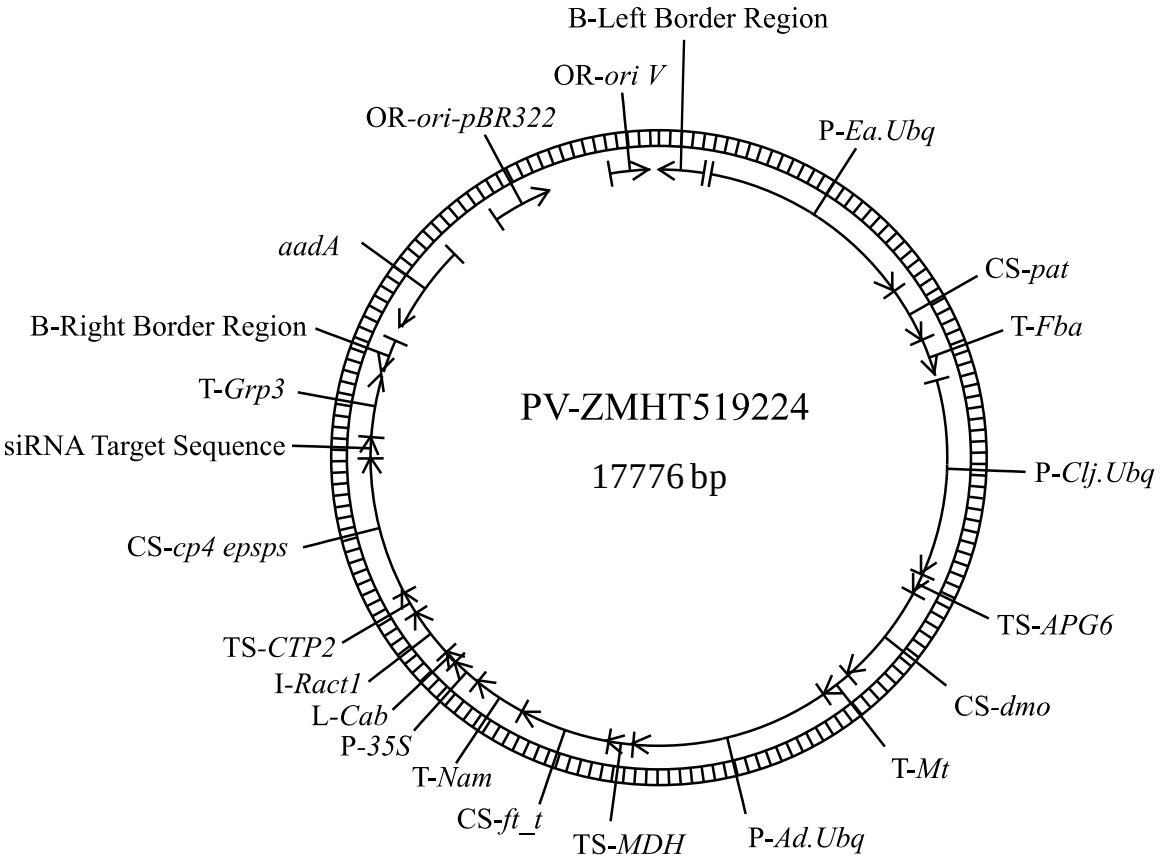


Figure III-2. Circular Map of PV-ZMHT519224

A circular map of PV-ZMHT519224 used to develop MON 87429 is shown. PV-ZMHT519224 contains one T–DNA. Genetic elements are shown on the exterior of the map.

Table III-1. Summary of Genetic Elements in PV-ZMHT519224

Genetic Element	Location in Plasmid Vector	Function (Reference)
T-DNA		
B[1]-Left Border Region	1-442	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983)
Intervening Sequence	443-513	Sequence used in DNA cloning
P[2]-*Ea.Ubq*	514-2695	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Erianthus ravennae* (plume grass) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	2696-2700	Sequence used in DNA cloning
CS[3]-*pat*	2701-3252	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)
T[4]-*Fba*	3253-3629	3' UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet) that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	3630-3691	Sequence used in DNA cloning
P-*Clj.Ubq*	3692-5617	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Coix lacryma-jobi* (adlay millet) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	5618-5627	Sequence used in DNA cloning

Table III-1. Summary of Genetic Elements in PV-ZMHT519224 (continued)

TS[5]-*APG6*	5628-5831	Codon optimized targeting sequence of the *Albino and pale green 6* (*Apg6)* gene from *Arabidopsis thaliana* encoding a chloroplast-targeted Hsp101 homologue transit peptide region that directs the protein to the chloroplast (GenBank Accession: NM_121549)
CS-*dmo*	5832-6854	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	6855-6862	Sequence used in DNA cloning
T-*Mt*	6863-7162	3' UTR sequence of the *OsMt* gene from *Oryza sativa* (rice) encoding metallothionein-like protein that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	7163-7170	Sequence used in DNA cloning
P-*Ad.Ubq*	7171-9127	Promoter, 5' UTR, and intron sequences for a *ubiquitin* gene (*Ubq*) from *Arundo donax* (giant reed) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	9128-9140	Sequence used in DNA cloning
TS-*MDH*	9141-9383	Targeting sequence from *Arabidopsis thaliana Mdh* gene encoding the malate dehydrogenase transit peptide region that directs the protein to the chloroplast (GenBank Accession: BT000621)
CS-*ft_t*	9384-10271	Modified version of R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa)* gene from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that confers tolerance to quizalofop and 2,4-D herbicides (Müller et al., 2006)

Table III-1. Summary of Genetic Elements in PV-ZMHT519224 (continued)

Intervening Sequence	10272-10286	Sequence used in DNA cloning
T-*Nam*	10287-10803	3' UTR sequence from the gene coding for a no apical meristem (*Nam*) protein domain containing protein from *Oryza sativa* (rice) (Hunt, 1994)
Intervening Sequence	10804-10809	Sequence used in DNA cloning
P-*35S*	10810-11133	Promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) that directs transcription in plant cells
Intervening Sequence	11134-11155	Sequence used in DNA cloning
L[6]-*Cab*	11156-11216	5' UTR leader sequence from the gene coding for chlorophyll a/b-binding (*CAB*) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression (Lamppa et al., 1985)
Intervening Sequence	11217-11232	Sequence used in DNA cloning
I[7]-*Ract1*	11233-11712	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	11713-11721	Sequence used in DNA cloning
TS-*CTP2*	11722-11949	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987)
CS-*cp4 epsps*	11950-13317	Codon optimized coding sequence of the 5-enolpyruvylshikimate-3-phosphate synthetase (*aroA*) gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	13318-13323	Sequence used in DNA cloning
siRNA Target Sequence	13324-13524	Modified partial 3' UTR sequence of *Zea mays* cDNA (Genbank Accession: EU974548) that contains male tissue specific siRNA target sequence (Brodersen and Voinnet, 2006)

Table III-1. Summary of Genetic Elements in PV-ZMHT519224 (continued)

Intervening Sequence	13525-13532	Sequence used in DNA cloning
T-*Grp3*	13533-14143	3' UTR sequence of the glycine-rich RNA binding- protein (*Grp3*) gene from *Oryza sativa* (rice) encoding the GRP3 protein that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	14144-14184	Sequence used in DNA cloning
B-Right Border Region	14185-14515	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)
Vector Backbone		
Intervening Sequence	14516-14659	Sequence used in DNA cloning
aadA	14660-15548	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	15549-16082	Sequence used in DNA cloning
OR[8]-*ori-pBR322*	16083-16671	Origin of replication from plasmid pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	16672-17293	Sequence used in DNA cloning
OR-*ori V*	17294-17690	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	17691-17776	Sequence used in DNA cloning

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

III.C. The *pat* Coding Sequence and PAT Protein

The *pat* expression cassette contains the *pat* gene encoding a protein of 183 amino acids (Figure III-3). MON 87429 expresses a 25.5 kDa PAT protein, which consists of a single polypeptide of 182 amino acids after the removal of the lead methionine that is cleaved during a co-translational process (Wehrmann et al., 1996; Wohlleben et al., 1988). The *pat* open reading frame in the expression cassette includes sequence from *S. viridochromogenes* that encodes the PAT protein. The expression of PAT protein confers glufosinate tolerance.

```
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-3. Deduced Amino Acid Sequence of the PAT Protein

The amino acid sequence of the MON 87429 PAT protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT519224 (See Table III-1 for more detail). The lead methionine (boxed with solid line) of the PAT protein produced in MON 87429 is cleaved during a co-translational process in MON 87429.

III.D. The *dmo* Coding Sequence and DMO Protein

The *dmo* expression cassette contains the *dmo* gene. The *dmo* expression cassette encodes a precursor protein of 408 amino acids (340 amino acids encoded by the *dmo* gene and 68 amino acids encoded by the *APG6* gene for targeting the DMO protein into chloroplasts) (Figure III-4). MON 87429 expresses two forms of mature DMO protein due to alternative processing of the chloroplast transit peptide (CTP). One form consists of 341 amino acids, which includes 340 amino acids encoded by the *dmo* gene and 1 amino acid (cysteine) encoded by the *APG6* gene. The other form of the DMO protein consists of 340 amino acids encoded by the *dmo* gene. The two forms of mature DMO protein expressed in MON 87429 are indistinguishable by Coomassie stain of SDS-PAGE and western blot analysis because the difference in molecular weight between these two forms is very small. Therefore, only a ~38.4 kDa DMO protein band 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 the DMO protein confers tolerance to dicamba herbicide.

```
1    MATATTTATA AFSGVVSVGT ETRRIYSFSH LQPSAAFPAK PSSFKSLKLK
51   QSARLTRRLD HRPFVVRCML TFVRNAWYVA ALPEELSEKP LGRTILDTPL
101  ALYRQPDGVV AALLDICPHR FAPLSDGILV NGHLQCPYHG LEFDGGGQCV
151  HNPHGNGARP ASLNVRSFPV VERDALIWIW PGDPALADPG AIPDFGCRVD
201  PAYRTVGGYG HVDCNYKLLV DNLMDLGHAQ YVHRANAQTD AFDRLEREVI
251  VGDGEIQALM KIPGGTPSVL MAKFLRGANT PVDAWNDIRW NKVSAMLNFI
301  AVAPEGTPKE QSIHSRGTHI LTPETEASCH YFFGSSRNFG IDDPEMDGVL
351  RSWQAQALVK EDKVVVEAIE RRRAYVEANG IRPAMLSCDE AAVRVSREIE
401  KLEQLEAA
```

Figure III-4. Deduced Amino Acid Sequence of APG6 Chloroplast Targeting Sequence and the DMO Protein

The amino acid sequence of the MON 87429 DMO precursor protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT519224 (See Table III-1 for more detail). The first 68 amino acids of the precursor protein (underlined) are the CTP from *APG6* gene. The CTP targets MON 87429 DMO precursor protein to the chloroplast and is partially cleaved in the chloroplast producing the mature 341 amino acid and 340 amino acid DMO proteins that begin with the cysteine at position 68 and methionine at position 69, respectively. The double underline shows the cysteine amino acid from APG6.

III.E. The *ft_t* Coding Sequence and FT_T Protein

The *ft_t* expression cassette contains the *ft_t* gene. The *ft_t* expression cassette encodes a precursor protein of 376 amino acids (295 amino acids encoded by the *ft_t* gene and 81 amino acids encoded by the *MDH* gene for targeting the FT_T protein into chloroplasts) (Figure III-5). MON 87429 expresses a ~36 kDa mature FT_T protein, which consists of a single polypeptide of 296 amino acids, 295 amino acids are encoded by the *ft_t* gene and 1 amino acid (alanine) is encoded by *MDH* gene due to the processing of the chloroplast transit peptide (CTP). The *ft_t* open reading frame in the expression cassette is the modified version of R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa)* gene from *Sphingobium herbicidovorans* that encodes a FOPs and 2,4-D dioxygenase protein (FT_T) (Müller et al., 2006). The expression of FT_T protein confers tolerance to quizalofop and 2,4-D herbicides.

```
1    MATATSASLF STVSSSYSKA SSIPHSRLQS VKFNSVPSFT GLKSTSLISG
51   SDSSSLAKTL RGSVTKAQTS DKKPYGFKIN AMHAALTPLT NKYRFIDVQP
101  LTGVLGAEIT GVDLREPLDD STWNEILDAF HTYQVIYFPG QAITNEQHIA
151  FSRRFGPVDP VPILKSIEGY PEVQMIRREA NESSRFIGDD WHTDSTFLDA
201  PPAAVVMRAI EVPEYGGDTG FLSMYSAWET LSPTMQATIE GLNVVHSATK
251  VFGSLYQATN WRFSNTSVKV MDVDAGDRET VHPLVVTHPV TGRRALYCNQ
301  VYCQKIQGMT DAESKSLLQF LYEHATKFDF TCRVRWKKDQ VLVWDNLCTM
351  HRAVPDYAGK FRYLTRTTVA GDKPSR
```

Figure III-5. Deduced Amino Acid Sequence of MDH Chloroplast Targeting Sequence and the FT_T Protein

The amino acid sequence of the MON 87429 FT_T precursor protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT519224 (See Table III-1 for more detail). The first 81 amino acids of the precursor protein (underlined) are the CTP from *MDH* gene. MDH targets FT_T protein to the chloroplast and is cleaved in the chloroplast producing the mature 296 amino acid FT_T protein that begins with the alanine at position 81. The double underline shows the alanine amino acid from MDH that is the N-terminus of the mature FT_T protein.

III.F. The *cp4 epsps* Coding Sequence and CP4 EPSPS Protein

The *cp4 epsps* expression cassette contains the *cp4 epsps* gene. The *cp4 epsps* expression cassette encodes a precursor protein of 531 amino acids (455 amino acids encoded by the *cp4 epsps* gene and 76 amino acids encoded by the *CTP2* gene for targeting the CP4 EPSPS protein into chloroplasts) (Figure III-6). MON 87429 expresses a 44 kDa CP4 EPSPS protein, consisting of a single polypeptide of 455 amino acids starting at the methionine position 77 (Padgette et al., 1996) after a complete cleavage of the chloroplast transit peptide (CTP2). The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS

(Barry et al., 2001; Padgette et al., 1996). The CP4 EPSPS protein is similar and functionally equivalent to endogenous plant EPSPS enzymes, but has a much-reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Barry et al., 2001; Padgette et al., 1996). The presence of this protein renders the plant tolerant to glyphosate.

```
1    MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL

51   LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA

101  TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD

151  RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR

201  DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF

251  PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED

301  VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL

351  RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT

401  HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS

451  DTKAA
```

Figure III-6. Deduced Amino Acid Sequence of the CP4 EPSPS Protein

The amino acid sequence of the MON 87429 CP4 EPSPS protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT519224 (See Table III-1 for more detail). Another mature form of CP4 EPSPS protein containing 454 amino acids, resulting from the cleavage of the lead methionine (boxed with dash line) was also observed.

III.G. Regulatory Sequences

The *pat* coding sequence in MON 87429 is under the regulation of the promoter, 5' untranslated region (UTR) and intron for a ubiquitin gene (*Ubq*) from *Erianthus ravennae* (plume grass) that direct transcription in plant cells (Cornejo et al., 1993). The *pat* coding sequence also utilizes the 3′ UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet) that directs polyadenylation of mRNA (Hunt, 1994).

The *dmo* coding sequence in MON 87429 is under the regulation of the promoter, 5' UTR and intron for a ubiquitin gene (*Ubq*) from *Coix lacryma-jobi* (adlay millet) that direct transcription in plant cells (Cornejo et al., 1993). The *dmo* coding sequence utilizes a codon optimized targeting sequence of the *Albino and pale green 6* (*Apg6)* gene from *Arabidopsis thaliana* encoding a chloroplast-targeted Hsp101 homologue transit peptide region that directs transport of the DMO protein to the chloroplast (GenBank Accession: NM_121549). The *dmo* coding sequence also utilizes the 3' UTR sequence of the *OsMt* gene from *Oryza sativa* (rice) encoding metallothionein-like protein that directs polyadenylation of mRNA (Hunt, 1994).

The *ft_t* coding sequence in MON 87429 is under the regulation of the promoter, 5' UTR, and intron for a *ubiquitin* gene (*Ubq*) from *Arundo donax* (giant reed) that directs transcription in plant cells (Cornejo et al., 1993). The *ft_t* coding sequence utilizes a chloroplast targeting sequence from *Arabidopsis thaliana Mdh* gene encoding the malate dehydrogenase transit peptide region that directs transport of the FT_T protein to the chloroplast (GenBank Accession: BT000621). The *ft_t* coding sequence also utilizes the 3' UTR sequence from the gene coding for a no apical meristem (*Nam*) protein domain containing protein from *Oryza sativa* (rice) that directs polyadenylation of mRNA (Hunt, 1994).

The *cp4 epsps* coding sequence in MON 87429 is under the regulation of the promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) that direct transcription in plant cells. The *cp4 epsps* coding sequence also utilizes the 5' UTR leader sequence from the gene coding for chlorophyll a/b-binding (CAB) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression (Lamppa et al., 1985), and the intron and flanking UTR sequence of the *act1* gene from *Oryza sativa* (rice) encoding rice Actin 1 (*Ract1*) protein that is also involved in regulating gene expression (McElroy et al., 1990). The *cp4 epsps* coding sequence utilizes a chloroplast targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the CP4 EPSPS protein to the chloroplast (Herrmann, 1995; Klee et al., 1987). The *cp4 epsps* coding sequence is regulated by a modified partial 3' UTR sequence of *Zea mays* cDNA (Genbank Accession: EU974548) that contains a target sequence recognized by endogenous male tissue specific siRNAs (Brodersen and Voinnet, 2006), which suppresses *cp4 epsps* gene expression in maize male tissue (Yang et al., 2018). The *cp4 epsps* coding sequence also utilizes a 3' UTR sequence of the glycine-rich RNA-binding protein (*Grp3*) gene from *Oryza sativa* (rice) encoding the GRP3 protein that directs polyadenylation of mRNA (Hunt, 1994).

III.H. T-DNA Border Regions

PV-ZMHT519224 contains Left and Right Border regions (Figure III-2 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 87429.

III.I. Genetic Elements Outside the T-DNA Border Regions

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-ZMHT519224 in bacteria and are referred to as plasmid backbone. The selectable marker, *aadA* is the coding sequence for an aminoglycoside-modifying enzyme, 3"(9) –*O*–nucleotidyltransferase from the transposon Tn*7* (Fling et al., 1985) that confers spectinomycin and streptomycin resistance in *E. coli* and *Agrobacterium* for use in molecular cloning. 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). The origin of replication, *ori V*, is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host range plasmid RK2 from *Agrobacterium* (Stalker et al., 1981). Because these elements are outside the T-DNA 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 87429 was confirmed by sequencing and bioinformatic analyses (Section IV).

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

This section describes the methods and results of a comprehensive molecular characterization of the genetic modification present in MON 87429. It provides information on the DNA insertion(s) into the plant genome of MON 87429, and additional information regarding the arrangement and stability of the introduced genetic material. The information provided in this section addresses the relevant factors in Codex Plant Guidelines, Section 4, paragraphs 30, 31, 32, and 33 (Codex Alimentarius, 2009).

IV.A. Description of Methodology Used to Characterize MON 87429

A schematic representation of the next generation sequencing (NGS) methodology and the basis of the characterization using NGS and PCR sequencing are illustrated in Figure IV-1 below. Appendix B defines the test, control and reference substances, and provides an additional overview of these techniques, their use in DNA characterization in maize plants and the materials and methods.

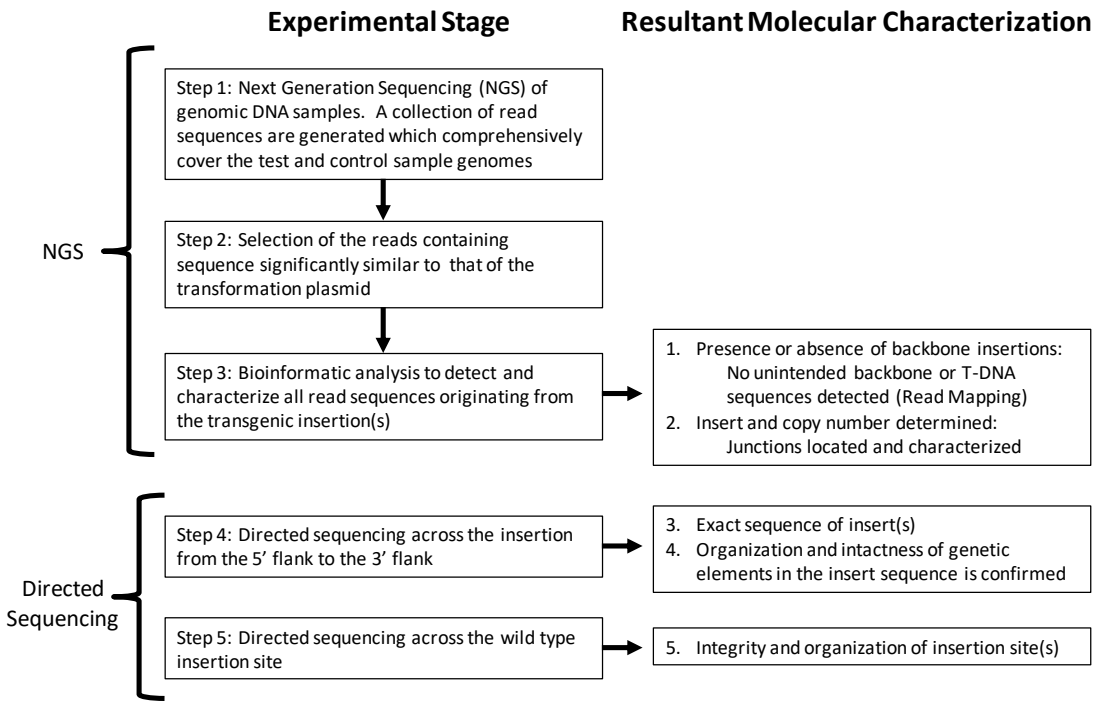


Figure IV-1. Molecular Characterization using Sequencing and Bioinformatics

Genomic DNA from MON 87429 (Test) and the conventional control was sequenced using technology that produces a set of short, randomly distributed sequence reads that comprehensively cover test and control genomes (Step 1). Utilizing these genomic sequence reads, bioinformatics searches are conducted to identify all sequence reads that are significantly similar to the transformation plasmid (Step 2). These identified captured reads are then mapped and analyzed to determine the presence/absence of transformation plasmid backbone sequences, identify insert junctions, and to determine the insert and copy number (Step 3). Using directed sequencing, overlapping PCR products are also produced which span any insert and the wild type insertion locus (Step 4 and Step 5 respectively); these overlapping PCR products are sequenced to allow for detailed characterization of the inserted DNA and insertion site.

The NGS method was used to characterize the genomic DNA from MON 87429 and the conventional control by generating short (~150 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 whole genome sequencing at 75× depth of coverage is adequate to provide comprehensive coverage and ensure detection of inserted DNA (Kovalic et al., 2012). To confirm sufficient sequence coverage of the genome, the 150 bp sequence reads are analyzed to determine the coverage of a known single-copy endogenous maize gene. This establishes the depth of coverage (the median number of times each base of the genome is independently sequenced). The level of sensitivity of this method was demonstrated by detection of a positive control plasmid DNA spiked at 1 and $1/10^{th}$ copy-per-genome equivalent. This confirms 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 analyzed in depth to determine the number of DNA inserts. NGS was run on five breeding generations of MON 87429 and the appropriate conventional controls. Results of NGS are shown in Sections IV.B and IV.E.

The DNA inserts of MON 87429 were characterized by mapping of sequencing reads to the transformation plasmid and identifying junctions and unpaired read mappings adjacent to the junctions. Examples of five types of NGS reads are shown in Figure IV-2. The junctions of the DNA insert and the flanking DNA are unique for each insertion (Kovalic et al., 2012). Therefore, insertion sites can be recognized by analyzing for sequence reads containing such junctions.

Directed sequencing (locus-specific PCR and DNA sequencing analyses, Figure IV-1, Step 4) complements the NGS method. It assesses the sequence identity of the insert relative to the corresponding sequence from the T-DNA in PV-ZMHT519224, and demonstrates that each genetic element in the insert was intact without rearrangement. It also characterizes the flank sequence beyond the insert corresponding to the genomic DNA of MON 87429. Directed sequencing results are described in Sections IV.B, IV.C and IV.D; methods are presented in Appendix B.

The stability of the T-DNA present in MON 87429 across multiple breeding generations was evaluated by NGS as described above by determining the number and identity of the DNA inserts in each generation. For a single copy T-DNA insert, two junction sequence classes are expected. In the case of an event where a single insertion 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.E; methods are presented in Appendix B.

Segregation analysis of the T-DNA was conducted to determine the inheritance and generational stability of the insert in maize. Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the genetic behavior of the T-DNA. Results are described in Section IV.F.

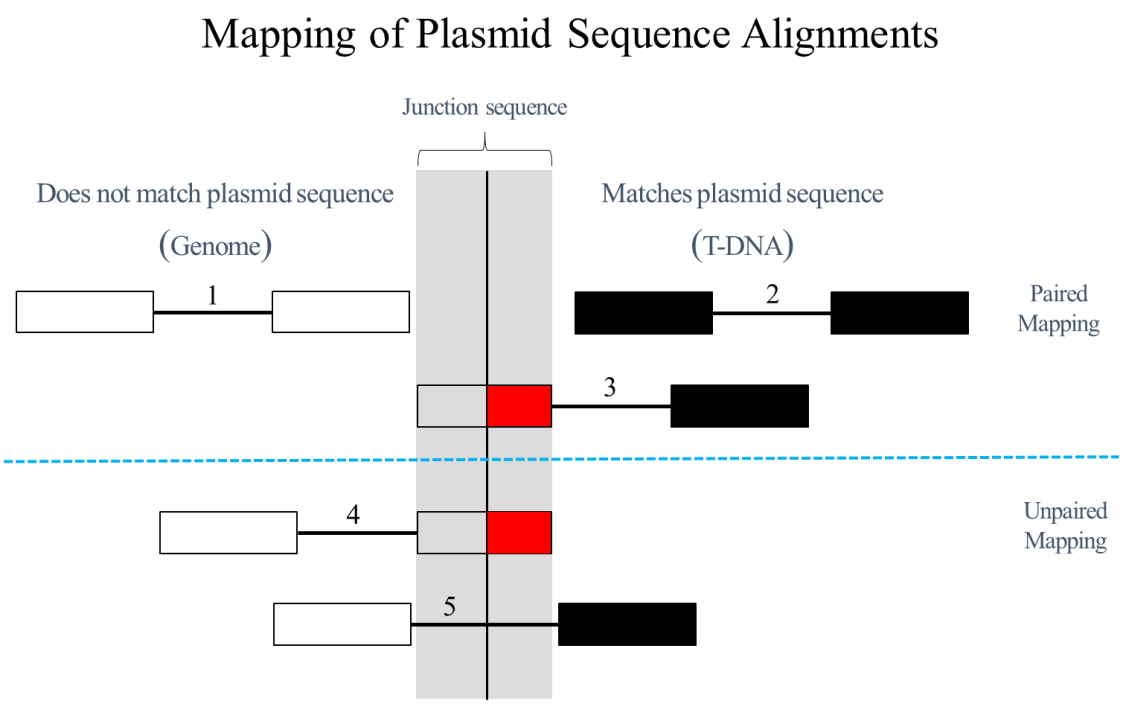


Figure IV-2. Five Types of NGS Reads

NGS yields data in the form of read pairs where sequence from each end of a size selected DNA fragment is returned. Depicted above are five types of sequencing reads/read pairs generated by NGS sequencing which can be found spanning or outside of junction points. Sequence boxes are filled red or black if it matches with plasmid sequence, and empty if it matches with genomic sequence. Grey highlighting indicates sequence reads spanning the junction. Junctions are detected by examining the NGS data for reads having portions of plasmid sequences that span less than the full read, as well as reads mapping adjacent to the junction points where their mate pair does not map to the plasmid sequence. The five types of sequencing reads/read pairs being (1) Paired and unpaired reads mapping to genomic sequence outside of the insert, greater than 99.999% of collected reads fall into this category and are not evaluated in this analysis, (2) Paired reads mapping entirely to the transformation plasmid sequence, such reads reveal the presence of transformation related sequence *in planta*, (3) Paired reads where one read maps entirely within the inserted DNA and the other read maps partially to the insert (indicating a junction point), (4) Single read mapping partially to the transformation plasmid DNA sequence (indicating a junction point) where its mate maps entirely to the genomic flanking sequence and (5) Single read mapping entirely to the transformation plasmid DNA sequence where its mate maps entirely to genomic flanking sequence, such reads are part of the junction signature.

IV.B. Characterization of the DNA Insert in MON 87429

The number of inserted DNA sequences from PV-ZMHT519224 in MON 87429 was assessed by generating a comprehensive collection of reads via NGS of MON 87429 genomic DNA using the R3 generation. A plasmid map of PV-ZMHT519224 is shown in Figure III-2. Table IV-1 provides descriptions of the genetic elements present in MON 87429. A schematic representation of the insert and flanking sequences in MON 87429 is shown in Figure IV-3. For full details on materials and methods see Appendix B.

IV.B.1. Next Generation Sequencing for MON 87429 and Conventional Control Genomic DNA

Genomic DNA from five breeding generations of MON 87429 (Figure IV-4) and conventional controls were isolated from seed and prepared for sequencing. For material and method details see Appendix B. These genomic DNA libraries were used to generate short (~150 bp) randomly distributed sequencing reads of the maize genome (Figure IV-1, Step 1).

To demonstrate sufficient sequence coverage the 150 bp sequence reads were analyzed by mapping all reads to a known single copy endogenous gene (*Zea mays* pyruvate decarboxylase (*pdc3*), GenBank Accession: 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 86× or greater for the five generations of MON 87429 (R3, R3F1, R4, R4F1, and R5) and the conventional control (Appendix B, Table B-1). It has been previously demonstrated that whole genome sequencing at 75× depth of coverage provides comprehensive coverage and ensures detection of inserted DNA (Kovalic et al., 2012).

To demonstrate the method's ability to detect any sequences derived from the PV-ZMHT519224 transformation plasmid, a sample of conventional control genomic DNA spiked with PV-ZMHT519224 DNA was analyzed by NGS and bioinformatics. The level of sensitivity of this method was demonstrated to a level of one genome equivalent and 1/10th genome equivalent, 100% nucleotide identity was observed over 100% of PV-ZMHT519224 (Appendix B, Table B-2). This result demonstrates that all nucleotides of PV-ZMHT519224 are observed by the sequencing and bioinformatic assessments performed and that a detection level of at least 1/10th genome equivalent was achieved for the plasmid DNA sequence assessment.

Table IV-1. Summary of Genetic Elements in MON 87429

Genetic Element[1]	Location in Sequence[2]	Function (Reference)
5' Flanking DNA	1-1029	DNA sequence flanking the 5' end of the insert
B[3]-Left Border Region[r1]	1030-1288	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983)
Intervening Sequence	1289-1359	Sequence used in DNA cloning
P[4]-*Ea.Ubq*	1360-3541	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Erianthus ravennae* (plume grass) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	3542-3546	Sequence used in DNA cloning
CS[5]-*pat*	3547-4098	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)
T[6]-*Fba*	4099-4475	3' UTR sequence of the *fructose-bisphosphate aldolase* (*Fba*) gene from *Setaria italica* (foxtail millet) that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	4476-4537	Sequence used in DNA cloning
P-*Clj.Ubq*	4538-6463	Promoter, 5' UTR, and intron sequences for a ubiquitin gene (*Ubq*) from *Coix lacryma-jobi* (adlay millet) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	6464-6473	Sequence used in DNA cloning
TS[7]-*APG6*	6474-6677	Codon optimized targeting sequence of the *Albino and pale green 6* (*Apg6)* gene from *Arabidopsis thaliana* encoding a chloroplast-targeted Hsp101 homologue transit peptide region that directs the protein to the chloroplast (GenBank Accession: NM_121549)

Table IV-1. Summary of Genetic Elements in MON 87429 (continued)

CS-*dmo*	6678-7700	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	7701-7708	Sequence used in DNA cloning
T-*Mt*	7709-8008	3' UTR sequence of the *OsMt* gene from *Oryza sativa* (rice) encoding metallothionein-like protein that directs polyadenylation of mRNA (Hunt, 1994)
Intervening Sequence	8009-8016	Sequence used in DNA cloning
P-*Ad.Ubq*	8017-9973	Promoter, 5' UTR, and intron sequences for a *ubiquitin* gene (*Ubq*) from *Arundo donax* (giant reed) that directs transcription in plant cells (Cornejo et al., 1993)
Intervening Sequence	9974-9986	Sequence used in DNA cloning
TS-*MDH*	9987-10229	Targeting sequence from *Arabidopsis thaliana Mdh* gene encoding the malate dehydrogenase transit peptide region that directs the protein to the chloroplast (GenBank Accession: BT000621)
CS-*ft_t*	10230-11117	Modified version of R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa)* gene from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase protein (FT_T) that confers tolerance to quizalofop and 2,4-D herbicides (Müller et al., 2006)
Intervening Sequence	11118-11132	Sequence used in DNA cloning
T-*Nam*	11133-11649	3' UTR sequence from the gene coding for a no apical meristem (*Nam*) protein domain containing protein from *Oryza sativa* (rice) (Hunt, 1994)
Intervening Sequence	11650-11655	Sequence used in DNA cloning

Table IV-1. Summary of Genetic Elements in MON 87429 (continued)

P-*35S*	11656-11979	Promoter and leader from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) that directs transcription in plant cells
Intervening Sequence	11980-12001	Sequence used in DNA cloning
L[8]-*Cab*	12002-12062	5' UTR leader sequence from the gene coding for chlorophyll a/b-binding (*CAB*) protein of *Triticum aestivum* (wheat) that is involved in regulating gene expression (Lamppa et al., 1985)
Intervening Sequence	12063-12078	Sequence used in DNA cloning
I[9]-*Ract1*	12079-12558	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	12559-12567	Sequence used in DNA cloning
TS-*CTP2*	12568-12795	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987)
CS-*cp4 epsps*	12796-14163	Codon optimized coding sequence of the 5-enolpyruvylshikimate-3-phosphate synthetase (*aroA*) gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides glyphosate tolerance (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	14164-14169	Sequence used in DNA cloning
siRNA Target Sequence	14170-14370	Modified partial 3' UTR sequence of *Zea mays* cDNA (Genbank Accession: EU974548) that contains male tissue specific siRNA Target Sequence (Brodersen and Voinnet, 2006)
Intervening Sequence	14371-14378	Sequence used in DNA cloning
T-*Grp3*	14379-14989	3' UTR sequence of the glycine-rich RNA binding- protein (*Grp3*) gene from *Oryza sativa* (rice) encoding the GRP3 protein that directs polyadenylation of mRNA (Hunt, 1994)

Table IV-1. Summary of Genetic Elements in MON 87429 (continued)

Intervening Sequence	14990-15030	Sequence used in DNA cloning
B-Right Border Region[r1]	15031-15037	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)
3' Flanking DNA	15038-16068	Flanking DNA

[1] Although flanking sequences and intervening sequences are not functional genetic elements, they comprise a portion of the sequence.
[2] Numbering refers to the sequence of the insert in MON 87429 and adjacent DNA
[3] B, Border
[4] P, Promoter
[5] CS, Coding Sequence
[6] T, Transcription Termination Sequence
[7] TS, Targeting Sequence
[8] L, Leader
[9] I, Intron
[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87429 was truncated compared to the sequences in PV-ZMHT519224

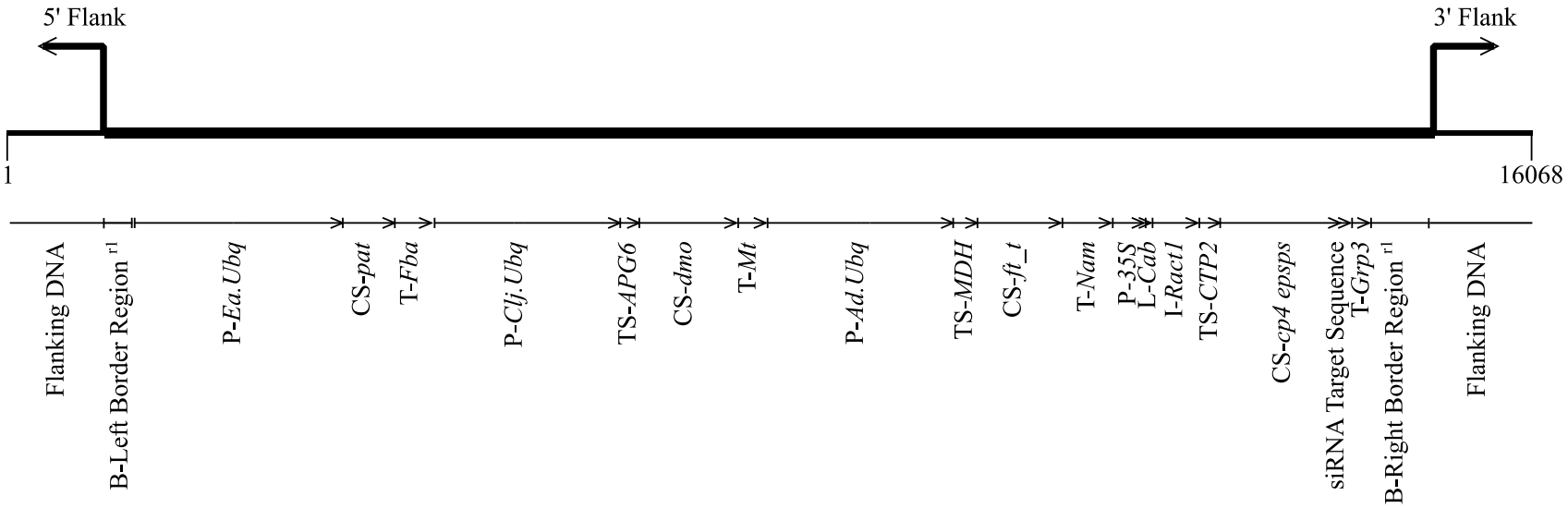


Figure IV-3. Schematic Representation of the Insert and Flanking Sequences in MON 87429

DNA derived from T-DNA of PV-ZMHT519224 integrated in MON 87429. 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 may not be drawn to scale.

r1 Superscript in Left and Right Border Regions indicate that the sequence in MON 87429 was truncated compared to the sequences in PV-ZMHT519224.

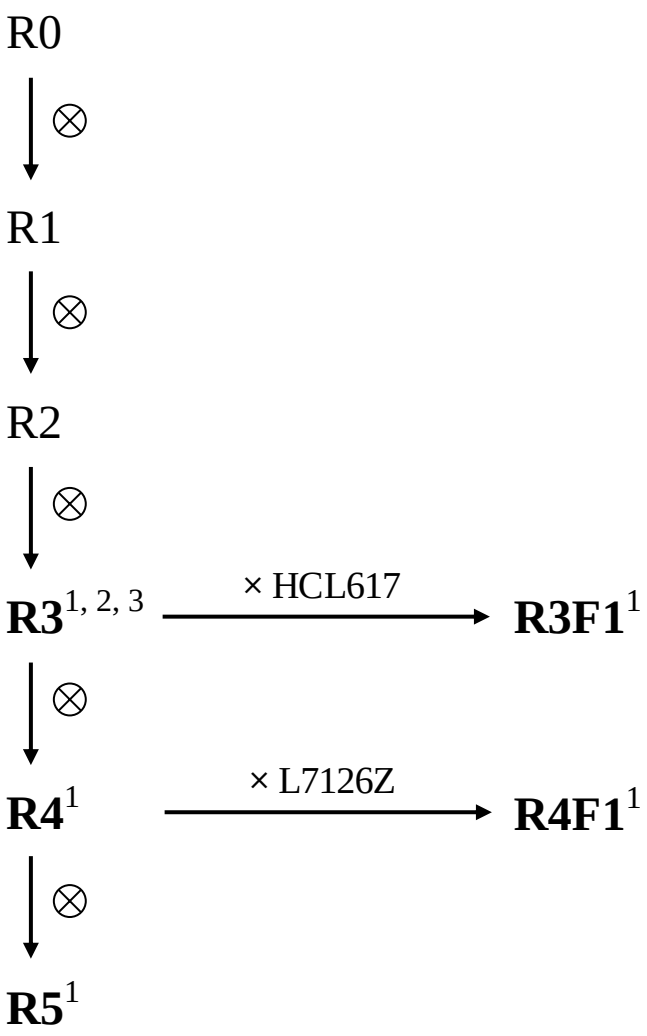


Figure IV-4. Breeding History of MON 87429

The generations used for molecular characterization and insert stability analyses are indicated in bold text. R0 (LH244) corresponds to the transformed plant, ⊗ designates self-pollination.

[1]Generations used to confirm insert stability

[2] Generation used for molecular characterization

[3] Generation used for commercial development of MON 87429

IV.B.2. Selection of Sequence Reads Containing Sequence of the PV-ZMHT519224

The transformation plasmid, PV-ZMHT519224 was transformed into the parental variety LH244 to produce MON 87429. Consequently, any DNA inserted into MON 87429 will consist of sequences that are similar to the PV-ZMHT519224 DNA sequence. Therefore, to fully characterize the DNA from PV-ZMHT519224 inserted in MON 87429, it is sufficient to analyze only the sequence reads that have significant similarity to PV-ZMHT519224 (Figure IV-1, Step 2).

Using established criteria (described in the materials and methods, Appendix B), sequence reads similar to PV-ZMHT519224 were selected from MON 87429 sequence datasets and were then used as input data for bioinformatic junction sequence analysis. PV-ZMHT519224 sequences were also compared against the conventional control sequence dataset.

IV.B.3. Determination of T-DNA Copy Number and Presence or Absence of Plasmid Vector Backbone

By mapping sequence reads to the transformation plasmid sequence and identifying junction signatures, the presence or absence of backbone sequence and the number of T-DNA insertions can be determined. For a single copy T-DNA insert sequence at a single genomic locus, a single junction signature pair and few if any reads aligning with the transformation plasmid backbone sequences are expected.

When reads from conventional maize LH244 were aligned with the transformation plasmid sequence, a number of reads mapped to the T-DNA element siRNA Target Sequence (Figure IV-5, see Panels 1 and 2; Panel 3 illustrates the read depth). The sequence alignments were expected since the siRNA Target Sequence in the T-DNA is derived from endogenous maize genomic sequence. Notably, the sequence alignment is isolated to that element alone and does not cross the element boundaries. No other sequence reads from LH244 conventional maize mapped to the transformation plasmid. Additional conventional controls (LH244 × HCL617 was the control for R3F1, and LH244 × L7126Z was the control for R4F1) evaluated in the generational stability analysis (see Table IV-3) produced comparable read maps.

When reads from the MON 87429 (R3) dataset were aligned with the transformation plasmid sequence, large numbers of reads mapped to T-DNA, and no reads were identified which aligned to the transformation plasmid backbone (Figure IV-6).

The mapping of a large number of sequencing reads from the MON 87429 (R3) dataset to the T-DNA was expected and fully consistent with the presence of the inserted DNA MON 87429. Since these sequences are identical, the mapped read mate pairs were used to distinguish their true mapping location. Also, no reads in the MON 87429 (R3) generation dataset were identified that align with the plasmid backbone. As a result, it is concluded that MON 87429 (R3) does not contain inserted sequence from the transformation plasmid backbone.

To determine the insert number in MON 87429 (R3), selected reads mapping to T-DNA as described above were analyzed to identify junctions. This bioinformatic analysis is used to find and classify partially matched reads characteristic of the ends of insertions. The number of unique junctions determined by this analysis are shown in Table IV-2.

Table IV-2. Unique Junction Sequence Class Results

Sample	Junctions Detected
MON 87429 (R3)	2
LH244	0
LH244 × HCL617	0
LH244 × L7126Z	0

Detailed mapping information of the junction sequences is shown in Figure IV-6. The location and orientation of the junction sequences relative to the T-DNA insert determined for MON 87429 are illustrated in Figure IV-6, panels 1 and 2. As shown in the figure, there are two junctions identified in MON 87429. Both junctions contain the T-DNA border sequence joined to flanking genomic sequence, indicating that they represent the sequences at the junctions of the intended T-DNA insert and the maize genome. As described earlier, no junctions were detected in any of the conventional maize control samples.

Considered together, the absence of plasmid backbone and the presence of two junctions (joining T-DNA borders and flanking sequences) indicate a single intended T-DNA at a single locus in the genome of MON 87429. Both of these junctions originate from the same locus of the MON 87429 genome and are linked by contiguous, known and expected DNA sequence. This is demonstrated by complete coverage of the sequenced reads spanning the interval between the junctions and the directed sequencing of overlapping PCR products described in Section IV.C.

Based on the comprehensive NGS and junction identification it is concluded that MON 87429 contains one copy of the T-DNA inserted into a single locus. This conclusion is confirmed by the sequencing and analysis of overlapping PCR products from this locus as described below.

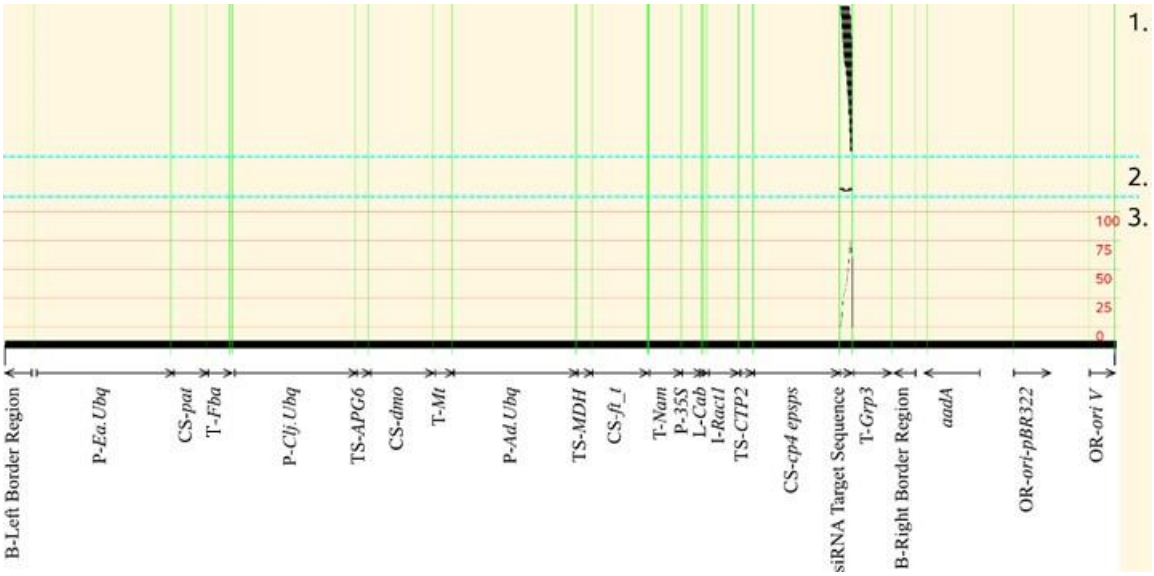


Figure IV-5. Read Mapping of Conventional Maize LH244 Versus PV-ZMHT519224

Panel 1 shows the location of unpaired mapped reads, Panel 2 shows paired mapped reads, and Panel 3 shows a representation of combined read depth for unpaired and paired reads. Vertical lines, in green, show genetic element boundaries. Comparable results were observed when read mapping LH244 × HCL617 versus PV-ZMHT519224, or read mapping LH244 × L7126Z versus PV-ZMHT519224. These additional conventional controls were used for the generational stability analysis (see Table IV-3).

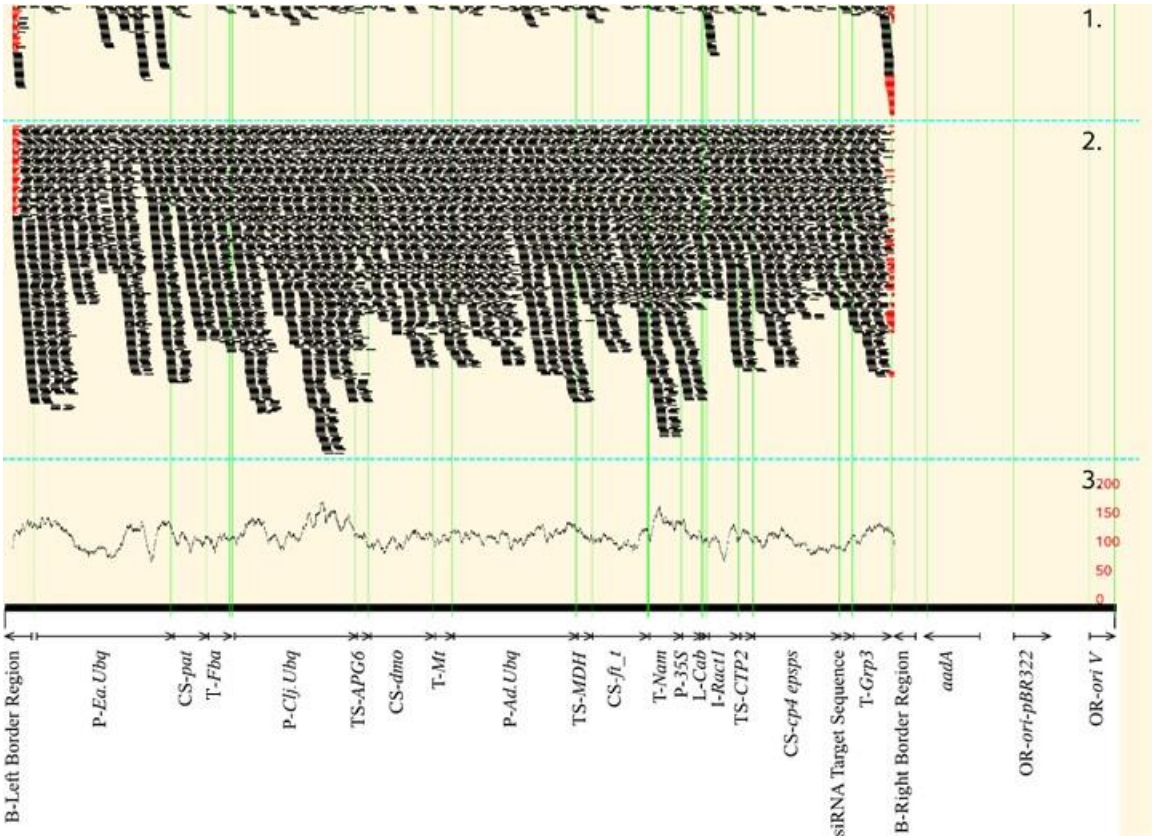


Figure IV-6. Read Mapping of MON 87429 (R3) Versus PV-ZMHT519224

Panel 1 shows the location of unpaired mapped reads. Panel 2 shows paired mapped reads and Panel 3 shows a representation of combined read depth for unpaired and paired reads. Vertical lines, in green, show genetic element boundaries. The region of flank junction sequences that aligns with transformation plasmid is shown in red. Comparable results were observed when read mapping the R3F1, R4, R4F1, and R5 generations of MON 87429 versus PV-ZMHT519224 (see Table IV-3 for the generational stability analysis).

IV.C. Organization and Sequence of the Insert and Adjacent DNA in MON 87429

The organization of the elements within the DNA insert and the adjacent genomic DNA was assessed using directed DNA sequence analysis (refer to Figure IV-1, Step 4). PCR primers were designed to amplify two overlapping regions of the MON 87429 genomic DNA that span the entire length of the insert and the adjacent DNA flanking the insert (Figure IV-7). The amplified PCR products were subjected to DNA sequencing analyses. The results of this analysis confirm that the MON 87429 insert is 14,008 bp and that each genetic element within the T-DNA is intact compared to PV-ZMHT519224. The border regions both contain small terminal deletions with the remainder of the inserted border regions being identical to the sequence in PV-ZMHT519224. The sequence and organization of the insert was also shown to be identical to the corresponding T-DNA of PV-ZMHT519224 as intended. This analysis also shows that only T-DNA elements (described in Table IV-1) were present within the inserted DNA. In addition, 1,029 base pairs flanking the 5′ end of the MON 87429 insert (Table IV-1, bases 1-1029) and 1031 base pairs flanking the 3′ end of the MON 87429 insert (Table IV-1, bases 15038-16068) were determined.

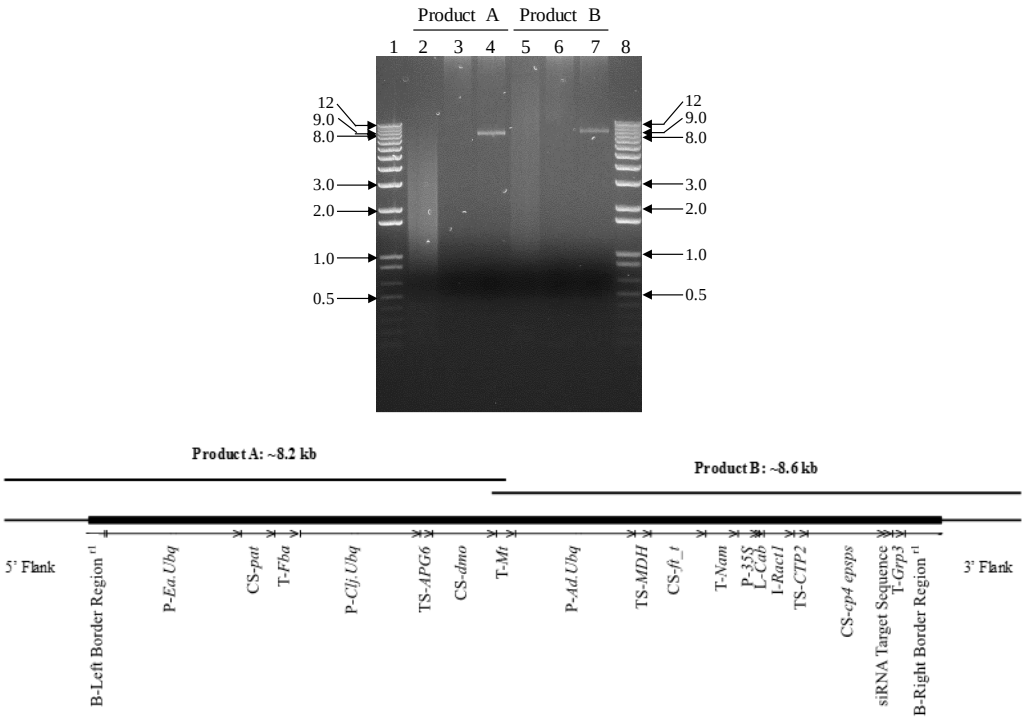


Figure IV-7. Overlapping PCR Analysis across the Insert in MON 87429

PCR was performed on both conventional control genomic DNA and genomic DNA of the R3 generation of MON 87429 using two pairs of primers to generate overlapping PCR fragments from MON 87429 for sequencing analysis. To verify size and specificity of the PCR products, 12 µl of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 87429 that appears at the bottom of the figure. This figure is a representative of the data generated in the study. Lane designations are as follows:

Lane	
1	1 Kb Plus DNA Ladder
2	No template control
3	LH244 Conventional Control
4	MON 87429
5	No template control
6	LH244 Conventional Control
7	MON 87429
8	1 Kb Plus DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1 Kb Plus DNA Ladder (Invitrogen) on the ethidium bromide stained gel.
[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87429 was truncated compared to the sequences in PV-ZMHT519224

IV.D. Sequencing of the MON 87429 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 87429 insert paired with a second primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-8). A sequence comparison between the PCR product generated from the conventional control and the sequence generated from the 5' and 3' flanking sequences of MON 87429 indicates that 54 bases of maize genomic DNA were deleted during integration of the T-DNA. There also was a 29 base insertion in the MON 87429 5′ flanking sequence and a 31 base insertion in the MON 87429 3' flanking sequence. Such changes are common during plant transformation (Anderson et al., 2016) and these changes presumably resulted from DNA repair mechanisms in the plant during *Agrobacterium*-mediated transformation processes (Salomon and Puchta, 1998).

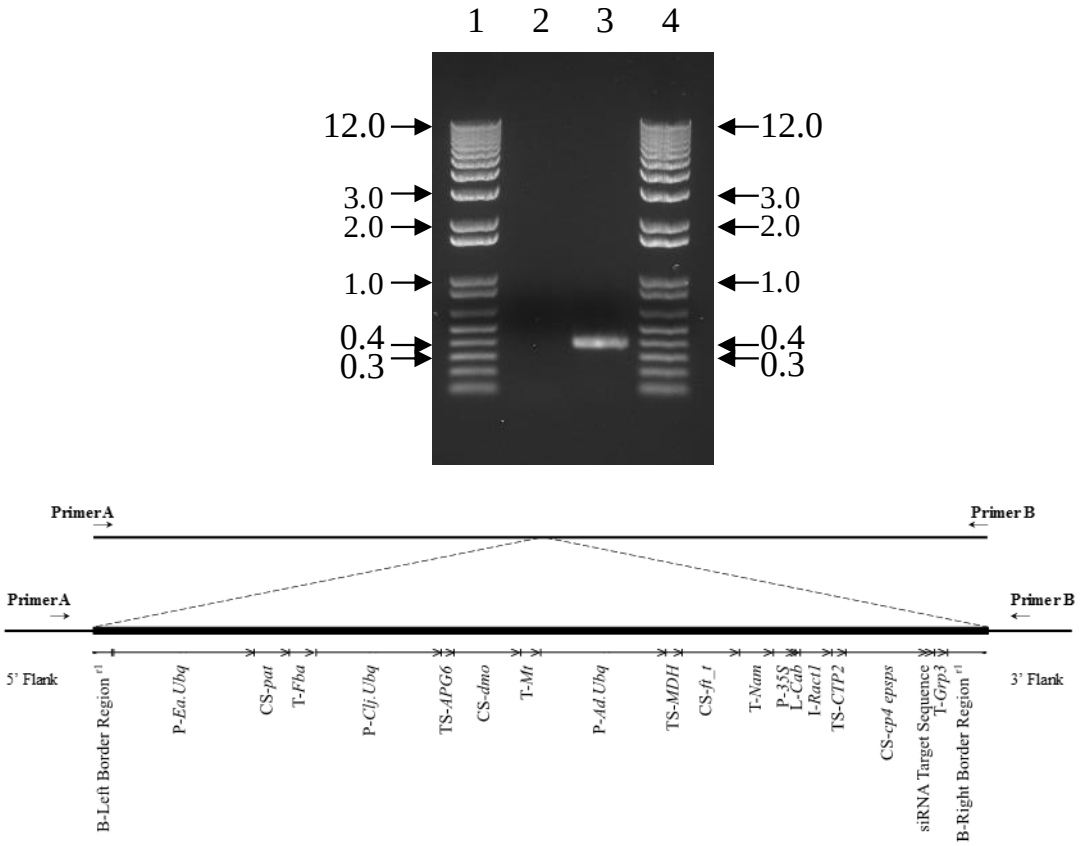


Figure IV-8. **PCR Amplification of the MON 87429 Insertion Site**

PCR analysis was performed to evaluate the insertion site. PCR was performed on conventional control genomic DNA using Primer A, specific to the 5′ flanking sequence, and Primer B, specific to the 3′ flanking sequence of the insert in MON 87429. The DNA generated from the conventional control PCR was used for sequencing analysis. This illustration depicts the MON 87429 insertion site in the conventional control (upper panel) and the MON 87429 insert (lower panel). Approximately 5 µl of each of the PCR reactions was loaded on the gel. This figure is representative of the data generated in the study. Lane designations are as follows:

Lane	
1	1 Kb Plus DNA Ladder
2	No template control
3	LH244 Conventional Control
4	1 Kb Plus DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1 Kb Plus DNA Ladder (Invitrogen) on the ethidium bromide stained gel.

[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 87429 was truncated compared to the sequences in PV-ZMHT519224.

IV.E. Determination of Insert Stability over Multiple Generations of MON 87429

In order to demonstrate the genetic stability of the T-DNA present in MON 87429 through multiple breeding generations, NGS reads from five breeding generations of MON 87429 were mapped to the transformation plasmid for junction identification. The breeding history of MON 87429 is presented in Figure IV-4, and the specific generations tested are indicated in the figure legend. The MON 87429 (R3) generation was used for the molecular characterization analyses discussed in Sections IV.B-IV.D and shown in Figure IV-4. To assess stability, four additional generations were evaluated by NGS as previously described in Section IV.B, and compared to the fully characterized MON 87429 R3 generation. The conventional controls used for the generational stability analysis included LH244, LH244 × HCL617 and LH244 × L7126Z which represent similar background genetics to each of the analyzed MON 87429 breeding generations. Genomic DNA isolated from each of the selected generations of MON 87429 and conventional controls were used for mapping and subsequent junction identification (Table IV-3).

To determine the insert number in the MON 87429 samples, the sequences generated and selected as described above in Section IV.B.2 were analyzed to identify junctions. The number of any resultant unique junctions containing the PV-ZMHT519224 DNA sequence determined by this analysis is shown in the table below.

Table IV-3. Junction Sequence Classes Detected

Sample	Junction Sequences Detected
MON 87429 (R3)	2
MON 87429 (R3F1)	2
MON 87429 (R4)	2
MON 87429 (R4F1)	2
MON 87429 (R5)	2
LH244	0
LH244 × HCL617	0
LH244 × L7126Z	0

As shown by alignment to the full flank/insert sequence obtained from directed sequencing, a single conserved pair of junctions linked by contiguous known and expected DNA sequence is present in MON 87429 (R3). Two identical junctions are found in each of the breeding generations (R3, R3F1, R4, R4F1, and R5), confirming the insertion of a single copy of PV-ZMHT519224 T-DNA at a single locus in the genome of MON 87429, and the consistency of these junctions in the mapping data across all generations tested demonstrates that this single locus is stably maintained throughout the MON 87429 breeding process.

These results demonstrate that the single locus of integration characterized in the R3 generation of MON 87429 is found in five breeding generations of MON 87429,

confirming the stability of the insert. This comprehensive NGS and bioinformatic analysis of NGS data from multiple generations supports the conclusion that MON 87429 contains a single, stable, inserted T-DNA.

IV.F. Inheritance of the Genetic Insert in MON 87429

The MON 87429 T-DNA resides at a single locus within the maize genome and therefore should be inherited according to Mendelian principles of inheritance. During development of lines containing MON 87429, phenotypic and genotypic segregation data were recorded to assess the inheritance and stability of the MON 87429 T-DNA using Chi square (χ^2) analysis over several generations. The χ^2 analysis is based on comparing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87429 breeding path for generating segregation data is described in Figure IV-9. The transformed R0 plant was self-pollinated to generate R1 seed. An individual plant homozygous for the MON 87429 T-DNA was identified in the R1 segregating population via a Real-Time TaqMan® PCR assay.

The homozygous positive R1 plant was self-pollinated to give rise to R2 seed. The R2 plants were self-pollinated to produce R3 seed. R3 plants homozygous for the MON 87429 T-DNA were crossed via traditional breeding techniques to a Monsanto proprietary elite inbred parent that did not contain the *dmo*, *pat*, *ft_t*, or *cp4 epsps* coding sequences to produce hemizygous R3F1 seed. The R3F1 plants were crossed again with a Monsanto proprietary elite inbred parent to produce BC1 seed. The BC1 generation was tested for the presence of the MON 87429 T-DNA by Real-Time TaqMan® PCR assay to select for hemizygous MON 87429 plants. At the BC1 generation, the MON 87429 T-DNA was predicted to segregate at a 1:1 ratio (hemizygous positive: homozygous negative) according to Mendelian inheritance principles.

Selection of hemizygous BC1 plants, followed by crossing with the Monsanto proprietary elite inbred parent, followed by testing for the presence of the T-DNA was repeated for two additional generations, to produce hemizygous BC2 seed and hemizygous BC3 seed, each at a predicted 1:1 (hemizygous positive: homozygous negative) segregation ratio according to Mendelian inheritance principles.

A Pearson's chi-square (χ^2) analysis was used to compare the observed segregation ratios of the MON 87429 T-DNA to the expected ratios. The χ^2 analysis was performed using the statistical program R Version 3.3.1 (2016-06-21).

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% ($\alpha = 0.05$).

The results of the χ^2 analysis of the segregating progeny of MON 87429 are presented in Table IV-4. The χ^2 value in the BC1, BC2, and BC3 generations indicated no statistically significant difference between the observed and expected segregation ratios of MON 87429 T-DNA. These results support the conclusion that the MON 87429 T-DNA resides at a single locus within the maize genome and is inherited according to Mendelian principles. These results are also consistent with the molecular characterization data indicating that MON 87429 contains a single intact copy of the T-DNA inserted at a single locus in the maize genome (Section IV.B-IV.E).

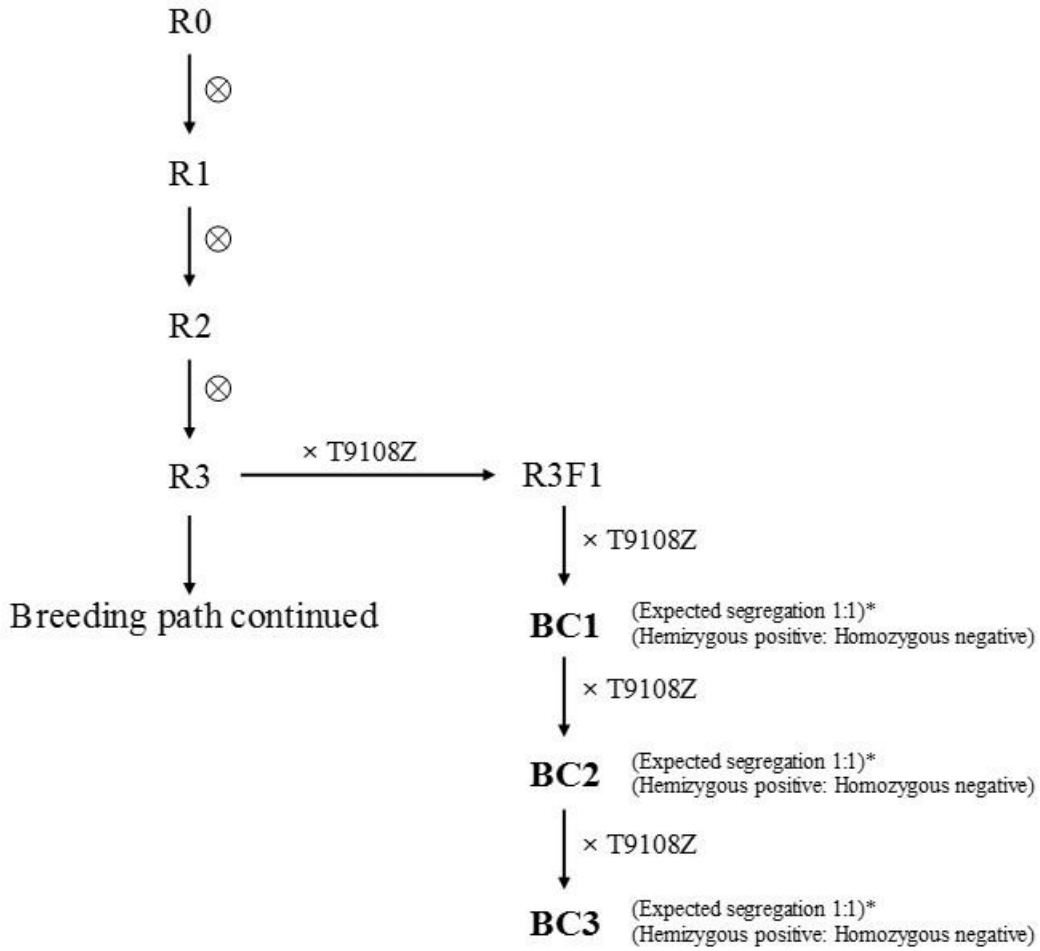


Figure IV-9. Breeding Path for Generating Segregation Data for MON 87429

*Chi-square analysis was conducted on segregation data from BC1, BC2, and BC3 generations (bolded text).
⊗: Self-Pollinated
BC: Back Cross

Table IV-4. Segregation of the Expression Cassette During the Development of MON 87429

				1:1 Segregation			
Generation	**Total Plants**	**Observed # Plant Positive**	**Observed # Plant Negative**	**Expected # Plant Positive**	**Expected # Plant Negative**	**χ 2**	**Probability**
BC1	309	148	161	154.50	154.50	0.55	0.460
BC2	236	112	124	118.00	118.00	0.61	0.435
BC3	216	97	119	108.00	108.00	2.24	0.134

IV.G. MON 87429 RHS Mechanism of Action

MON 87429 maize produces the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to provide maize lines with tissue-specific glyphosate tolerance to facilitate the production of hybrid maize seed. MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen (Table V-5). Specifically-timed glyphosate applications will produce a non-viable pollen phenotype through male tissue-specific glyphosate sensitivity in MON 87429 maize. Glyphosate is a systemic herbicide that is readily translocated via the phloem in plants (Devine et al., 1993). Once glyphosate is in the phloem, it moves to areas of high meristematic activity, following a typical source to sink distribution (Devine et al., 1993). Pollen development in a maize plant takes approximately four weeks to complete (Ma et al., 2008). Early tassel development begins at approximately V9 growth stage (Ritchie et al., 1997), therefore glyphosate applications made at around this time will allow maximum translocation of glyphosate to the male reproductive tissues, and selectively cause cell death only in those cells that are not tolerant to glyphosate (e.g., pollen cells).

The first-generation RHS product, MON 87427 maize, utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS expression in vegetative and female reproductive tissues. This specific promoter and intron combination in MON 87427 results in limited or no production of CP4 EPSPS protein in key male reproductive tissues; resulting in the non-viable pollen phenotype following late applications of glyphosate (USDA-APHIS Petition #10-281-01p p. 26). While the RHS trait in MON 87429 maize results in the same tissue-specific CP4 EPSPS expression seen in MON 87427, the mechanism by which this is achieved is enhanced in MON 87429.

Expression of CP4 EPSPS in MON 87429 plants is driven by the *CaMV 35S* promoter, a known constitutive promoter (Holtorf et al., 1995; Terada and Shimamoto, 1990). In maize and other monocot plants, *CaMV 35S* has been shown to drive weak gene expression in pollen tissue (Hamilton et al., 1992; Heck et al., 2005). Additionally, MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation specifically in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen.

RNA interference (RNAi) is a natural process in eukaryotic organisms for the regulation of endogenous gene expression (Fire et al., 1998; Jones-Rhoades et al., 2006). Both microRNAs (miRNAs) and small interfering RNAs (siRNAs) can trigger the RNAi pathway (Carthew and Sontheimer, 2009). Endogenous maize male tissue specific small interfering RNAs (mts-siRNA) described in Yang et al. (2018), are expected to be involved in regulation of endogenous gene expression in male tissue such as the tassel. MON 87429 takes advantage of endogenous mts-siRNAs to degrade the CP4 EPSPS mRNA in male tissue. MON 87429 CP4 EPSPS mRNA contains a 201 bp siRNA Target Sequence in the 3' UTR (Figure IV-10) which is recognized by the endogenous mts-siRNAs resulting in degradation of the CP4 EPSPS mRNA, and reduced expression of CP4 EPSPS protein in male tissue.

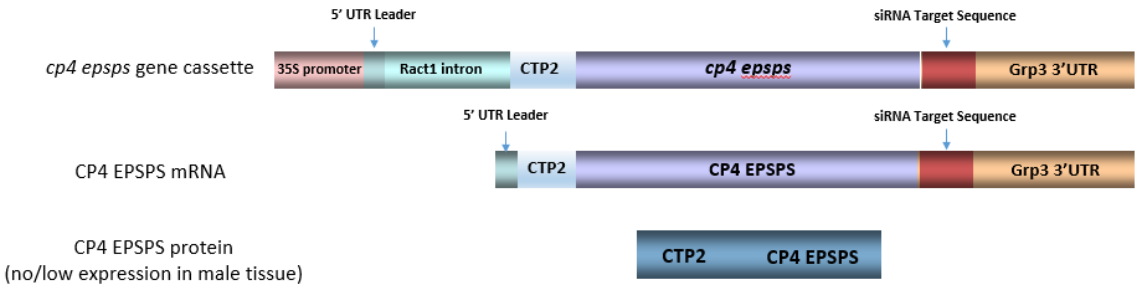


Figure IV-10. Schematic Diagram of *cp4 epsps* Gene Cassette, CP4 EPSPS mRNA and CP4 EPSPS Protein

The identification, cloning and testing of the 201 bp siRNA Target Sequence in MON 87429 is described in detail in Yang et al. (2018). The process is summarized as follows: 1) Identification of male tissue specific small interfering RNAs (mts-siRNA) primarily expressed in tassel, 2) Identification of Target Sequences corresponding to these endogenous mts-siRNAs present in maize, 3) Identification and selection of the target gene, EU974548, based on the 3' UTR region of this gene containing clustered, overlapping alignments of multiple siRNA Target Sequences, 4) Confirmation that the endogenous target, EU974548, is conserved across a wide range of maize germplasms, 5) Selection of the 201 bp siRNA Target Sequence, derived from EU974548, based on the intended non-viable pollen phenotype.

The model representing the mechanism of action by which mts-siRNAs trigger degradation of the CP4 EPSPS mRNA in MON 87429 tassel tissue is summarized below and shown in Figure IV-11. The first five steps represent the endogenous mts-siRNA pathway in conventional maize, based on current knowledge of siRNA driven RNAi systems (Gorski et al., 2017):

- Step 1: mts-siRNA precursor(s) RNA is produced from endogenous mts-siRNA gene.
- Step 2: The mts-siRNA precursor RNA is cleaved by the Dicer (DCL4) complex to produce mts-siRNAs.
- Step 3: Independently, endogenous target genes are transcribed that contain siRNA Target Sequences (shown as red line in the endogenous target gene double helix).
- Step 4: The resulting mts-siRNA molecules, from step 2, are then incorporated into multiprotein RNA-induced silencing complexes (RISC), which facilitate siRNA Target Sequence recognition and mRNA cleavage which leads to specific suppression of the target mRNA.
- Step 5: It is expected that endogenous mRNAs containing siRNA Target Sequences are cleaved by the RISC complex and then subjected to further degradation by 3'-5' and 5'-3' exoribonucleases due to the lack of a 3' poly adenine tail or a 5' cap structure at the cleavage site, inhibiting mRNA translation into protein.

The following three steps occur only in MON 87429 male tassel tissue as MON 87429 uses the endogenous siRNA machinery to specifically target CP4 EPSPS mRNA for degradation:

- Step 6: Upon CP4 EPSPS mRNA transcription, endogenous mts-siRNAs trigger cleavage of CP4 EPSPS mRNA via recognition of the 201bp siRNA Target Sequence in the 3' UTR of the CP4 EPSPS mRNA.
- Step 7: The CP4 EPSPS mRNA cleavage products are then subjected to degradation by 3'-5' and 5'-3' exoribonucleases due to the lack of a 3' poly adenine tail or a 5' cap structure at the cleavage site.
- Step 8: As a result of above processes, CP4 EPSPS mRNA is reduced, resulting in reduction in translation. Thus, little to no CP4 EPSPS protein is expressed in male tissue resulting in glyphosate sensitivity.

The same CP4 EPSPS mRNA is expressed throughout MON 87429 maize tissue (e.g., root, leaf, ear and tassel), however due to the male tissue specific expression of the mts-siRNAs, the CP4 EPSPS mRNA is only targeted for degradation in tassel tissue resulting in little to no expression of CP4 EPSPS protein in pollen (Table V-5).

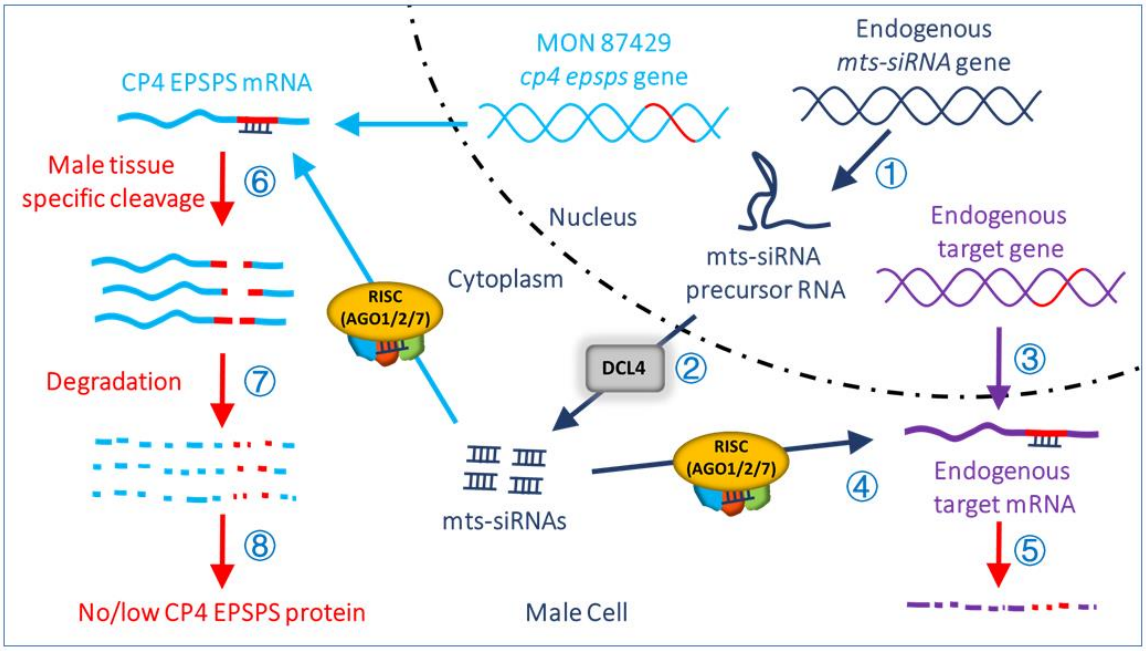


Figure IV-11. MON 87429 CP4 EPSPS siRNA Target Sequence Molecular Mechanism of Action

IV.H. Absence of Unintended Effects due to the MON 87429 RHS Mechanism of Action

IV.H.1. Lack of Secondary siRNA from CP4 EPSPS mRNA Suppression

In plants, primary mRNA degradation products, produced via siRNA mediated degradation, may be used as an RNA template for the generation of secondary siRNAs. These secondary siRNAs could initiate suppression of genes other than the intended target of the primary siRNA. Additionally, it has been reported that secondary siRNAs can move short distances (cell-to-cell) or long distances (between organs) from the site of origin and potentially silence genes with complementary sequence (Borges and Martienssen, 2015) resulting in the suppression of mRNAs other than the intended primary target. To determine whether secondary siRNAs are derived from the CP4 EPSPS transcript in MON 87429 plants, low molecular weight RNA Northern blotting was conducted (Figure IV-12). The upper panel shows high quality and equal loading of total RNA samples from various MON 87429 and conventional wildtype (WT) tissues. The RNA molecules were then transferred onto a membrane and hybridized with three probes that cover the entire CP4 EPSPS transcript except for the siRNA Target Sequence. The siRNA Target Sequence was intentionally excluded from the probes to avoid cross hybridization with the endogenous mts-siRNAs (Appendix C).

An *in vitro* synthesized 25-nt RNA oligo with a sequence identical to a portion of the CP4 EPSPS mRNA was detected as a positive control (Figure IV-12, middle left), indicating that the probes worked, and hybridization and visualization were successful. No hybridizing small RNAs were observed in any samples tested (including leaves, roots, tassels at different developmental stages, and ears) for either MON 87429 or the WT plants (Figure IV-12, middle), demonstrating that no secondary siRNAs were generated from the CP4 EPSPS transcripts in plants containing MON 87429. After stripping off the CP4 EPSPS probes, the blot was reprobed with a DNA oligo complementary to Zm-miRNA159, which is expressed in all maize tissues (Zhang et al., 2009) and used as a positive control here. Zm-miRNA159 was detected in all samples (Figure IV-12, bottom), indicating separation and electroblotting of small RNAs were successful (Yang et al., 2018).

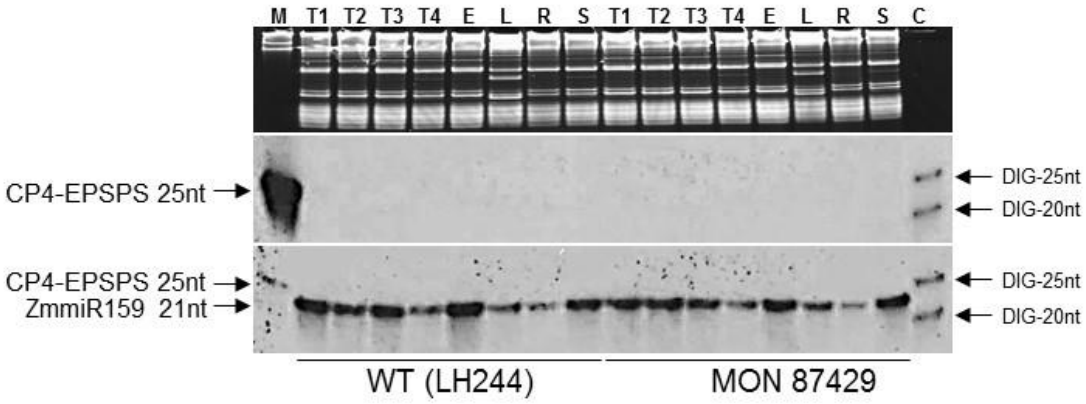


Figure IV-12. Absence of CP4 EPSPS Derived Secondary siRNAs.

Total RNA from various tissues of WT (LH244 conventional control; left side of blot) or MON 87429 maize (right side of blot), respectively, was used for Northern blotting. The upper panel shows equal loading and quality of the RNA samples by sharp, distinct RNA bands. M: a mix of the 1kb RNA ladder (1 µg) and a synthetic 25-nt CP4 EPSPS RNA oligo (1 ng) as a positive control; T1: V6 tassel; T2: V8 tassel; T3: V10 tassel; T4: V12 tassel; E: VT ear; L: V6 leaf; R: VE root; S: VE shoot; C: synthetic DIG-labeled 20- and 25-nt RNAs as small size markers. The middle panel is the low molecular weight RNA blot that was hybridized with three DIG-labeled probes covering the whole CP4 EPSPS transcript except the 201-bp target region. The lower panel is the same small RNA blot that was reprobed for miR159 after stripping off the CP4 EPSPS probes to demonstrate the quality of small RNA in the samples.

IV.H.1.1. Expression of Endogenous Genes with Sequence Homology to the siRNA Target Sequence

Bioinformatic analysis identified seven putative endogenous genes in the maize genome that contain sequences with at least 90% identity with the siRNA Target Sequence present in the CP4 EPSPS mRNA 3' UTR. All these putative genes are located in one region on chromosome 6. This is consistent with reports demonstrating that the maize genome is clustered with 60-80% highly repetitive sequences that are often non-functional pseudogenes and long terminal repeat (LTR) retrotransposons (Meyers et al., 2001; SanMiguel et al., 1996; Whitelaw et al., 2003). These putative genes share amino acid sequence homology with putative membrane proteins resembling serine incorporators that facilitate the synthesis of two serine-derived lipids, phosphatidylserine and sphingolipids (Yang et al., 2018). To confirm that the inclusion of the 201 bp siRNA Target Sequence in the *cp4 epsps* expression cassette did not impact the regulation of these seven putative endogenous genes, the expression levels of the putative gene transcripts in tassels were assessed.

Two quantitative reverse transcription PCR assays were developed: a TaqMan assay designed for detecting transcripts from two of the seven putative genes and a SYBR Green assay designed for six of the seven putative genes in the gene family (expression

of one putative gene can be detected by both assays) (Appendix C). Due to low transcript abundance and high sequence similarities between these seven putative genes, assays targeted to the individual transcripts could not be developed. Overall, these putative genes were expressed at very low levels in both transgenic MON 87429 and conventional wildtype (WT) control tassels. Furthermore, the expression levels of the putative endogenous genes in MON 87429 plants were comparable to those in WT control plants, and there were no significant differences in transcript expression between MON 87429 and WT control plants (Figure IV-13). Four additional representative conventional maize lines (Lines 1-4) were included to assess the natural variability in expression of these putative genes across conventional maize lines. The natural variability in expression of these putative genes proved to be greater than the difference between MON 87429 and WT control plants. These data demonstrate there is no impact to the regulation of the seven putative genes that share sequence homology with the siRNA Target Sequence in MON 87429. Furthermore, there were no observed off-type phenotypes in MON 87429 maize plants (e.g. flowering, pollen characteristics or grain yield) that would be indicative of adverse unintended effects (Section VII).

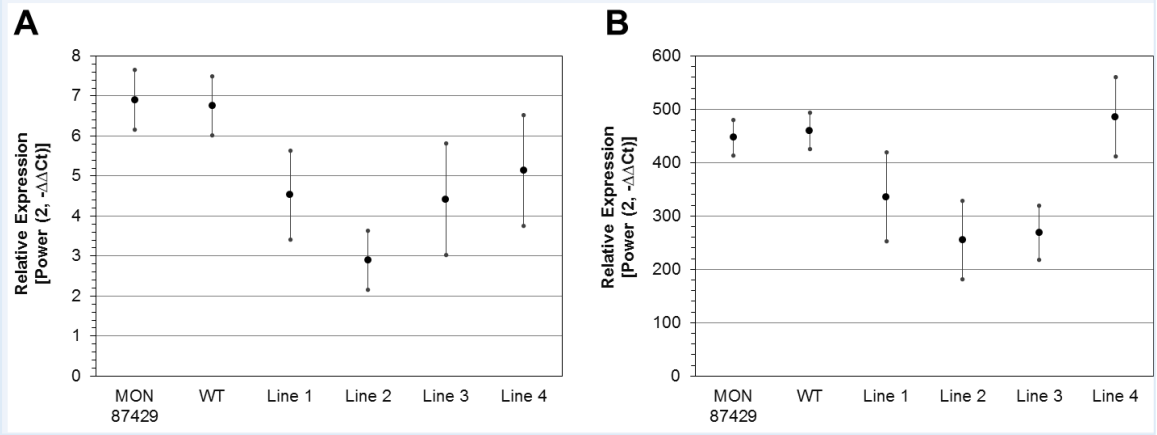


Figure IV-13. Expression of Endogenous Genes with Sequence Homology to the siRNA Target Sequence in Tassels

(A) Expression levels of two endogenous genes as determined by a TaqMan assay. (B) Expression levels of six endogenous genes as determined by a SYBR Green assay. Plants containing MON 87429 (test) and WT (LH244 conventional control) and additional four conventional maize lines (reference) were grown in a randomized complete block design with 100 replications for the test and control materials, and 25 replications for each reference material in a greenhouse. Each replication included three technical replicates (subsamples) per material. Real-time PCR was conducted in triplicate for each subsample. The statistical analysis using a linear mixed model for a randomized complete block design reveals there are no significant differences between test and WT control at the 5% level. Large dots: mean relative expression level; small dots: upper or lower 95% confidence interval, respectively.

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

As described above, characterization of the genetic modification in MON 87429 was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 87429 contains a single copy of the intended T-DNA containing the *pat, dmo, ft_t,* and *cp4 epsps* expression cassettes that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions are based on the following:

- Molecular characterization of MON 87429 by Next Generation Sequencing (NGS) demonstrated that MON 87429 contains a single intended DNA insert. These whole-genome analyses provided a comprehensive assessment of MON 87429 to determine the presence and identity of sequences derived from PV-ZMHT519224 and demonstrated that MON 87429 contains a single T-DNA insert with no detectable plasmid backbone sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) performed on MON 87429 was used to determine the complete sequence of the single DNA insert from PV-ZMHT519224, 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-ZMHT519224 T-DNA. Furthermore, the genomic organization at the insertion site in MON 87429 was assessed by comparing the sequences flanking the T-DNA insert in MON 87429 to the sequence of the insertion site in conventional maize. This analysis determined that 54 bases were deleted upon T-DNA integration. There also was a 29 base insertion in the MON 87429 5′ flanking sequence and a 31 base insertion in the MON 87429 3′ flanking sequence.

- Generational stability analysis by Next Generation Sequencing (NGS) demonstrated that the single PV-ZMHT519224 T-DNA insert in MON 87429 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87429.

- Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA as a single chromosomal locus that shows an expected pattern of inheritance.

- MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen with no impact on endogenous plant gene expression.

Taken together, the characterization of the genetic modification in MON 87429 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 87429.

In addition, MON 87429 maize utilizes an endogenous maize regulatory element (an siRNA Target Sequence) to target CP4 EPSPS mRNA for degradation in tassel tissues. A focused study on the MON 87429 siRNA Target Sequence demonstrated the absence of unintended effects on endogenous gene regulation.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE DMO, PAT, CP4 EPSPS AND FT_T PROTEINS PRODUCED IN MON 87429

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 87429 contains *dmo, pat, cp4 epsps* and *ft_t* expression cassettes that, when transcribed and translated, result in the expression of the DMO, PAT, CP4 EPSPS and FT_T proteins, respectively. Based on the previous characterizations and safety assessments, as well as the history of safe use for the DMO, PAT and CP4 EPSPS proteins, information pertaining to the characterization, equivalence, allergenicity and toxicity of these proteins will not be discussed in detail in this petition. Summaries of safety assessments for DMO, PAT, and CP4 EPSPS proteins that have been previously evaluated by the USDA-APHIS are found in Sections V.A., V.B. and V.C. The characterization and safety assessment of the FT_T protein is found in section V.D. These assessments support a conclusion that the DMO, PAT, CP4 EPSPS, and FT_T proteins produced in MON 87429 are safe for human and animal consumption and do not impact the plant pest risk of MON 87429 maize.

V.A. DMO Protein Safety Assessment Summary

MON 87429 maize contains a demethylase gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein. As a mono-oxygenase protein, the DMO protein is part of a the larger oxygenase family of enzymes that incorporate one or two oxygen atoms into substrates and are widely distributed in many universal metabolic pathways (Harayama et al., 1992). In MON 87429 maize, the DMO protein enzymatically catalyzes the demethylation of the broadleaf herbicide dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde, thus conferring dicamba resistance (Chakraborty et al., 2005). Expression of the DMO protein in MON 87429 is targeted to the chloroplast by a chloroplast transit peptide (CTP), which facilitates its co-localization with the endogenous reductase and ferredoxin enzymes required to supply electrons for the DMO demethylation reaction (Behrens et al., 2007). The DCSA product of the reaction catalyzed by the DMO protein is a known metabolite of dicamba in conventional cotton, soybean, soil, and livestock, and its safety has been evaluated by the FAO-WHO and EPA (FAO-WHO, 2011a; U.S. EPA, 2009). The other reaction product, formaldehyde, is found naturally in many plants and edible fungi at levels up to several hundred ppm (Adrian-Romero et al., 1999; Tashkov, 1996).

In the construction of the plasmid vector used in the development of MON 87429, PV-ZMHT519224, the *Albino and pale green 6* chloroplast transit peptide (CTP) coding sequence from *Arabidopsis thaliana* (*APG6*, Table IV-1) was joined to the *dmo* coding sequence, resulting in the production of a precursor protein consisting of the DMO protein and an N-terminal 68 amino acid chloroplast transit peptide APG6, which is used 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). Data from N-terminal sequencing analysis of the MON 87429-produced DMO indicate that processing of the DMO precursor protein expressed in MON 87429 produced two isoforms of the mature MON 87429 DMO protein that differ by a single, additional amino acid at the N-terminus that is derived from the CTP. The single amino difference between the two isoforms of the DMO protein expressed in MON 87429 (designated DMO+1 and DMO+0) results in the presence of two polypeptide chains of 341 and 340 amino acids, respectively, with an apparent molecular weight ~38 kDa, as the two forms are indistinguishable by Coomassie stain and western blot analysis of SDS-PAGE gels. Thus, MON 87429 DMO protein will be used to refer to both forms of the protein collectively in this petition, except where stated otherwise.

The MON 87429 DMO protein shares a high level of sequence identity with DMO proteins previously assessed and present in biotechnology-derived crops that were deregulated by USDA-APHIS (MON 87708 soybean, USDA-APHIS Petition #10-188-01p, MON 88701 cotton, USDA-APHIS Petition #12-185-01p and MON 87419 maize, USDA-APHIS Petition #15-113-01p). Additionally, these biotechnology-derived crops expressing DMO proteins completed consultation with U.S. FDA (MON 87708 soybean, BNF 000125, MON 88701 cotton, BNF 000135 and MON 87419 maize, BNF 000135), where it was demonstrated that food and feed derived from these events are not materially different than the respective conventional crops. The minor amino acid substitutions between the wild-type DMO protein from the DI-6 strain of *S. maltophilia*, the MON 87429 DMO protein and the DMO proteins expressed in these other biotechnology-derived crops are localized to the N-terminus of the proteins and at position 112 of the amino acid sequences (Herman et al., 2005) (Figure V-1). Additionally, based upon the crystal structure of the wild-type DMO proteins, these amino acid substitutions are structurally distant from the active site and are not expected to impact catalytic site coordination, functional activity, immunoreactivity or specificity (D'Ordine et al., 2009; Dumitru et al., 2009; Wang et al., 2016). Thus, prior safety assessments of the DMO protein expressed in other biotechnology-derived crops are directly applicable to the DMO protein expressed in MON 87429.

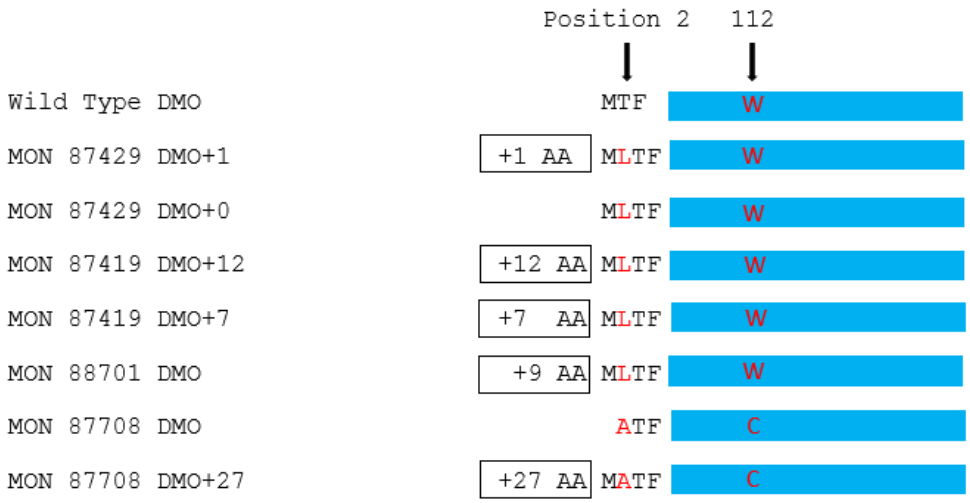


Figure V-1. Forms of DMO Protein and Their Relation to the Wild-Type DMO Protein

The diagram represents the various DMO forms discussed in this section. Position refers to amino acid residues as wild-type DMO and the N-terminal boxed region indicates residues from CTPs. 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 87429 DMO proteins are identical to wild-type DMO, except for the insertion of a leucine at position 2 and an addition of 1 amino acid encoded by the *AGP6* gene at the N-terminus for MON 87429 DMO+1. The MON 87419 DMO proteins are identical to wild-type DMO, except for the insertion of a leucine at position 2 and the amino acids derived from the C-terminal transit peptides at the N-terminus, which are 12 and 7 amino acids encoded by the *CTP4* gene at the N-terminus for MON 87419 DMO+12 and MON 87419 DMO+7, respectively. The MON 88701 DMO protein is identical to wild-type DMO, except for an insertion of a leucine at position 2, and an addition of 9 amino acids encoded by the *CTP2* gene at the N-terminus. The MON 87708 DMO proteins are identical to wild-type DMO, except for the insertion of an alanine at position 2, a single amino acid change at position 112 (tryptophan to cysteine) and an additional 27 amino acids encoded by the *RbcS* gene at the N-terminus for MON 87708 DMO+27. The MON 87708 DMO (fully processed) protein additionally lacks a lead methionine residue.

The DMO protein is specific for the oxidative demethylation of dicamba, forming DCSA. 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). 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 87429 DMO will catalyze the conversion of endogenous compounds. In order to confirm the specificity of the MON 87429 DMO protein for dicamba, and to demonstrate that the minor differences in amino acid sequences present in the MON 87429 DMO protein relative to the DMO proteins expressed in previous biotechnology-derived crops protein do not impact the activity or selectivity for dicamba herbicide as compared to potential endogenous substrates, the potential for MON 87429 DMO to catabolize dicamba and *o*-anisic acid was evaluated using the same qualitative assay used to evaluate the selectivity of

MON 87708 DMO (USDA-APHIS Petition #10-188-01p p. 76). Although *o*-anisic acid is not known to be present in corn, this substance was chosen for this confirmatory experiment since, among the five substrates used in the original study, which included ferulic acid, *o*-anisic acid, sinapic acid, syringic acid, and vanillic acid, *o*-anisic acid is the substrate that is most structurally similar to dicamba. The results from this assessment were similar to the previously reported results for MON 87708 DMO in that the DCSA product was observed using dicamba as the substrate whereas no demethylated products were observed with *o*-anisic acid as the substrate (Appendix E), confirming that the MON 87429 DMO did not catabolize *o*-anisic acid. Thus, MON 87429 DMO is active and has a high specificity for dicamba as a substrate.

The data and information summarized in this section confirm that the molecular mechanism of the MON 87429 DMO protein that confers dicamba tolerance is well understood, that the MON 87429 DMO protein is structurally and functionally homologous to the DMO proteins present in biotechnology-derived crops that have been deregulated by USDA-APHIS and demonstrated to not be materially different than the respective conventional crops in consultation with the US FDA, and that the MON 87429 DMO protein is specific for dicamba. Thus, prior environmental and safety assessments for the DMO protein demonstrating the lack of impact on plant pest potential, the lack of homology to known protein toxins or allergens, digestibility in *in vitro* digestion assays and lack of acute oral toxicity are directly applicable to the MON 87429 DMO protein and are not detailed further herein.

V.B. PAT Protein Safety Assessment Summary

MON 87429 maize contains an acetyltransferase gene from *Streptomyces viridochromogenes* that expresses phosphinothricin N-acetyltransferase (PAT) protein. The molecular mechanism of the PAT protein, which acetylates glufosinate in the presence of acetyl CoA to form N-acetyl glufosinate, is well understood (Thompson et al., 1987). Glufosinate is a racemic mixture of the D- and L-forms of the amino acid phosphinothricin. The herbicidal activity of glufosinate results from the binding of L-phosphinothricin to glutamine synthetase (OECD, 1999; 2002a). Expression of the PAT protein in MON 87429 maize results in the ability to covert L-phosphinothricin to the non-herbicidal N-acetyl glufosinate, thus conferring glufosinate resistance to the crop.

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 to each other 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). Expression of the *pat* gene in MON 87429 results in a single polypeptide of 182 amino acids with an apparent molecular weight of ~25 kDa. Data from N-terminal sequencing analysis of the MON 87429-produced PAT protein indicate that it is identical to the wild type PAT

protein encoded by *S. viridochromogenes* and to the PAT proteins produced in several commercially available glufosinate tolerant crops, including events T25, TC1507, A2704-12, A5547-127 and DAS-59122-7 (USDA-APHIS Petitions #94-357-01p, #00-136-01p, #96-068-01p, #98-014-1p, #03-353-01p respectively) (Hérouet et al., 2005; ILSI-CERA, 2011), except for the first methionine that is removed due to co-translational processing in MON 87429. N-terminal methionine cleavage is common and naturally occurs in the vast majority of proteins (Meinnel and Giglione, 2008).

Numerous glufosinate-tolerant crops including maize, canola, soybean, sugar beet, rice and cotton have been deregulated by USDA-APHIS (USDA-APHIS Petitions #94-357-01p, #00-136-01p, #03-353-01p and #15-113-01p maize; #98-278-01p and 01-206-01p canola, #98-014-01p, #12-215-01p, #11-234-01p and #09-349-01p soybean; #97-336-01p sugar beet, #98-329-01p rice; and #12-185-01p, #13-262-01p and #08-340-01p cotton) and completed consultations with US FDA (U.S. FDA, 1995b; a; 1996; 1997; 1998a; b; 1999; 2002), where it was demonstrated that food and feed derived from these crops are not materially different than the respective conventional crops. The safety of PAT proteins has been confirmed following extensive reviews by regulatory agencies in at least 15 different countries for more than 30 biotechnology-derived events in several different crop species (e.g., maize, soybean, cotton, canola and sugar beet). Additionally, the EPA has issued a tolerance exemption for PAT protein (U.S. EPA, 1997). Prior safety assessments of the PAT proteins expressed in these other biotechnology-derived crops are directly applicable to the MON 87429 PAT protein because the amino acid sequence of the MON 87429 PAT protein is identical to the PAT proteins in these biotechnology-derived crops that are derived from the *pat* gene and because the PAT proteins produced from the *bar* and *pat* genes are equivalent in terms of function and safety. The PAT proteins have a robust history of safe consumption and safe use in agriculture that is supported by the lack of any documented reports of adverse human or animal affects since the introduction of biotechnology-derived crops expressing PAT proteins in 1995 (Duke, 2005).

The PAT protein expressed in MON 87429 is highly specific for glufosinate. Enzyme assays have demonstrated that the PAT protein is unable to acetylate other common L-amino acids that are structurally similar to L-phosphinothricin, and substrate competition assays showed no inhibition of glufosinate acetylation in the presence of high concentrations of L-amino acids that are structurally similar to L-phosphinothricin (including the glufosinate analog L-glutamate) (Wehrmann et al., 1996). Recent metabolic profiling reported non-specific PAT (*bar*) mediated acetylation of two amino acids (aminoadipate and tryptophan) in senescent leaf extracts from *A. thaliana*, however this observation has not been reported in maize (Christ et al., 2017). 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, MON 87419 and DAS-59122-7) that PAT does not affect maize metabolism.

The data and information summarized in this section confirm that the molecular mechanism of the MON 87429 PAT protein that confers glufosinate tolerance is well understood, that the MON 87429 PAT protein is identical to the PAT proteins present in biotechnology-derived crops that have been deregulated by USDA-APHIS and

demonstrated to not be materially different than the respective conventional crops in consultation with the US FDA, that the MON 87429 PAT protein is highly specific for glufosinate and that the PAT protein does not affect maize metabolism. Thus, prior environmental and safety assessments for the PAT protein demonstrating lack of impact on plant pest potential, lack of homology to known protein toxins or allergens, digestibility in *in vitro* digestion assays and lack of acute oral toxicity are directly applicable to the MON 87429 PAT protein and are not detailed further herein.

V.C. CP4 EPSPS Protein Safety Assessment Summary

MON 87429 contains a 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4 (*cp4 epsps*) that expresses a EPSPS protein. EPSPS is a key enzyme involved in aromatic amino acid biosynthesis and catalyzes a reaction where the enolpyruvyl group from phosphoenol pyruvate (PEP) is transferred to the 5-hydroxyl of shikimate-3-phosphate (S3P) to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate (Alibhai and Stallings, 2001). Shikimic acid is a substrate for the biosynthesis of aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules that are necessary for plant growth. The shikimic acid pathway and EPSPS enzymes are ubiquitous to plants and microorganisms, but are absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). The CP4 EPSPS protein expressed in MON 87429 is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Sikorski and Gruys, 1997). In conventional plants, glyphosate blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (Haslam, 1993; Steinrücken and Amrhein, 1980). In Roundup Ready® plants, which are tolerant to Roundup® agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996).

The *cp4 epsps* expression cassette in MON 87429 encodes a precursor protein of 531 amino acids (455 amino acids encoded by the *cp4 epsps* gene and 76 amino acids encoded by the *CTP2* gene for targeting the CP4 EPSPS protein into chloroplasts). Expression of the *cp4 epsps* gene in MON 87429 results in a single polypeptide chain of 455 amino acids with an apparent molecular weight of ~44 kDa. Data from N-terminal sequencing analysis of the MON 87429-produced CP4 EPSPS protein indicate that the polypeptide chain starts at methionine position 77 after complete cleavage of the chloroplast transit peptide (CTP2) (Padgette et al., 1996). The CP4 EPSPS protein expressed in MON 87429 is identical in structure and function to the CP4 EPSPS protein expressed in Roundup Ready® products across several crops, including soybeans, maize, canola, cotton, sugar beet, and alfalfa.

The safety and mode-of-action of CP4 EPSPS protein is well documented and is the subject of many publications (Harrison et al., 1996; Hoff et al., 2007; ILSI-CERA, 2010; U.S. EPA, 1996a). Numerous glyphosate-tolerant, commercially available CP4 EPSPS containing crops have been deregulated by USDA-APHIS (USDA-APHIS Petitions #10-281-01p and 04-125-01p maize; 11-188-01p and 98-216-01p canola; 04-110-01p

alfalfa; 06-178-01p and 93-258-01p soybean) and have completed consultations with the FDA (e.g., MON 87427 BNF 000126 in 2012 and MON 88017 BNF 000097 in 2005), where it was demonstrated that food and feed derived from these crops are not materially different than the respective conventional crops. The safety of the CP4 EPSPS protein has been reviewed by regulatory agencies around the world (ILSI-CERA, 2011; OECD, 1999; 2002a). Additionally, in 1996 the U.S. EPA established an exemption from the requirement of a tolerance for residues of the plant pesticide inert ingredient CP4 EPSPS and the genetic material necessary for its production in all plants (40 CFR § 174.523, redesignated from § 180.1174, effective April 25, 2007). Prior safety assessments of the CP4 EPSPS protein expressed in these other biotechnology-derived crops are directly applicable to the MON 87429 CP4 EPSPS protein because the amino acid sequence and function of the MON 87429 CP4 EPSPS protein is identical to the CP4 EPSPS proteins in these biotechnology-derived crops. The CP4 EPSPS protein has a robust history of safe consumption and safe use in agriculture that is supported by the lack of any documented reports of adverse human or animal effects since the introduction of biotechnology-derived crops expressing CP4 EPSPS protein.

EPSPS enzymes, including the MON 87429 CP4 EPSPS protein, are highly specific for their substrates. The only known substrates of any biological significance for EPSPS enzymes are S3P and PEP. Glyphosate is not enzymatically modified by EPSPS. Shikimic acid was shown to be a very poor substrate for EPSPS enzyme, requiring much higher concentrations to observe turnover by the enzyme than for S3P (Gruys et al., 1992). Methyl shikimate, quinic acid, and dihydroshikimic acid do not serve as substrates for the EPSPS enzyme (Franz et al., 1997). As with most physiological pathways, there is tight regulation of metabolic flux through the shikimic acid pathway. Pathway flux is regulated both transcriptionally and post-transcriptionally (Maeda and Dudareva, 2012; Tzin et al., 2012). The first enzyme in the pathway, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS), has been identified as the key regulatory checkpoint for the flux through the pathway, with possible secondary checkpoints at shikimate kinase and chorismate synthase (Maeda and Dudareva, 2012; Tzin et al., 2012). Thus, plants, have mechanisms to regulate flux through the shikimate pathway irrespective of EPSPS synthase activity levels. Due to both the high substrate specificity of EPSPS enzymes and lack of a role as a regulatory enzyme in the shikimic acid pathway, there is no plausible mechanism for the modification of endogenous plant constituents due to the expression of CP4 EPSPS.

The data and information summarized in this section confirm that the molecular mechanism of the MON 87429 CP4 EPSPS protein that confers glyphosate tolerance is well understood, that the MON 87429 CP4 EPSPS protein is identical to the CP4 EPSPS proteins present in biotechnology-derived crops that have been deregulated by USDA-APHIS and demonstrated to not be materially different than the respective conventional crops in consultation with the US FDA, that the MON 87429 CP4 EPSPS protein is selective for glyphosate and that the CP4 EPSPS protein does not affect maize metabolism. Thus, prior environmental and safety assessments for the CP4 EPSPS protein demonstrating a lack of impact on plant pest potential, lack of homology to known protein toxins or allergens, digestibility in *in vitro* digestion assays and lack of

acute oral toxicity are directly applicable to the MON 87429 CP4 EPSPS protein and are not detailed further herein.

V.D. FT_T Protein Characterization and Safety Assessment

A multistep approach to the safety assessment of the MON 87429 FT_T proteins was conducted according to guidance established by the Codex Alimentarius Commission (Codex Alimentarius, 2009) and OECD, which embody the principles and guidance of the FDA's 1992 policy on foods from new plant varieties. The assessment includes: 1) documenting the history of safe consumption of the expressed protein or its structural and functional homology to proteins that lack adverse effects on human or animal health; 2) characterization of the physicochemical and functional properties of each expressed protein; 3) quantification of each expressed proteins' expression in plant tissues; 4) examination of the similarity of each expressed protein to known allergens, toxins or other biologically active proteins known to have adverse effects on humans and animals; 5) evaluation of the susceptibility of each expressed protein to the digestive enzymes pepsin and pancreatin; 6) a confirmatory evaluation of potential animal toxicity through an animal assay. The safety assessment supports the conclusion that exposure to the FT_T protein derived from MON 87429 would not pose any meaningful risk to human or animal health or the environment.

V.D.1. Identity and Function of the FT_T protein from MON 87429

The FT_T protein produced in MON 87429 is encoded by the *ft_t* gene that provides tolerance to aryloxyalkanoate herbicides. Aryloxyalkanoate herbicides include the aryloxyphenoxypropionate acetyl coenzyme A carboxylase (ACCase) inhibitors (so called "FOPs" herbicides such as quizalofop) and some synthetic auxins, such as 2,4-D. The *ft_t* gene in MON 87429 is a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdp*a) gene from the soil bacteria *Sphingobium herbicidovorans*. The amino acid sequence of the FT_T protein shares ~ 89% sequence identity with wild type RdpA (Figure V-2). A total of 30 amino acid substitutions throughout the protein sequence resulted in FT_T displaying improved enzyme kinetics (increased V_{max}) and substrate affinity (reduced K_m) for 2,4-D, relative to RdpA (Appendix G, Table G-1). The amino acid modifications also resulted in FT_T protein displaying retained activity at temperatures experienced during the summer months in maize growing areas compared to RdpA protein (Appendix G, Table G-1).

```
1    AMHAALTPLT NKYRFIDVQP LTGVLGAEIT GVDLREPLDD STWNEILDAF
51   HTYQVIYFPG QAITNEQHIA FSRRFGPVDP VPILKSIEGY PEVQMIRREA
101  NESSRFIGDD WHTDSTFLDA PPAAVVMRAI EVPEYGGDTG FLSMYSAWET
151  LSPTMQATIE GLNVVHSATK VFGSLYQATN WRFSNTSVKV MDVDAGDRET
201  VHPLVVTHPV TGRRALYCNQ VYCQKIQGMT DAESKSLLQF LYEHATKFDF
251  TCRVRWKKDQ VLVWDNLCTM HRAVPDYAGK FRYLTRTTVA GDKPSR
```

Figure V-2 Deduced Amino Acid Sequence of the FT_T Protein

The amino acid sequence of the MON 87429 FT_T protein was deduced from the full-length coding nucleotide sequence present in PV-ZMHT519224 (See Table III-1 for more detail). The double underline shows the alanine amino acid from the CTP MDH that is the N-terminus of the mature FT_T protein. The single underline shows the 30 amino acid substitutions in FT_T. The number counts start from methionine corresponding to start codon. The substitutions are S6T, S9T, Q10N, R11K, F12Y, E13R, R14F, A16D, L82I, G103S, V105F, D130E, H134Y, T145S, R169K, Q178T, R180W, G209V, S210T, K213R, G214A, V217C, R224K, E226Q, P235S, R246K, G289A, V291D, R292K, and A294S, where the first letter denotes the original amino acids followed by the position and the new amino acid.

RdpA protein is an alpha-ketoglutarate-dependent non-heme iron dioxygenase (Müller et al., 2006), and given their structural similarity, the FT_T protein is also an alpha-ketoglutarate-dependent non-heme iron dioxygenase. Alpha-ketoglutarate-dependent non-heme iron dioxygenases belong to a diverse superfamily of Fe(II)/alpha-ketoglutarate dependent hydroxylases that catalyze a range of oxygenation reactions in synthesis and decomposition reactions that include hydroxylation reactions, desaturations, demethylations, ring expansions, ring formations and other oxidative reactions (Hausinger, 2004). This protein superfamily is broadly distributed across the plant, animal and bacterial kingdoms, therefore environmental exposure to Fe(II)/alpha-ketoglutarate dependent hydroxylases is ubiquitous. Members of this superfamily share a common double-stranded, beta-helix protein fold with three metal-binding ligands found in a His1-X-Asp/Glu-Xn-His2 motif (Hausinger, 2004). In oxygenation reactions, alpha-ketoglutarate (αKG) chelates Fe(II) using its C1 carboxylate and C2-ketone. Decarboxylation of αKG results in the formation of succinate and carbon dioxide, which leads to the generation of an Fe(IV)-oxo or other activated oxygen species that subsequently hydroxylate the primary substrate, e.g., quizalofop (Bugg, 2003; Carolis and Luca, 1994; Hausinger, 2004). Thus, the FT_T protein catalyzes a dioxygenase reaction in the presence of αKG and oxygen to metabolize quizalofop, a FOP herbicide, into the herbicidally-inactive quizalofop phenol and pyruvate (Figure V-3). The FT_T protein also catalyzes the dioxygenase reaction that degrades 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin herbicide, into herbicidally-inactive 2,4-dichlorophenol (2,4-DCP) and glyoxylic acid in the presence of alpha-ketoglutarate and oxygen. Succinate and carbon dioxide are released as products of this reaction (Figure V-3). The safety of 2,4-D, quizalofop, and their relevant metabolites

have been assessed by US EPA. US EPA concluded that there is a reasonable certainty that no harm will result to the general population, or to infants and children from aggregate exposure to 2,4-D (U.S. EPA, 2017a) or quizalofop (U.S. EPA, 2018) residues or their metabolites.

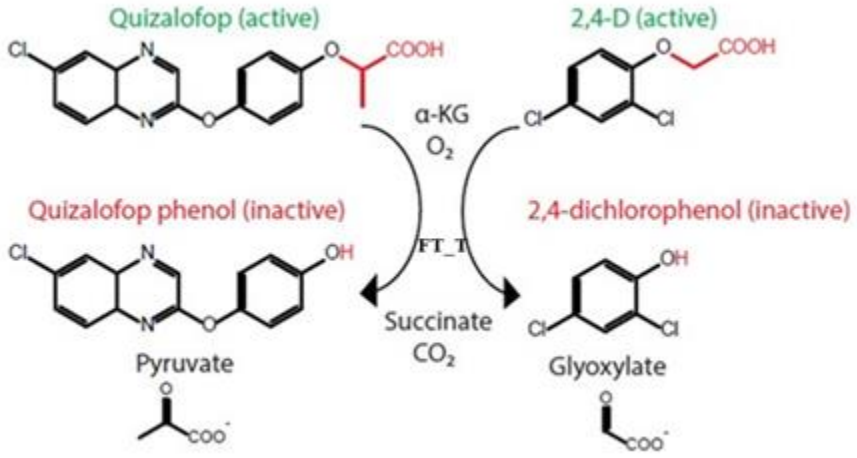


Figure V-3. Substrate and Metabolites of FT_T Protein Reaction with Quizalofop (left) and 2,4-D (right)

V.D.2. Characterization and Equivalence of FT_T protein from MON 87429

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties of the introduced protein(s) produced from the inserted DNA and accompanied by various assessments to confirm 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 MON 87429-produced FT_T protein purified from grain of MON 87429 was characterized and the equivalence of the physicochemical and functional properties between the MON 87429-produced FT_T and *E. coli*-produced MON 87429 FT_T proteins was established using a panel of analytical tests: 1) N-terminal sequence analysis of MON 87429-produced FT_T determined the expected N-terminal sequence; 2) Nanoscale liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the MON 87429-produced FT_T sequence; 3) western blot analysis with an antibody specific for FT_T protein demonstrated that the immunoreactive properties of the MON 87429-produced FT_T and *E. coli*-produced MON 87429 FT_T were equivalent; 4) SDS-PAGE analysis showed that the electrophoretic mobility and apparent molecular weight of the MON 87429-produced FT_T and *E. coli*-produced MON 87429 FT_T proteins were equivalent; 5) MON 87429-produced FT_T and *E. coli*-produced MON 87429 FT_T proteins were both determined to be non-glycosylated; and 6) functional activity analysis demonstrated that MON 87429-produced FT_T and

E. coli-produced MON 87429 FT_T proteins had equivalent enzymatic activity (See Appendix D).

Taken together, these data provide a detailed and thorough characterization of the MON 87429-produced FT_T protein and establish its equivalence to *E. coli*-produced MON 87429 FT_T protein. This equivalence justifies the use of the *E. coli*-produced MON 87429 FT_T protein in studies to assess the safety of the FT_T protein expressed in MON 87429.

V.D.3. FT_T Protein Donor Organism and History of Safe Use

MON 87429 contains the *ft_t* gene, a modified version of the *Rdpa* gene from *Sphingobium herbicidovorans*, that expresses the FT_T protein. FT_T is a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (RdpA) protein (Müller et al., 2006). *S. herbicidovorans* is a common gram-negative, rod-shaped, non-motile, non-spore-forming soil bacterium (Takeuchi et al., 2001; Zipper et al., 1996), which is strictly aerobic and chemo-organotrophic, and not known to be associated with human disease. The taxonomy of *S. herbicidovorans* is:

Kingdom: Bacteria
Phylum: Proteobacteria
Class: *Alphaproteobacteria*
Order: *Sphingomonadales*
Family: *Sphingomonadaceae*
Genus: *Sphingobium*

Members of the genus *Sphingobium* have been isolated from a wide variety of habitats including soil and freshwater (Chaudhary et al., 2017). *Sphingobium* species have also been isolated from foods such as corn (Rijavec et al., 2007), papaya (Thomas et al., 2007) and tomato (Enya et al., 2006). Thus, there is wide-spread human and animal exposure to the *Sphingobium* species in the environment without any known adverse safety or allergenicity reports.

As noted in Section V.D.1, the FT_T protein shares the common high order structure of alpha-ketoglutarate-dependent dioxygenases that contains a classical dioxygenase active pocket including an iron atom coordinated by two histidine residues and one aspartic acid or glutamate residue (Hausinger, 2004). These alpha-ketoglutarate-dependent dioxygenases have been identified in a broad range of organisms including bacteria, fungi, plants, and vertebrates, which have been extensively consumed by both humans and animals (Hausinger, 2004; Kundu, 2012) without any reports of adverse effects.

V.D.4. FT_T Protein Catalyzes a Specific Enzyme Reaction

Whereas the amino acid modifications described in Section V.D.1 present in the FT_T protein, relative to wild type RdpA from *S. herbicidovorans*, improved enzymatic activity, substrate affinity and retained activity at increased temperatures, they did not alter the specificity of the FT_T protein for its substrate. To confirm FT_T protein substrate specificity was not impacted by the optimization, an endogenous substrate

specificity assessment was conducted. Endogenous plant small molecules with similar structures to known FT_T substrates, which are compatible with the FT_T enzymatic active site, were investigated as potential substrates.

The screening approach, detailed in Appendix G, utilized a three-step process: Step 1, the NAPRALERT[8] database, which includes plant specific small molecule datasets collected from several crop plant species including corn (Bisson et al., 2016), was utilized to identify small molecules with structural similarity to dichlorprop, a synthetic auxin herbicide that has the most basic structure of the herbicidal aryloxyalkanoate compounds. In Step 2, selected compounds were then subjected to *in silico* protein-small molecule docking simulations using the structure of the coordinated FT_T active site that was determined by crystallography. This step resulted in the identification of 38 compounds that showed potential docking to the FT_T active site *in silico*. In Step 3, 32 commercially available compounds out of the 38 compounds identified in Step 2, 11 herbicide control compounds and cinnamate (a compound identified as a marginal substrate in a similar enzyme family (Griffin et al., 2013)) were screened as potential substrates *in vitro* by measuring the functional response of purified FT_T protein. The list of compounds assayed, and results can be found in Table V-1.

Table V-1. *In Vitro* FT_T Enzymatic Activity Assay Compound List

Pub Chem ID[1]	Chemical Name[1]	Common Name[1]	Tag[2]	Rel. Activity[3]
5484172	(2R)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoic acid	Quizalofop-P	Herbicide	100%
185588	(2R)-2-(4-chloro-2-methylphenoxy)propanoic acid	Mecoprop-P	Herbicide	69%
91701	2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid	Fluazifop	Herbicide	53%
15118048	(2R)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid	Cyhalofop	Herbicide	35%
8427	2-(2,4-dichlorophenoxy)propanoic acid	Dichlorprop	Herbicide	21%
1486	2-(2,4-dichlorophenoxy)acetic acid	2,4-D	Herbicide	19%
50895	2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid	Haloxyfop	Herbicide	19%
86134	2-[4-[(6-chloro-1,3-benzoxazol-2-yl)oxy]phenoxy]propanoic acid	Fenoxaprop	Herbicide	15%
7204	2-(4-chloro-2-methylphenoxy)acetic acid	MCPA	Herbicide	15%

[8] https://napralert.org/

Pub Chem ID[1]	Chemical Name[1]	Common Name[1]	Tag[2]	Rel. Activity[3]
50465	2-[(4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid	Fluroxypyr	Herbicide	8%
41428	2-[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid	Triclopyr	Herbicide	3%
4947	3,4,5-trihydroxybenzoic acid propyl ester	Propyl gallate	Endogenous	1%
637775	(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)-2-propenoic acid	Sinapate	Endogenous	< 1%
7127	1,2-dimethoxy-4-prop-2-enylbenzene	Methyl Eugenol	Endogenous	< 1%
1548883	(Z)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid	Ferulic acid	Endogenous	< 1%
21685	2,6-di(butan-2-yl)phenol	N/A	Endogenous	< 1%
445858	(E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid	Ferulic acid	Endogenous	< 1%
75318	2-ethoxycarbonylbenzoic acid	Monoethylphalate	Endogenous	< 1%
5281166	2-[(1R,2R)-3-oxo-2-[(Z)-pent-2-enyl]cyclopentyl]acetic acid	Jasmonic acid	Endogenous	< 1%
13988328	8-Hydroxy-2-oxo-1,2-dihydroquinoline-4-carboxylic acid	2,8-dihydroxy-4-quinolinecarboxylic acid	Endogenous	< 1%
6140	(2S)-2-amino-3-phenylpropanoic acid	Phenylalanine	Endogenous	< 1%
736186	(E)-3-(3-hydroxy-4-methoxyphenyl)-2-propenoic acid	Isoferulic acid	Endogenous	< 1%
10742	4-hydroxy-3,5-dimethoxybenzoic acid	Syringic acid	Endogenous	< 1%
802	2-(1H-indol-3-yl)acetic acid	Indole-3-acetic acid	Endogenous	< 1%
637542	(E)-3-(4-hydroxyphenyl)-2-propenoic acid	4-Hydroxycinnamic acid	Endogenous	< 1%
730037	4-(1H-indol-3-yl)-4-oxobutanoic acid	N/A	Endogenous	< 1%
325	(4-propan-2-ylphenyl)methanol	4-Isopropylbenzyl alcohol	Endogenous	< 1%
8554	benzene-1,2-dicarboxylic acid dimethyl ester	Dimethyl phthalate	Endogenous	< 1%
60961	(2R,3R,4S,5R)-2-(6-aminopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol	Adenosine	Endogenous	< 1%

Pub Chem ID[1]	Chemical Name[1]	Common Name[1]	Tag[2]	Rel. Activity[3]
12474015	3-ethyl-5-methoxy-1H-indole	N/A	Endogenous	< 1%
896	N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide	Melatonin	Endogenous	< 1%
13067	2-(1H-indol-3-yl)acetic acid ethyl ester	Ethyl 3-indoleacetate	Endogenous	< 1%
637758	(E)-3-phenyl-2-propenoic acid ethyl ester	Ethyl cinnamate	Endogenous	< 1%
6781	benzene-1,2-dicarboxylic acid diethyl ester	Diethyl phthalate	Endogenous	< 1%
17355	4-phenyl-2-butanone	Benzylacetone	Endogenous	< 1%
3314	2-methoxy-4-prop-2-enylphenol	Eugenol	Endogenous	< 1%
62428	2-(1-naphthalenyl)acetic acid ethyl ester	Ethyl 1-naphthaleneacetate	Endogenous	< 1%
6057	(2S)-2-amino-3-(4-hydroxyphenyl)propanoic acid	Tyrosine	Endogenous	< 1%
10364	2-methyl-5-propan-2-ylphenol	Carvacrol	Endogenous	< 1%
6305	(2S)-2-amino-3-(1H-indol-3-yl)propanoic acid	Tryptophan	Endogenous	< 1%
444539	(E)-3-phenylprop-2-enoic acid	Trans-cinnamate	Literature	< 1%
77021	2,7-dimethoxynaphthalene	N/A	Endogenous	< 1%
689043	(E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid	Caffeic acid	Endogenous	< 1%
3080590	2-(2-oxo-1,3-dihydroindol-3-yl)acetic acid	2-oxoindole-3-acetate	Endogenous	< 1%

[1] The PubChem ID, Chemical Name, Common Name (if available) and Tag are provided (N/A is not available). https://pubchem.ncbi.nlm.nih.gov/.
[2] The column "Tag" indicates which compounds are herbicides (Herbicide), endogenous compound (Endogenous) or literature reported (Literature).
[3]The activity of triplicate assays (each with 3 replicates; n= 9) for FT_T activity reported as relative activity, which is the observed activity of the compound relative to quizalofop-P reported as a percentage. Initial velocities were first normalized in each test set against 2,4-D (positive control in each assay) and then across experiments against quizalofop-P.

Eleven herbicide controls were included in the assay. As expected, FT_T protein activity was detected in the presence of the 11 herbicide control compounds. The measured FT_T activity for all 32 endogenous plant compounds and cinnamate was ≤ 1% relative to quizalofop. These data suggest that FT_T protein is specific to substrate molecules with the following structural features: (1) existence of phenoxy group (2) presence of terminal carboxylate, and (3) available site for oxidation between the phenoxy group and terminal carboxylate. Endogenous plant small molecules with all three features did not exist in the NAPRALERT database, including the six compounds identified in the *in silico* Step 2 above that were not commercially available to test *in vitro*. Therefore, the FT_T enzyme is unlikely to metabolize endogenous small molecules in maize plants at biologically relevant activity levels.

V.D.5. FT_T Protein in MON 87429 is Not Homologous to Known Allergens or Toxins

Bioinformatics analyses were performed to assess the potential for allergenicity, toxicity, or biological activity of FT_T protein. The allergen bioinformatic results demonstrated there were no biologically relevant sequence similarities to allergens when the FT_T protein sequence was used as a query for a FASTA search of the publicly available allergen (AD_2018) database. Furthermore, no short (eight amino acid) polypeptide matches were shared between the FT_T protein sequence and proteins in the allergen database. These data show that FT_T protein sequence lacks both structurally and immunologically relevant similarities to known allergens, gliadins, and glutenins.

FASTA bioinformatic alignment searches using the FT_T amino acid sequence were performed with a toxin database to identify possible homology with proteins that may be harmful to human and animal health. The toxin database, TOX_2018, is a subset of sequences derived from the PRT_2018 database, that was selected using a keyword search and filtered to remove likely non-toxin proteins. The TOX_2018 database contains 28,344 sequences. The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the FT_T protein and any sequence in the TOX_2018 database.

These analyses demonstrated that FT_T protein does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects on human or animal health.

V.D.6. FT_T Protein in MON 87429 is Susceptible to Degradation in *in vitro* Digestion Assay

The susceptibility to degradation by pepsin and pancreatin of MON 87429-produced FT_T was assessed using the *E. coli*-produced MON 87429 FT_T, which was shown to be equivalent to the MON 87429-produced FT_T (see Appendix D). The results indicated that the full-length, *E. coli*-produced FT_T protein is readily degraded by pepsin and pancreatin. Transient peptide fragments of ~4 kDa that were resistant (i.e., present over the course of the 60 min digestive reaction) to pepsin degradation were observed. To better understand the fate of the transiently-stable peptide fragments, sequential degradation of the FT_T protein in pepsin followed by pancreatin was

conducted. The results indicated that the transient fragments are readily degraded by sequential digestion (see Appendix N). Thus, evidence supports the conclusion that gastrointestinal digestion is sufficient to degrade the intact FT_T protein and any fragments thereof making it highly unlikely that intact or large peptide fragments of FT_T protein would be absorbed in the small intestine and have the potential to impact human or animal health.

V.D.7. FT_T Protein in MON 87429 is Not Acutely Toxic

An acute oral toxicology study with FT_T protein was conducted to provide further confirmation of the safety of this protein. There was no evidence of acute toxicity in mice when dosed orally at 2000 mg/kg body weight with FT_T protein. Based on an absence of toxicity in the acute oral toxicity study with the FT_T protein and the relatively low dietary exposure to the protein (i.e., low expression levels of FT_T protein present in MON 87429 grain (Section V.E.4 Table V-6), the risks to humans and animals following dietary exposure to the FT_T protein from consumption of food or feeds derived from MON 87429 is very low.

V.D.8. Assessment of Potential Allergenicity of the FT_T 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.

The FT_T protein has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) FT_T protein originates from *S. herbicidovorans*, an organism that has not been reported to be a source of known allergens.

2) FT_T protein represents a small portion of the total protein in the grain that could be consumed from MON 87429 maize due to very low expression in grain.

3) Bioinformatics analyses demonstrated that FT_T 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 FT_T protein demonstrated that the protein is rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin) under physiological conditions.

Taken together, these data support the conclusion that FT_T protein produced in MON 87429 does not pose a significant allergenic risk to humans or animals.

V.D.9. Human and Animal Exposure to the FT_T Protein

Based on the non-toxic mode-of-action of the FT_T protein, its susceptibility to the digestive enzymes pepsin and pancreatin, the relatively low dietary exposure to the protein (i.e., low expression levels of FT_T protein present in MON 87429 grain), and based on an absence of toxicity in a confirmatory acute oral toxicity study, it is unlikely that exposure to the FT_T protein in food and feed products derived from MON 87429 pose a risk to human and animal health.

V.E. Expression Levels of DMO, PAT, CP4 EPSPS and FT_T Proteins Produced in MON 87429

The protein expression levels determined in MON 87429 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, PAT, CP4 EPSPS and FT_T protein levels are forage, leaf, root and grain tissue samples. Levels of the introduced proteins were determined in forage, leaf, root and grain tissue and were used as appropriate to evaluate potential food, feed and environmental exposures.

MON 87429 DMO, PAT, CP4 EPSPS and FT_T protein levels in various tissues of MON 87429 relevant to the characterization and risk assessment were determined using a multiplexed immunoassay. In addition, to further support the MON 87429 RHS trait mode-of-action (MOA), levels of CP4 EPSPS expression in pollen tissue was determined to demonstrate the differential expression between vegetative and pollen tissue. Tissues of MON 87429 were collected from four replicate plots planted in a randomized complete block design during the 2017 growing season at five field sites in the U.S. The field sites were representative of maize-producing regions suitable for commercial production. Forage, leaf, root, grain and pollen tissue samples were collected from plants at each replicated plot at all field sites treated with dicamba, glufosinate, quizalofop and 2,4-D herbicides (Appendix F).

V.E.1. Expression Level of DMO Protein

MON 87429 DMO protein levels were determined in forage, leaf, root and grain tissues. The results obtained from the multiplexed immunoassay are summarized in Table V-2 and the details of the materials and methods are described in Appendix F. The mean DMO protein level in MON 87429 across all sites was highest in leaf at 35 µg/g dw and lowest in root at 2.3 µg/g dw. The mean DMO protein level in MON 87429 grain was 2.4 µg/g dw.

Table V-2. Summary of DMO Protein Levels in Maize Tissues Collected from MON 87429 Produced in United States Field Trials During 2017

Tissue Type	Development Stage	Mean (SE) Range (µg/g dw)[1]	LOQ[2] (µg/g dw)
Forage	R5	21 (1.6) 9.1-32	0.14
Leaf	V2-V4	35 (2.3) 16-55	0.14
Grain	R6	2.4 (0.15) 1.3-3.6	0.14
Root	V2-V4	2.3 (0.27) 1.0-5.2	0.14

[1]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20).
[2] LOQ=limit of quantitation.

V.E.2. Expression Level of PAT Protein

PAT protein levels were determined in forage, leaf, root and grain tissues. The results obtained from the multiplexed immunoassay are summarized in Table V-3 and the details of the materials and methods are described in Appendix F. The mean PAT protein level in MON 87429 across all sites was highest in leaf at 5.8 µg/g dw and lowest in grain at 0.84 µg/g dw.

Table V-3. Summary of PAT Protein Levels in Maize Tissues Collected from MON 87429 Produced in United States Field Trials During 2017

Tissue Type	Development Stage	Mean (SE) Range (µg/g dw)[1]	LOQ[2] (µg/g dw)
Forage	R5	1.3 (0.067) 0.71-1.8	0.03
Leaf	V2-V4	5.8 (0.40) 2.9-9.8	0.03
Grain	R6	0.84 (0.066) 0.32-1.5	0.03
Root	V2-V4	2.0 (0.15) 0.40-3.1	0.03

[1]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20).
[2] LOQ=limit of quantitation.

V.E.3. Expression Level of CP4 EPSPS Protein

CP4 EPSPS protein levels were determined in forage, leaf, root and grain tissues. The results obtained from the multiplexed immunoassay are summarized in Table V-4. CP4 EPSPS protein levels were also determined in pollen to further demonstrate the differential expression between vegetative and pollen tissue resultant from the MON 87429 RHS trait mode-of-action (MOA). The results obtained from the multiplexed immunoassay are summarized in Table V-5. The details of the materials and methods for these analyses are described in Appendix F. The mean CP4 EPSPS protein level in MON 87429 across all sites was the highest in leaf at 54 µg/g dw and lowest in grain at 0.63 µg/g dw. The mean CP4 EPSPS protein level in MON 87429 pollen across all sites was below the limit of quantitation (<LOQ).

Table V-4. Summary of CP4 EPSPS Protein Levels in Maize Tissues Collected from MON 87429 Produced in United States Field Trials During 2017

Tissue Type	Development Stage	Mean (SE) Range (µg/g dw)[1]	LOQ[2] (µg/g dw)
Forage	R5	7.6 (0.50) 4.0 - 11	0.30
Leaf	V2-V4	54 (3.2) 30- 82	0.11
Grain	R6	0.63 (0.028) 0.41- 0.85	0.34
Root	V2-V4	10 (1.7) 3.4 - 29	0.11

[1]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20).
[2] LOQ=limit of quantitation.

Table V-5. Summary of CP4 EPSPS Protein Levels in Maize Pollen Tissue Collected from MON 87429 Produced in United States Field Trials During 2017

Tissue Type	Development Stage	Mean (SE) Range (µg/g dw)[1]	LOQ[2] (µg/g dw)
Pollen	R1	<LOQ	0.11

[1]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20). Over 50% of the samples (12 of 20) were <LOQ and so < LOQ is reported here.
[2]LOQ=limit of quantitation.

V.E.4. Expression Level of FT_T Protein

FT_T protein levels were determined in forage, leaf, root and grain tissues. The results obtained from the multiplexed immunoassay are summarized in Table V-6 and the details of the materials and methods are described in Appendix F. The mean FT_T protein level in MON 87429 across all sites was the highest in leaf at 440 µg/g dw and lowest in root at 41 µg/g dw. The mean FT_T protein level in MON 87429 grain was 47 µg/g dw.

Table V-6. Summary of FT_T Protein Levels in Maize Tissues Collected from MON 87429 Produced in United States Field Trials During 2017

Tissue Type	Development Stage	Mean (SE) Range (µg/g dw)[1]	LOQ[2] (µg/g dw)
Forage	R5	97 (5.2) 56 - 140	0.036
Leaf	V2-V4	440 (25) 210 - 670	0.036
Grain	R6	47 (3.6) 19 - 79	0.036
Root	V2-V4	41 (4.1) 7.2 - 82	0.036

[1]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20).
[2] LOQ=limit of quantitation.

V.F. MON 87429 DMO, PAT, CP4 EPSPS and FT_T Proteins Characterization and Safety Conclusion

MON 87429 DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde. DMO proteins have been previously characterized, and the safety of these proteins and crops expressing these proteins has been well established. Expression studies using immunoassay demonstrated that DMO was expressed at low levels in grain. Taken together, the evidence indicates that the consumption of the DMO protein from MON 87429 or its progeny is 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 87429, have been previously characterized, and the safety of these proteins and crops expressing these proteins has been well established. Expression studies using immunoassay demonstrated

that PAT was expressed at low levels in grain. Taken together, the evidence indicates that the consumption of the PAT protein from MON 87429 or its progeny is safe for humans and animals.

Members of the EPSPS protein family, including CP4 EPSPS, are enzymes which catalyze a key step in aromatic amino acid biosynthesis and catalyzes the reaction where the enolpyruvyl group from phosphoenol pyruvate (PEP) is transferred to the 5-hydroxyl of shikimate-3-phosphate to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate. CP4 EPSPS proteins, including the CP4 EPSPS protein isolation from MON 87429, have been previously characterized, and the safety of these proteins and crops expressing these proteins has been well established. Expression studies using immunoassay demonstrated that CP4 EPSPS was expressed at low levels in grain. Taken together, the evidence indicates that the consumption of the CP4 EPSPS protein from MON 87429 or its progeny is safe for humans and animals.

The FT_T protein is an alpha-ketoglutarate-dependent non-heme iron dioxygenase that catalyzes a dioxygenase reaction in the presence of alpha-ketoglutarate (αKG) and oxygen by incorporating oxygen into quizalofop, thus degrading it into the herbicidally-inactive quizalofop phenol and pyruvate, while maintaining specificity for its substrate. The physicochemical characteristics of the MON 87429-produced FT_T protein were determined and equivalence between MON 87429-produced FT_T and *E. coli*-produced FT_T proteins was demonstrated. This equivalence justifies the use of the *E. coli*-produced FT_T as a test substance in the protein safety studies (acute oral toxicity and digestibility). Expression studies using immunoassay demonstrated that FT_T was expressed at low levels in grain. The FT_T protein lacks structural similarity to allergens, toxins or other proteins known to produce adverse effects in mammals. In addition, the ubiquitous presence of *Sphingobium* species in the environment has resulted in widespread human and animal exposure and is not commonly known for allergenicity and human or animal pathogenicity. The FT_T protein is rapidly digested by proteases found in the human gastrointestinal tract (pepsin and pancreatin) and demonstrated no acute oral toxicity in mice at the high dose tested. Based on the above information, the consumption of the FT_T protein in foods derived from MON 87429 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 87429 or derived from MON 87429 are as safe for human and animal consumption as maize currently on the market and expression of the DMO, PAT, CP4 EPSPS and FT_T proteins in MON 87429 does not impact the weediness or plant pest risk of MON 87429 maize.

VI. COMPOSITIONAL ASSESSMENT OF MON 87429

Food and feed 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. Maize is not known to have any endogenous toxicants or antinutrients associated with overall plant pest potential (OECD, 2002b). For MON 87429, the introduced proteins, DMO, PAT, CP4 EPSPS and FT_T, confer herbicide tolerance and lack catalytic activity that is intended to or expected to affect the plant's metabolism. Given the nature of these introduced traits and the overall lack of meaningful unintended compositional characteristics observed for biotechnology-derived products characterized to date (Herman and Price, 2013; Venkatesh et al., 2015), compositional changes that would affect the levels of components in MON 87429 maize were not expected.

Monsanto is currently in consultation with the Food and Drug Administration following their policy, "Foods Derived from New Plant Varieties," on the food and feed safety of MON 87429 maize (Submitted 05-Feb-2019). Samples were collected from 5 field sites grown in 2017 that were representative of U.S. maize growing regions. Composition data for 25 components including major nutrients in grain (protein, amino acids, total fat, linoleic acid, carbohydrates, acid detergent fiber, neutral detergent fiber and ash), major nutrients in forage (protein, total fat, carbohydrates, acid detergent fiber, neutral detergent fiber and ash) and anti-nutrients in grain (phytic acid and raffinose) were submitted to FDA as part of the voluntary food/feed safety and nutritional assessment for MON 87429 maize. The results of the compositional assessment found that there were no biologically meaningful differences between MON 87429 and conventional control and support the conclusion that MON 87429 maize is compositionally equivalent to the conventional control.

This section provides analyses of concentrations of key nutrients and anti-nutrients in grain and forage of MON 87429 compared to that of a conventional control maize hybrid grown and harvested under similar conditions. The production of materials for compositional analyses used a sufficient variety of field trial sites, reflecting a range of environmental conditions under which MON 87429 is expected to be grown and robust field designs (randomized complete block design with four replicates). Samples were subjected to sensitive analytical methods that allow quantitative and accurate measurements of key components. See Appendix M for details on composition methods.

VI.A. Compositional Equivalence of MON 87429 Grain and Forage to Conventional Maize

Grain and forage samples were harvested from MON 87429 and a conventional control grown at five sites in the United States during 2017. The field sites were planted in a randomized complete block design with four replicates per site. MON 87429 and the conventional control were grown under agronomic field conditions typical for each of the different growing regions. MON 87429 plots were treated with dicamba, glufosinate,

quizalofop and 2,4-D to generate samples under conditions of the intended use of the product.

The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients and anti-nutrients in grain and forage of MON 87429 and the conventional control.

Compositional analyses of grain and forage samples are reported for a subset of components listed in the maize OECD consensus document (OECD, 2002b). Harvested grain samples were assessed for moisture and levels of key nutrients including proximates (protein, total fat and ash), essential amino acids (10 components), linoleic acid (essential fatty acid), carbohydrates by calculation and fiber (acid detergent fiber (ADF) and neutral detergent fiber (NDF)). Grain samples were also assessed for levels of antinutrients (phytic acid and raffinose). Harvested forage samples were assessed for moisture and levels of nutrients including proximates (protein, total fat and ash), carbohydrates by calculation and fiber (ADF and NDF). In all, 27 different components were analyzed. Moisture values for grain and forage were measured to enable the conversion of components from fresh to dry weight but were not statistically analyzed. Therefore, 25 components were statistically analyzed.

The statistical comparison of MON 87429 and the conventional control was based on compositional data combined across all five field sites. Statistically significant differences were identified at the 5% level ($\alpha = 0.05$). A statistically significant difference between MON 87429 and the conventional control does not necessarily imply biological relevance from a food and feed safety perspective. Therefore, any statistically significant differences observed between MON 87429 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 (MON 87429) and Conventional Control Means

The difference in means between MON 87429 and the conventional control was determined for use in subsequent steps.

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

The difference between MON 87429 and the conventional control was evaluated in the context of variation within the conventional control germplasm grown across multiple sites (i.e., variation due to environmental influence) by determining the range of replicate values for the conventional control (range value = maximum value minus the minimum value). A mean difference less than the variability seen due to natural environmental variation within the single, closely related germplasm 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 87429 on composition was evaluated in the context of sources of natural variation such as environmental and germplasm influences. This assessment determined whether the component mean value of MON 87429 was within the natural variability defined by the literature values or the ILSI Crop Composition Database (ILSI-CCDB) values. This natural variability is important in assessing the biological relevance to food and feed safety of statistically significant differences in composition between MON 87429 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 87429 and the conventional control mean values does not necessarily imply a meaningful contribution by MON 87429 to compositional variability. Only if the impact of MON 87429 on levels of components is large relative to natural variation inherent to conventional maize would the difference in composition be potentially meaningful from a food and feed safety and nutritional perspective. Differences between MON 87429 and the conventional control that are within the observed natural variation for maize would support a conclusion of compositional equivalence.

Compositional Equivalence of MON 8729 Grain and Forage to that of Conventional Maize

There were no statistically significant differences ($p<0.05$) for 23 of the 25 components analyzed from MON 87429 grain and forage (Table VI-1–Table VI-4). There were two components (total fat and linoleic acid in grain) that showed a statistically significant difference ($p<0.05$) between MON 87429 and the conventional control (Table VI-1). For total fat, the mean value was 3.76 % dw for MON 87429 and 3.88 % dw for the conventional control, a difference of -0.15 % dw. For linoleic acid, the mean value was 55.53 % Total Fatty Acid (FA) for MON 87429 and 55.21 % Total FA for the conventional control, a difference of 0.32 % Total FA. For these components, the mean difference between MON 87429 and the conventional control was less than the conventional control range values and the MON 87429 mean component values were also within the range of values observed in the literature and/or the ILSI-CCDB values (Table VI-5).

These data indicated that the statistically significant differences observed in total fat and linoleic acid in grain were not biologically meaningful from a food and feed safety perspective. These results support the conclusion that MON 87429 was not a major contributor to variation in component levels in maize grain or forage and confirmed the compositional equivalence of MON 87429 to the conventional control in levels of key nutrients and anti-nutrients in grain and forage.

Table VI-1. Summary of Maize Grain Proximates, Linoleic Acid, Carbohydrates by Calculation and Fiber for MON 87429 and Conventional Control

Component	MON 87429 Mean (S.E.)[1] Range	Control Mean (S.E.) Range	Control Range Value[2]	Difference (Test minus Control) Mean (S.E.)	p-Value
Protein (% dw)[3]	9.20 (0.30) 7.95 - 10.99	9.35 (0.30) 8.04 - 11.01	2.96	-0.15 (0.14)	0.289
Total fat (% dw)	3.76 (0.050) 3.46 - 4.01	3.88 (0.050) 3.58 - 4.16	0.57	-0.12 (0.045)	0.049
Linoleic acid (% Total FA)[4]	55.53 (0.63) 53.61 - 57.87	55.21 (0.63) 53.28 - 57.38	4.10	0.32 (0.12)	0.018
Ash (% dw)	1.15 (0.057) 0.97 - 1.34	1.15 (0.057) 0.91 - 1.41	0.50	-0.0013 (0.024)	0.958
Carbohydrates by calculation (% dw)	85.87 (0.38) 83.97 - 87.27	85.61 (0.38) 83.62 - 87.34	3.73	0.25 (0.15)	0.104
ADF (% dw)	2.63 (0.078) 1.94 - 3.67	2.53 (0.078) 2.28 - 3.03	0.74	0.098 (0.11)	0.378

Table VI-1. Summary of Maize Grain Proximates, Linoleic Acid, Carbohydrates by Calculation and Fiber for MON 87429 and Conventional Control (continued)

Component	MON 87429 Mean (S.E.)[1] Range	Control Mean (S.E.) Range	Control Range Value[2]	Difference (Test minus Control) Mean (S.E.)	p-Value
NDF (% dw)[3]	7.82 (0.16) 6.97 - 8.63	8.05 (0.16) 6.44 - 9.80	3.36	-0.22 (0.23)	0.339

[1]Mean (S.E.) = least-square mean (standard error)
[2]Maximum value minus minimum value for the control maize hybrid
[3]dw=dry weight
[4]FA=Fatty Acid

Table VI-2. Summary of Maize Grain Essential Amino Acids for MON 87429 and Conventional Control

Component (% dw)[1]	MON 87429 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	Difference (Test minus Control) p-Value
Arginine	0.45 (0.014) 0.40 - 0.54	0.45 (0.014) 0.38 - 0.51	0.13	0.0047 (0.0068)	0.498
Histidine	0.26 (0.0091) 0.22 - 0.31	0.25 (0.0091) 0.22 - 0.30	0.085	0.0018 (0.0053)	0.739
Isoleucine	0.34 (0.013) 0.30 - 0.43	0.34 (0.013) 0.29 - 0.41	0.12	0.0013 (0.0069)	0.850
Leucine	1.20 (0.054) 0.98 - 1.56	1.22 (0.054) 1.00 - 1.51	0.52	-0.012 (0.027)	0.672
Lysine	0.26 (0.0077) 0.22 - 0.35	0.26 (0.0077) 0.21 - 0.28	0.067	0.0062 (0.0084)	0.499
Methionine	0.20 (0.0067) 0.17 - 0.25	0.20 (0.0067) 0.17 - 0.24	0.065	-0.0040 (0.0026)	0.135

Table VI-2. Summary of Maize Grain Essential Amino Acids for MON 87429 and Conventional Control (continued)

Component (% dw)[1]	MON 87429 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Phenylalanine	0.48 (0.020) 0.40 - 0.59	0.48 (0.020) 0.40 - 0.60	0.20	0.0022 (0.010)	0.845
Threonine	0.34 (0.0097) 0.30 - 0.42	0.34 (0.0097) 0.30 - 0.39	0.096	0.0010 (0.0056)	0.853
Tryptophan	0.074 (0.0016) 0.065 - 0.080	0.075 (0.0016) 0.066 - 0.083	0.017	-0.0012 (0.0016)	0.479
Valine	0.44 (0.015) 0.39 - 0.55	0.44 (0.015) 0.38 - 0.52	0.14	0.0032 (0.0072)	0.656

[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-3. Summary of Maize Grain Anti-Nutrients for MON 87429 and Conventional Control

Component (% dw)[1]	MON 87429 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Phytic acid	0.64 (0.060) 0.39 - 0.91	0.67 (0.060) 0.44 - 0.91	0.47	-0.033 (0.032)	0.355
Raffinose	0.15 (0.020) 0.097 - 0.23	0.16 (0.020) 0.11 - 0.26	0.15	-0.010 (0.0056)	0.083

[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 Forage Proximates, Carbohydrates by Calculation and Fiber for MON 87429 and Conventional Control

Component (% dw)[1]	MON 87429 Mean (S.E.)[2] Range	Control Mean (S.E.) Range	Control Range Value[3]	Difference (Test minus Control) Mean (S.E.)	p-Value
Protein	7.10 (0.20) 4.57 - 8.93	7.00 (0.20) 6.03 - 8.21	2.18	0.10 (0.22)	0.654
Total fat	2.93 (0.14) 2.20 - 3.54	2.81 (0.14) 1.58 - 3.41	1.83	0.12 (0.17)	0.507
Carbohydrates by calculation	86.32 (0.24) 84.46 - 89.56	86.51 (0.24) 85.04 - 88.11	3.07	-0.19 (0.33)	0.594
ADF	20.59 (0.92) 15.74 - 24.53	21.06 (0.92) 14.63 - 28.26	13.63	-0.47 (0.79)	0.562
NDF	33.63 (1.20) 26.99 - 44.93	34.76 (1.20) 26.95 - 42.49	15.54	-1.13 (1.39)	0.422
Ash	3.65 (0.22) 3.06 - 4.58	3.68 (0.22) 2.80 - 4.72	1.92	-0.034 (0.10)	0.743

[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. Literature and ILSI-CCDB Database Ranges for Components in Maize Grain and Forage

Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Grain Nutrients		
Proximates		
protein (% dw)	8.27-13.33[a]; 9.17-12.19[b]	5.72-17.26
total fat (% dw)	2.95-4.40[a]; 3.18-4.23[b]	1.363-7.830
ash (% dw)	1.17-2.01[a]; 1.27-1.63[b]	0.616-6.282
Amino Acids		
arginine (% dw)	0.34-0.52[a]; 0.34-0.50[b]	0.12-0.71
histidine (% dw)	0.25-0.37[a]; 0.27-0.34[b]	0.14-0.46
isoleucine (% dw)	0.30-0.48[a]; 0.32-0.44[b]	0.18-0.69
leucine (% dw)	1.02-1.87[a]; 1.13-1.65[b]	0.64-2.49
lysine (% dw)	0.26-0.33[a]; 0.28-0.31[b]	0.129-0.668
methionine (% dw)	0.17-0.26[a]; 0.16-0.30[b]	0.11-0.47
phenylalanine (% dw)	0.43-0.72[a]; 0.45-0.63[b]	0.24-0.93
threonine (% dw)	0.29-0.45[a]; 0.31-0.39[b]	0.22-0.67
tryptophan (% dw)	0.047-0.085[a]; 0.042-0.07[b]	0.027-0.215
valine (% dw)	0.42-0.62[a]; 0.45-0.58[b]	0.27-0.86
Fatty Acids		
linoleic acid (% Total FA)	49.31-64.70[a]; 56.51-65.65[b]	34.27-67.68
Carbohydrates By Calculation		
carbohydrates by calculation (% dw)	81.31-87.06[a]; 82.10-85.98[b]	77.4-89.7
Fiber		
ADF (% dw)	1.82-4.48[a]; 1.83-3.39[b]	1.41-11.34
NDF (% dw)	6.51-12.28[a]; 6.08-10.36[b]	4.28-22.64
Anti-Nutrients		
phytic acid (% dw)	0.69-1.09[a]; 0.60-0.94[b]	0.111-1.940
raffinose (% dw)	0.079-0.22[a]; 0.061-0.15[b]	0.020-0.466
Forage Nutrients		
Proximates		
protein (% dw)	5.80-10.24[a]; 5.56-9.14[b]	3.14-16.32
total fat (% dw)	1.28-3.62[a]; 0.20-1.76[b]	0.296-6.755
ash (% dw)	2.67-8.01[a]; 4.59-6.9[b]	0.66-13.20
Carbohydrates By Calculation		
carbohydrates by calculation (% dw)	81.88-89.26[a]; 84.11-87.54[b]	73.3-92.9
Fiber		
ADF (% dw)	19.11-30.49[a]; 20.73-33.39[b]	9.90-47.39
NDF (% dw)	27.73-49.62[a]; 31.81-50.61[b]	20.29-67.80

[1]dw=dry weight; FA=Fatty Acid.
[2]Literature range references: [a](Harrigan et al., 2009) (see U.S. Field data);[b](Harrigan et al., 2009) (see Chile field data).
[3]ILSI range is from ILSI Crop Composition Database, 2016 (Accessed February 21, 2017).

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides a comparative assessment of the phenotypic, agronomic, and environmental interactions characteristics of MON 87429 compared to the conventional control. The data support a conclusion that MON 87429 is unlikely to pose a plant pest risk. This conclusion is based on the results of multiple evaluations including field and laboratory assessments. MON 87429 has been planted in the U.S. under permit/notification since 2014 (Appendix A).

Phenotypic, agronomic, and environmental interactions characteristics of MON 87429 were evaluated in a comparative manner to assess plant pest potential. These assessments included seed germination and dormancy characteristics and pollen characteristics in the laboratory as well as phenotypic and agronomic characteristics and plant responses to abiotic stressors, diseases, and arthropod pests in the field. Results from these assessments demonstrate that MON 87429 does not possess 1) increased weediness characteristics; 2) increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests; or 3) characteristics that would confer a plant pest risk compared to conventional maize.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87429, 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 87429, data were collected to provide a detailed description of the phenotypic, agronomic, and environmental interactions characteristics of MON 87429. A subset of these data represents specific characteristics that are typically associated with altered plant pest potential (e.g., seed dormancy, lodging, seed loss, and environmental interactions data).

The plant characterization of MON 87429 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; and 5) plant responses to abiotic stressors, diseases, and arthropod pests. 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 87429 was compared to an appropriate conventional control that had a genetic background similar to MON 87429 but did not possess the inserted traits. In addition, multiple commercial maize hybrids developed through conventional breeding and selection (see Appendices H-J and Tables

H-1, I-1, and J-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 maize hybrids provide context for interpreting experimental results.

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

Data category	Characteristic measured (section where discussed)	Evaluation timing (setting of evaluation)	Evaluation description (measurement endpoints)
Germination, dormancy, and emergence	Germinated (VII.C.1)	Day 4 and 7 at OT[1] Day 4, 7, and 11 at SOT[1] (Laboratory)	Percentage of seed with a radicle protruding through the seed coat and greater than 1 mm in length
	Dead (VII.C.1)	Day 4 and 7 at OT Day 4, 7, and 11 at SOT (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 firm-swollen (VII.C.1)	Day 7 at OT Day 11 at SOT (Laboratory)	Percentage of seed that imbibed water and were firm to the touch but lacked any evidence of growth (viability determined by a tetrazolium test)
	Viable hard (VII.C.1)	Day 7 at OT Day 11 at SOT (Laboratory)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test)
	Early stand count (VII.C.2.1)	V2 – V5 growth stage (Field)	Number of plants per m^2
Vegetative growth	Plant height (VII.C.2.1)	R1 – R6 (Field)	Distance from the soil level to the flag leaf collar
	Days to maturity (VII.C.2.1)	R6 (Field)	Number of days from planting to kernel black layer
	Final stand count (VII.C.2.1)	Pre-harvest (Field)	Number of plants per m^2

[1] Optimum temperature OT = 20/30°C, Suboptimum temperature SOT = 10°C for seven days followed by 25°C for four days.

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

Data category	Characteristic measured (section where discussed)	Evaluation timing (setting of evaluation)	Evaluation description (measurement endpoints)
Reproductive development	Days to flowering (VII.C.2.1)	R1 (Field)	Days from planting until 50% of the plants have begun to shed pollen
	Pollen viability (VII.C.3)	R1 (Laboratory)	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen diameter (VII.C.3)	R1 (Laboratory)	Diameter of representative pollen grains
	Grain moisture (VII.C.2.1)	Harvest (Field)	Percentage moisture of harvested grain
	Seed weight (VII.C.2.1)	Harvest (Field)	Mass of 100 mature seeds, adjusted to 15.5% moisture content
	Yield (VII.C.2.1)	Harvest (Field)	Mass of harvested grain per hectare, adjusted to 15.5% moisture content
Lodging and seed retention	Lodging (VII.C.2.1)	Pre-harvest (Field)	Percentage of plants leaning >45° from vertical or broken below the ear
	Seed loss (VII.C.2.1)	Pre-harvest (Field)	Number of ears completely detached from plants in two rows
Environmental interactions	Abiotic stress response (VII.C.2.2)	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)	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)	Four times during growing season (Field)	Qualitative assessment of each plot, with categorical scale of increasing severity (none, slight, moderate, severe)

[1] Optimum temperature OT = 20/30°C, Suboptimum temperature SOT = 10°C for seven days followed by 25°C for four days.

VII.B. Data Interpretation

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 maize whose biological properties and plant pest potential are well-known. Familiarity considers the biology of the crop, the introduced traits, 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 87429 was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interactions characteristics. Based on all of the data collected, an assessment was made to determine if MON 87429 could be expected to pose an increased plant pest risk compared to conventional maize.

VII.B.1. Interpretation of Phenotypic and Agronomic Data

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

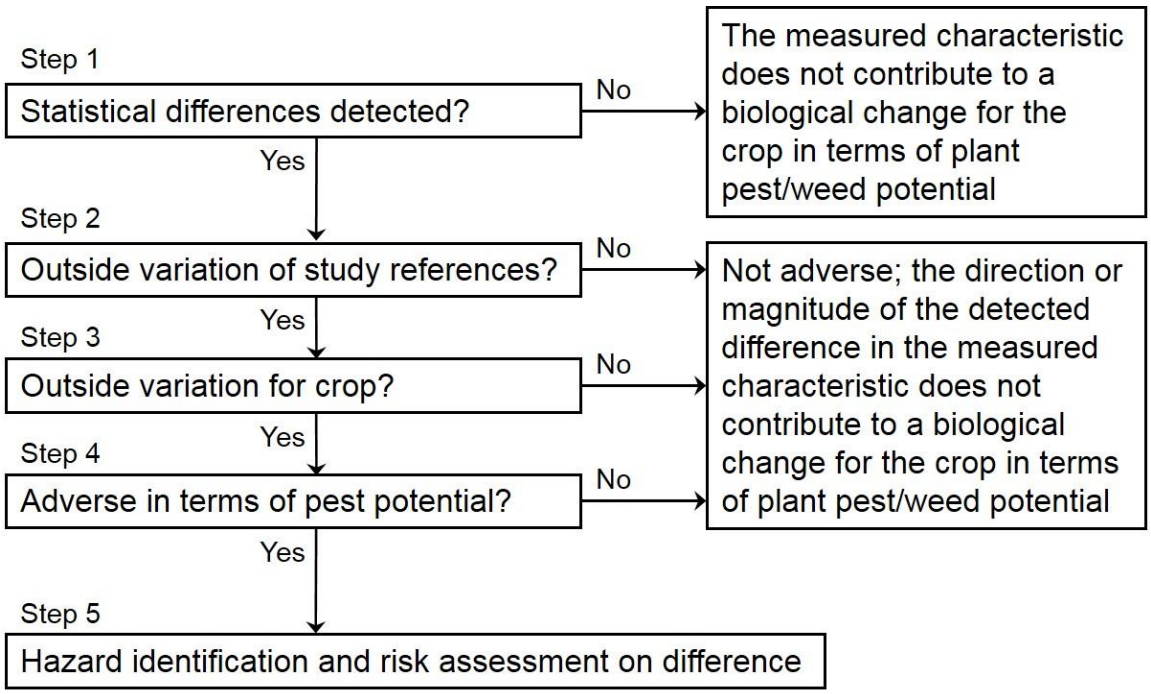


Figure VII-1. Interpretation of Statistical Differences

Step 1 – Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed. A combined-site analysis is used for multi-site data. All statistically significant differences are evaluated and considered in the context of a change in weediness or plant pest risk. Any difference detected is further assessed.

Step 2 – Evaluate Differences in the Context of Commercial Reference Materials Included in the Study

If a difference for a characteristic is detected then the mean value of the biotechnology-derived crop for the characteristic is assessed relative to the range of variation of the commercial reference materials included in the study (e.g., reference range).

Step 3 – 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 commercial reference materials 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 4 – Relevance of Difference to Weediness or plant pest risk

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 weediness or plant pest risk.

Step 5 – Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, a risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced weediness or plant pest risk 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 Environmental Interactions Data

The environmental interactions data consisting of plant responses to abiotic stressors, diseases, and arthropod pests are categorical and 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 four replications within an observation at a site.

Observations for which no differences are detected support a conclusion of no increased weediness or plant pest risk. Observations for which differences are detected are not considered to increase weediness or plant pest risk if the biotechnology-derived crop stressor responses and damage ratings are within the reference range or are not consistently observed in multiple environments in which the same stressor occurred.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interactions Characteristics of MON 87429

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 87429. The characteristics for MON 87429 evaluated in these assessments included: seed germination and dormancy characteristics (Section VII.C.1), plant phenotypic, agronomic, and environmental interactions observations under field conditions (Section VII.C.2), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendix H, Appendix I, and Appendix J.

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 regime of alternating 20°C and 30°C for testing the germination and dormancy characteristics of maize seed (AOSA, 2017a; b). The AOSA further recognizes a temperature regime of constant 10°C for seven days followed by 25°C for four days for cold testing of maize (AOSA, 2009).

A comparative assessment of seed germination and dormancy characteristics was conducted for MON 87429 and the conventional control. The seed lots for MON 87429 and the conventional control were harvested from one 2017 field production site in Kihei, Hawaii. Four reference maize hybrids were obtained from commercial sources. MON 87429 was compared to the conventional control for percentages of germinated, dead, viable firm-swollen, and viable hard seed using two temperature regimes: optimum (alternating 20°C and 30°C) and suboptimum (AOSA cold test: constant 10°C for seven days followed by 25°C for four days). The assay for each temperature regime was conducted using a randomized complete block design with four replications. Descriptions of the evaluated germination and dormancy characteristics and the timing of the evaluations are listed in Table VII-1. The materials and experimental methods are further discussed in Appendix H

In the analyses of the dormancy and germination data, no statistically significant differences ($\alpha = 0.05$) were detected between MON 87429 and the conventional control in either the optimum (20/30°C) or suboptimum (10/25°C) temperature regimes for any of the evaluated characteristics, including viable hard seed (Table VII-2). These results support the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

Table VII-2. Germination and Dormancy Characteristics of MON 87429 and the Conventional Control

Temperature Regime (°C)[1]	Characteristic[2]	Mean % (SE)[3]		Reference Range
		MON 87429	Control	
Optimum	Germinated	93.0 (1.47)	96.3 (0.48)	99.0 – 100.0
	Dead	7.0 (1.47)	3.8 (0.48)	0.0 – 1.0
	Viable firm-swollen	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Viable hard	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
Suboptimum	Germinated	94.5 (1.26)	96.3 (1.25)	99.3 – 100.0
	Dead	5.5 (1.26)	3.8 (1.25)	0.0 – 0.8
	Viable firm-swollen	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0
	Viable hard	0.0 (0.00)	0.0 (0.00)	0.0 – 0.0

Notes: No statistically significant differences were detected between MON 87429 and the conventional control (α =0.05).

[1] The optimum temperature regime was 20/30ºC for seven days; The suboptimum temperature regime was 10ºC for seven days followed by 25ºC for four days.

[2] Statistical comparisons were performed using ANOVA (optimum temperature regime) or Fisher's Exact Test (suboptimum temperature regime). No statistical comparisons were made if MON 87429 and control values were 0.

[3] N= 4 for means. S.E. = standard error.

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

Phenotypic and agronomic characteristics were evaluated under field conditions as part of the plant characterization assessment of MON 87429. These data were developed to provide USDA-APHIS with a detailed description of MON 87429 relative to the conventional control and reference maize 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 87429 and the conventional control that may impact its weediness or plant pest risk. Specific characteristics that are typically associated with weediness (e.g., lodging and seed loss) were used to assess whether there is a potential increase in weediness of MON 87429 compared to conventional maize.

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 87429. In the 2017 U.S. field trials conducted to evaluate the phenotypic and agronomic characteristics of MON 87429, data were also collected on plant responses to abiotic stressors, diseases, and arthropod pests.

Data were collected from eight 2017 field sites within maize production regions of the U.S. (Table VII-3). The test material MON 87429, the conventional control, and four commercial reference hybrids were planted at each site in a randomized complete block design with four replications. Sixteen unique references were included among the sites. Additional details on the materials and methods are presented in Appendix I.

VII.C.2.1. Phenotypic and Agronomic Assessments

MON 87429 was compared to the conventional control in a combined-site analysis for nine phenotypic and agronomic characteristics: early stand count, days to flowering, plant height, days to maturity, lodging, final stand count, moisture, seed weight, and yield. Descriptive statistics are provided for an additional characteristic, seed loss, that had insufficient variability for formal statistical analysis. Descriptions of the evaluated phenotypic and agronomic characteristics and the timing of the evaluations are listed in Table VII-1. The materials and methods are further discussed in Appendix I.

The means for MON 87429 and the conventional control for seed loss, which was excluded from formal analysis, were low (0.2 and 0.1 ears/two rows, respectively) and the mean for MON 87429 was within the reference range. These results suggest that this characteristic does not contribute to a biological change for MON 87429 in terms of plant pest potential.

In a combined-site analysis of phenotypic and agronomic characteristics of MON 87429 compared to the conventional control, no statistically significant differences ($\alpha = 0.05$) were detected between MON 87429 and the conventional control for any of the analyzed characteristics (Table VII-4). These results, together with those for seed loss, support the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

Table VII-3. Phenotypic and agronomic and Environmental Interactions Sites for MON 87429 during 2017

Site Code	County, State
IAAU	Audubon, Iowa
IARL	Jefferson, Iowa
ILCX	Vermilion, Illinois
ILMN	Warren, Illinois
INSH	Boone, Indiana
NESW	Seward, Nebraska
NEYO	York, Nebraska
OHTR	Miami, Ohio

Table VII-4. Combined-Site Analysis of Phenotypic and Agronomic Characteristics of MON 87429 Compared to the Conventional Control in 2017 U.S. Field Trials

Characteristic (units)	Mean (S.E.)[1]		Reference Range[2]
	MON 87429	Control	
Early stand count (plants/m^2)	9.0 (0.08)	9.1 (0.07)	8.5 – 9.5
Days to flowering	63.6 (0.86)	63.5 (0.81)	57.3 – 69.0
Plant height (cm)	241.2 (3.41)	243.3 (3.06)	181.5 – 251.1
Days to maturity	131.7 (1.34)	130.5 (1.61)	118.8 – 139.6
Lodging (%)	8.3 (2.03)	10.3 (3.60)	0.0 – 16.2
Seed loss (ears/two rows)	0.2 (0.13)†	0.1 (0.09)	0.0 – 0.2
Final stand count (plants/m^2)	8.4 (0.11)	8.4 (0.12)	7.8 – 9.3
Moisture (%)	18.7 (0.43)	18.6 (0.45)	15.1 – 23.6
Seed weight (g)	37.2 (0.74)	36.8 (0.75)	31.1 – 43.1
Yield (t/ha)	15.1 (0.32)	14.8 (0.34)	13.1 – 18.9

Notes: No statistically significant differences were detected between MON 87429 and the conventional control (α =0.05) in the combined-site, linear mixed model analysis. All plots at sites INSH, NESW, and NEYO were thinned to uniform density following early stand count.

† Indicates that p values could not be generated due to insufficient variability for formal statistical analysis.

[1] N = 32 for means. S.E. = standard error.

[2] Minimum and maximum mean values among 16 references, where each mean was combined over all the sites at which the reference was planted.

VII.C.2.2. Environmental Interactions Assessments

Plant responses to abiotic stressors, diseases, and arthropod pests 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. These 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, diseases, and arthropod pests potentially occurring across the season and across sites. These data were summarized and not subjected to ANOVA. For a particular stressor, all comparisons of the range of responses for MON 87429 to the range of responses for the conventional control across all observation times and sites are reported. Descriptions of the evaluated environmental interactions characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, additional details concerning the qualitative environmental interactions assessments, and detailed results of the qualitative data comparisons are further discussed and presented in Appendix I.

In an assessment of plant responses to abiotic stressors, diseases, and arthropod pests, no differences were observed between MON 87429 and the conventional control for any of the 288 observations (including 96 abiotic stressor, 96 disease, and 96 arthropod pest observations) among all observations at eight sites (Table VII-5). These results support the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

Table VII-5. Summary of Qualitative Environmental Interactions Assessments for MON 87429 during 2017

Stressor	Number of observations across all sites	Number of observations with no differences between MON 87429 and the conventional control across all sites
Abiotic stressors	96	96
Diseases	96	96
Arthropod pests	96	96
Total	288	288

No differences were observed between MON 87429 and the conventional control during any of the 96 observations (8 sites, 4 timepoints, 3 stressors) for each stressor category. MON 87429 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 87429 and the conventional control.

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 be a factor in determining 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 87429. In addition, morphological characterization of pollen produced by MON 87429 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87429 compared to the conventional control.

The viability and morphology of pollen collected from MON 87429 compared to that of the conventional control were assessed. Pollen was collected from MON 87429, the conventional control, and four commercial references grown under similar agronomic conditions at a field site in Warren County, Illinois; 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. Pollen was collected from three non-systematically selected plants per plot and stained for assessment. MON 87429 was compared to the conventional control for percentage viable pollen and pollen diameter. Descriptions of the evaluated pollen viability and morphology characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, and general pollen morphology results are further discussed and presented in Appendix J.

No statistically significant differences (α=0.05) were detected between MON 87429 and the conventional control for percentage viable pollen or pollen diameter (Table VII-6). Furthermore, no visual differences in general pollen morphology were observed between MON 87429 and the conventional control (Appendix J, Figure J-1). These results support the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

Table VII-6. Viability and Diameter of Pollen Collected from MON 87429, the Conventional Control, and the Reference Materials

Pollen Characteristic (unit)	Mean (S.E.)[1]		Reference Range[2]
	MON 87429	Control	
Viability (%)	98.9 (0.37)	99.1 (0.32)	98.2 – 99.1
Diameter (µm)	85.1 (0.45)	85.1 (0.34)	83.4 – 86.7

Note: No significant differences were detected between the MON 87429 and the conventional control (α=0.05).

[1] MON 87429 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 reference materials.

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 statistically significant 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 phenotypic, agronomic, and environmental interactions data were used to assess whether the introduction of the dicamba, glufosinate, quizalofop, and 2,4-D-tolerance and RHS traits altered the plant pest potential of MON 87429 compared to the conventional control, considered within the context of the variation among the reference maize 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 responses to abiotic stressors, diseases, and arthropod pests. Within these data categories, specific characteristics typically associated with weediness were also assessed to determine whether there was a potential increase in weediness of MON 87429 compared to conventional maize.

Results from these assessments comparing MON 87429 and the conventional control support the conclusion that MON 87429 does not possess: 1) increased weediness characteristics; 2) increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests; or 3) characteristics that would confer a plant pest risk compared to conventional maize. Therefore, based on the results of multiple assessments discussed above and presented in the appendices, the weight of evidence supports the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize and is unlikely to pose a plant pest risk.

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 87429 maize 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.C demonstrated that MON 87429 maize is no more susceptible to diseases or pests than commercially cultivated maize. Additionally, data presented support that, with the exception of the introduced traits, MON 87429 maize is phenotypically similar to conventional maize and is not expected to pose a greater plant pest risk compared to conventional maize. Thus, except for greater diversity in herbicide chemistry tolerance that will provide greater flexibility in weed control options, there are no expected changes to the inputs needed for MON 87429 maize production, 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 U.S. 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, plant population density, soil fertility, water availability 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. Maintaining weed populations below economic thresholds in maize requires 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 date, plant population and row spacing), 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 an herbicide application (USDA-NASS, 2019b).

As shown in Sections VI and VII, with the exception of introduced traits, no biologically meaningful phenotypic, compositional, or environmental interaction differences between MON 87429 maize and conventional maize have been observed. Moreover, herbicide-tolerant maize is currently grown on approximately 90% of U.S. maize acres (USDA-ERS, 2018b). Dicamba, glufosinate, quizalofop, 2,4-D and glyphosate herbicides are

currently labeled for preplant and postemergence applications in maize. In support of Monsanto's third generation herbicide tolerant product MON 87419 maize, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first (U.S. EPA, 2019). The combined (pre- and post-emergence) maximum annual application rate of dicamba in MON 87419 maize would be 2.0 lbs. a.e. dicamba per acre per year, increased from the current maximum annual application rate of 0.75 lbs. a.e. in conventional maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. Glufosinate, quizalofop and 2,4-D herbicides are currently labeled for preplant applications on conventional and herbicide-tolerant maize hybrids and for in-crop postemergence applications on herbicide-tolerant (HT) hybrids. The intended preplant and postemergence uses of these herbicides with MON 87429 maize would not be any different than current labeled uses in HT maize and therefore does not require a label change. The MON 87429 event confers glyphosate tolerance in specific plant tissues (i.e., not in tassels) and will be used to facilitate the production of hybrid seed. MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate tolerance (e.g., NK603). The combination of herbicide-tolerance traits will allow the preplant and postemergence use of dicamba, glufosinate, quizalofop, 2,4-D and glyphosate herbicides in an integrated weed management program in maize with multiple sites-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 87429 maize in the U.S. would have a notable impact on current maize production practices, beyond the intended benefits of effective management of common, troublesome weeds, and/or herbicide-resistant weeds and additional options for growers to rotate and/or use combinations of herbicides with multiple sites-of-action for preplant and in-crop postemergence herbicide applications.

Hybridization is a fundamental concept used in maize breeding and production programs in the U.S. and most of the world. The fixation of alleles in pure lines (i.e., inbreds) causes a general reduction in maize vigor and productivity, but maize grown from the F1 hybrid seed produced through crossing two inbred lines has improved vigor (e.g., heterosis) compared to the open pollinated varieties. Modern maize breeding is based on selecting inbred lines and producing crosses that possess desirable traits. Techniques such as marker-assisted selection can also reduce the time and cost required to achieve breeding goals (Yousef and Juvik, 2001).

Tissue-specific expression of CP4 EPSPS protein in MON 87429, allowing for glyphosate induced non-viable pollen phenotype, is the second generation of Monsanto's Roundup® Hybridization System (RHS) for hybrid seed production. The first-generation RHS event, MON 87427 maize, was deregulated by USDA in 2013 (USDA-APHIS Petition #10-281-01p). Similar to the MON 87427 RHS trait, the second-generation RHS trait in MON 87429 allows inbred MON 87429 lines, treated with glyphosate at the appropriate timings (V8 to V13) to serve as female parents in the production of hybrid seed. Inbred MON 87429 lines receive two glyphosate applications at vegetative growth stages ranging from V8 to V13, resulting in the intended non-viable pollen phenotype due

to tissue-specific glyphosate tolerance. An additional benefit of including the RHS trait in MON 87429 maize at a single locus with dicamba, glufosinate, quizalofop and 2,4-D tolerance traits, is the reduction in the number of trait loci that would otherwise need to be managed and combined, via traditional breeding methods.

In hybrid maize production systems, inbred MON 87429 plants treated with glyphosate during tassel development will be pollinated by male pollen donor plants that contain a glyphosate tolerance trait (e.g., NK603). Male donor inbred plants are cultivated in proximity to female inbred MON 87429 plants and the male donor inbred plants are not impacted by the glyphosate applications due to full glyphosate tolerance in all tissues. This cross results in hybrid offspring (e.g., MON 87429 × NK603) with full-plant tolerance to glyphosate, both in vegetative and reproductive tissues, as well as tolerance to dicamba, glufosinate, quizalofop, and 2,4-D herbicides.

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

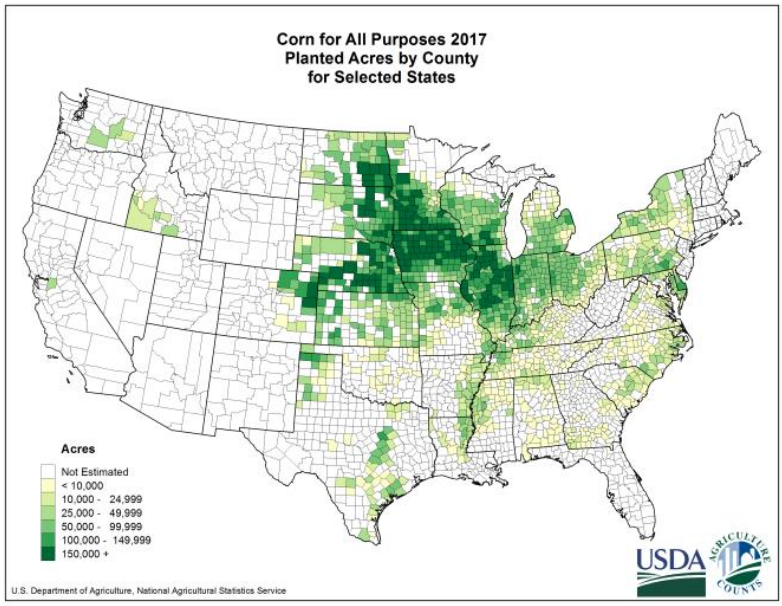
VIII.B.1. Maize Grain Production

The U.S., China, Brazil, European Union and Argentina are the top five countries/regions producing maize globally (USDA-FAS, 2019). The U.S. is the largest producer of maize (*Zea mays*), producing approximately 35% of the world maize production in 2017/18 (USDA-FAS, 2019). China follows with approximately 24% of the maize production. Maize for all purposes was planted on approximately 89 million acres in 2018 in the U.S. (Table VIII-1), consistent with soybean acres and more than the 47.8 million wheat acres (USDA-NASS, 2019c). Much of that production occurs in the upper Midwest States (Figure VIII-1). The 2018 maize acreage was down 1.04 million acres from 2017 (Table VIII-1). Approximately 81.7 million acres were harvested for grain in 2018 and 6.1 million acres were harvested for silage in 2018 (USDA-NASS, 2019c). Total maize grain production was approximately 14.6 billion bushels in 2018 with an average yield of 178.6 bushels per acre (Table VIII-1). The value of maize grain production reached $48.46 billion in the U.S. in 2017 (Table VIII-1). The value of maize production in the U.S. has ranged from $46.73 to $76.94 billion in the past 10 years. The principal uses of maize are feed and residuals, ethanol fuel, exports, food, seed and industrial uses (Capehart et al., 2018).

Table VIII-1. Field Maize Production in the U.S., 2007-2018[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2018	89,140	81,767	178.9	14,625,974	-[2]
2017	90,167	82,703	176.6	14,604,067	48.46
2016	94,004	86,748	174.6	15,148,038	51.30
2015	88,019	80,753	168.4	13,601,964	49.34
2014	90,597	83,136	171.0	14,215,532	52.95
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

[1]Source: (USDA-NASS, 2018a)
[2]Data not available



Source:(USDA-NASS, 2018b)
Figure VIII-1. U.S. Maize Acreage by County in 2017

VIII.B.2. Maize Seed Production

Commercial hybrid maize seed production is a labor intensive process. Single cross hybrid maize seed production involves planting male and female parent inbreds in separate rows or blocks in an isolated field. The female parent inbred is prevented from shedding pollen to prevent self-pollination and ensure pollination only by the intended male parent inbred. The male parent inbred is usually destroyed by mechanical means

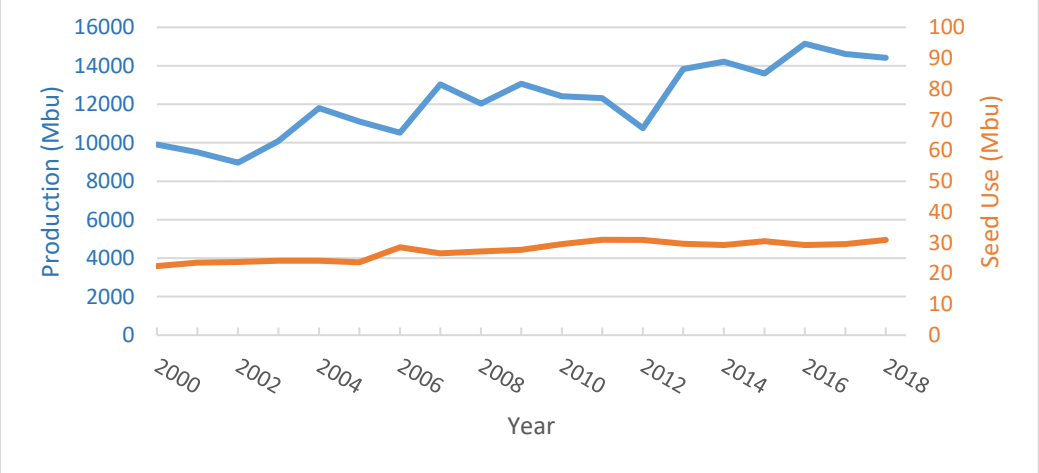
following pollination to prevent seed mixing during harvest. Ears that are produced from the cross-pollinated female parent inbred are harvested, processed, and the seed sold to farmers for planting as hybrid maize seed.

Maintaining an adequate supply of the parental inbred lines is vital to producing an adequate supply of hybrid maize seed. Often referred to as foundation seed, parental inbred lines are produced and maintained under strict isolation in the production field to preserve the identity and integrity of the genetics within each inbred. Quality control checks performed during the production of inbreds include visual inspections of the plants grown in isolation, and the use of molecular tools to verify the genetics of each inbred line (Gowda et al., 2017).

Maize seed production is not expected to be affected by the introduction of MON 87429. Over the last 18 years, the volume of hybrid maize seed planted in the U.S. has increased from 22.45 million bushels (MBu) planted in 2000 to 30.90 MBu planted in 2018 (USDA-ERS, 2018a). Grain yields have increased significantly over this same period from 9,915 MBu in the year 2000 to 14,420 MBu in 2018 (Figure VIII-2).

A number of factors must be considered in hybrid maize seed production, including: 1) forecasting the quantity of specific hybrid maize seed that will be needed at least a year in advance; 2) selection of a production area that mitigates risks and maximizes the yield of hybrid maize seed; and 3) agronomic practices. Key considerations and practices for producing hybrid maize seed are described in detail in MON 87427 USDA-APHIS Petition #10-281-01p (p. 123), the first generation RHS event and include: input considerations, control of weeds, disease and pests, plant density, plot isolation, parent delay to synchronize flowering, pollen control methods such as detasseling and cytoplasmic male sterility, harvesting and labeling requirements. These practices may differ from those used in commercial maize grain production.

The practices for producing hybrid maize seed using MON 87429 are generally similar to those using manual or mechanical detasseling methods or cytoplasmic male sterility (CMS), except for the use of glyphosate at early tassel development timings to confer the male sterile phenotype through tissue-specific glyphosate tolerance.



Source: (USDA-ERS, 2018a)

Figure VIII-2. Hybrid maize seed planted, and grain produced in the U.S. from 2000-2018.

VIII.C. Production Management Considerations

MON 87429 provides tolerance to the same herbicides (glufosinate, quizalofop and 2,4-D, and tissue-selective glyphosate) as several other events present in herbicide tolerant maize hybrids being grown in the U.S. (e.g., Genuity® SmartStax®, Genuity® VT Double PRO®, LibertyLink®, Enlist® maize, and first generation RHS MON 87427 maize), or for dicamba, events previously deregulated by USDA-APHIS (Petition #15-113-01p). With the widespread use of herbicide tolerant maize hybrids since 1997 (USDA-ERS, 2018b), it is anticipated that no major changes in production management practices will occur beyond the intended benefits of more effective and improved management of common, troublesome and/or herbicide-resistant weeds and the opportunity for growers to rotate and/or use combinations of herbicides with multiple sites-of-action for preemergence and in-crop postemergence herbicide applications. Cultivation practices for hybrid maize, containing the RHS trait in MON 87429, used in grain production will not differ from other commercially available, glyphosate tolerant maize.

The RHS trait in MON 87429 maize offers the same benefits to hybrid maize seed production as the RHS trait in MON 87427, described in detail in USDA-APHIS Petition #10-281-01p (p. 5). Briefly, these benefits include enabling maize hybrid seed producers to discontinue the practice of manually or mechanically detasseling female inbred plants in their production field, which must occur during a critical 3-4 day time period of maize tassel development that can be influenced by changes in weather (e.g., extreme heat). The ability to treat MON 87429 maize inbreds with glyphosate (between V8 to V13), in

® Genuity, SmartStax, and Genuity VT DoublePRO are registered trademarks of Monsanto Technology LLC.
® LibertyLink is a registered trademark of BASF.
®Enlist is a registered trademark of Dow AgroSciences, DuPont or Pioneer and their affiliated companies or respective owners.

place of detasseling, provides flexibility to hybrid maize seed producers as well as reduces the cost of hybrid seed production by removing the reliance on costly, labor intensive manual/mechanical detasseling. For weed management during the production of hybrid seeds using MON 87429, seed producers will be provided best management practices for herbicide resistance management, including the use of herbicides with multiple sites-of-action. An additional benefit of including the RHS trait in MON 87429 maize at a single locus with herbicide tolerance traits is the reduction in the number of trait loci that would otherwise need to be managed and combined, via traditional breeding methods. Further, the amount of glyphosate recommended for use with MON 87429 maize for hybrid seed or grain production will not exceed the total amount of glyphosate already approved for in crop use with other commercially available, glyphosate tolerant maize events.

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 87429 maize does not contain insect protection traits, therefore the information on this subject is incorporated here by reference (USDA-APHIS Petition #13-290-01p p. 166). 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 foliar application of insecticides, or use of crop rotation or other 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 87429 was developed to improve the management of weeds and has no unique insect pest control attributes. Environmental observations in field studies indicated that the presence of the dicamba, glufosinate, quizalofop, and 2,4-D-tolerance and RHS traits did not meaningfully alter the susceptibility of MON 87429 maize to arthropod-related damage (Section VII.C). Therefore, no changes in current insect pest management practices in maize are anticipated from the introduction of MON 87429.

VIII.E. Management of Diseases and Other Pests

Monsanto summarized major issues associated with the management of diseases and non-insect pests in its petition for nonregulated status for Corn Rootworm-Protected Maize MON 87411 (USDA-APHIS Petition #13-290-01p). MON 87429 does not contain disease protection traits, therefore the information on this subject is incorporated here by reference (USDA-APHIS Petition #13-290-01p p. 171). Briefly, management of diseases and pests of maize are important to protecting the yield of harvested grain. Disease and pest incidence vary from year to year and growers may choose to use pesticides or a variety of management practices to control problematic diseases or pests.

MON 87429 was developed to improve the management of weeds and has no unique attributes for control of diseases or other pests. Environmental observations in field studies indicated that the presence of the dicamba, glufosinate, quizalofop, and 2,4-D-

tolerance and RHS traits did not meaningfully alter the susceptibility of MON 87429 maize to diseases or other pests (Section VII.C). Therefore, no changes in current management practices for diseases or other pests in maize are anticipated from the introduction of MON 87429 maize.

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 in 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; Knezevic and Datta, 2015). Some weeds can tolerate cold, wet conditions better than maize, and can gain an advantage prior to planting. A number of weeds compete with maize and reduce yield significantly with delay in weed control. Some of these weeds are difficult to control since they have similar life cycle and growth habit as those of maize plant (Jhala et al., 2014). Annual weed species such as giant foxtail (*Setaria faberi*), barnyardgrass (*Echinochloa crus-galli* (L.) *Beauv*.) and Palmer amaranth (*Amaranthus palmeri*) have been shown to reduce maize yields by up to 13% (Fausey et al., 1997; Knake and Slife, 1965), 35% (Bosnic and Swanton, 1997) and 91% (Massinga et al., 2003), respectively. A compilation of data from 2007-2013 on weeds impacting yield in North America showed that uncontrolled weeds in maize could reduce yield at an average of 50% annually (Soltani et al., 2016). 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).

A case study on herbicide tolerant field maize within a larger survey involving 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, at least twelve annual broadleaf, nine annual grass, and seven perennial species were identified as troublesome weeds (Table VIII-2) (Gianessi et al., 2002). Estimates of yield loss in this case study ranged from a low of 15% due to wirestem muhly and sandburs to a high of 48% from bur cucumber.

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in maize. A national weed survey conducted in 2015 has identified Palmer amaranth (*Amaranthus palmeri*), common lambsquarters (*Chenopodium album*), Canada thistle (*Cirsium arvense*), kochia (*Bassia scoparia*), common waterhemp (*Amaranthus rudis*), giant ragweed (*Ambrosia trifida*), morningglory spp (*Ipomoea* spp.), yellow nutsedge (*Cyperus esculentus*), common ragweed (*Ambrosia artimisiifolia*) and

downy brome (*Bromus tectorum*) as top 10 most troublesome weeds in the US (Van Wychen, 2015). Waterhemp, Palmer amaranth, morningglory, giant ragweed, horseweed and johnsongrass are common troublesome weeds in Midwest fields. The most frequently reported common weeds in the Southeast region are pigweeds (*Amaranthus* spp.), morningglory, crabgrass (*Digitaria* spp.), nutsedge (*Cyperus* spp.) and broadleaf signalgrass (*Brachiaria platyphylla*) (Webster et al., 2012). Morning glory, pigweeds, nutsedge, johnsongrass (*Sorghum halepense*), crabgrass and horseweed (*Conyza canadensis*) are the most frequently mentioned troublesome weeds in the Southeast region (Webster et al., 2012).

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 (Timmons, 2005). In 2018, herbicides were applied to 98% of the planted maize acreage (USDA-NASS, 2019b). Glyphosate is the most widely applied herbicide in maize being applied on [ ] of the planted acreage (Table VIII-3). Triketones and Isoxazole are the second most widely used group of herbicides at [ ] followed by chloroacetamide and isoxazoline herbicides at [ ] and Triazines (atrazine, metribuzin, simazine) at [ ] [ ]. CBI-Deleted CBI-Deleted CBI-Deleted, CBI-Deleted CBI-Deleted

In 2018, approximately 90% of the total maize acreage in the U.S. was planted with hybrids possessing biotechnology-derived herbicide-tolerance traits (USDA-ERS, 2018b). Maize hybrids possessing both herbicide-tolerance and insect-protected traits were planted on 80% of the maize acreage in 2018 (USDA-ERS, 2018b). The introduction of herbicide-tolerant crops such as Roundup Ready®, LibertyLink® and recently Enlist® maize, have offered growers an alternative and effective solution for the weeds control in maize by enabling in-crop postemergence use of glyphosate, glufosinate, quizalofop and 2,4-D herbicides. Although these herbicides (glyphosate, glufosinate, quizalofop and 2,4-D) provide control of numerous annual and perennial weed species, preemergence herbicides are a key component of weed control programs in maize. This is evident in the widespread use of triketones, chloroacetamide and triazine herbicides in maize (Table VIII-3, Table VIII-4 and Table VIII-5). 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.

Table VIII-4 and Table 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 an herbicide program in maize, which offers the

® LibertyLink is a registered trademark of BASF.
®Enlist is a registered trademark of Dow AgroSciences, DuPont or Pioneer and their affiliated companies or respective owners.

best overall control of the weed species present. Generally, a mixture or premixture of two or more herbicide active ingredients is needed to achieve broad spectrum control of both grasses and broadleaf weed species. However, glyphosate, glufosinate, tembotrione, and topramezone each provide control of numerous 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 (*Bassia scoparia*)	NP/NW	10-70	33
Lambsquarters (*Chenopodium album*)	MW/SE/NE/CA	15-80	33
Morningglory (*Ipomoea* spp.)	MW/SE/SP	20-75	33
Nightshade (*Solanum* spp.)	MW/NP/CA	25-50	26
Pigweeds/Waterhemp (*Amaranthus* spp.)	US	30-90	36
Ragweed, Common (*Ambrosia artemisiifolia*)	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*)	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*)	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 = Northwest, SE = Southeast, SW = Southwest, SP = Southern Plains.

Table VIII-3. Herbicide Applications in Maize in 2018 in the U.S.[1]

Herbicide	Chemical Family	Site-of-Action[2] (SOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per SOA	
Glyphosate	Glycine	EPSPS inhibitor	[ ]	[ ]	CBI-Deleted, CBI-Deleted
Atrazine	Triazine	PSII inhibitor	[ ]	[ ]	CBI-Deleted
Metribuzin	Triazine		[ ]		CBI-Deleted, CBI-Deleted
Simazine	Triazine		[ ]		CBI-Deleted
Acetochlor	Chloroacetamide	Long-chain fatty acid inhibitor	[ ]	[ ]	CBI-Deleted
Alachlor	Chloroacetamide		[ ]		CBI-Deleted
Dimethenamid	Chloroacetamide		[ ]		CBI-Deleted, CBI-Deleted
Metolachlor	Chloroacetamide		[ ]		CBI-Deleted
Pyroxasulfone	Isoxazoline		[ ]		CBI-Deleted
Isoxaflutole	Isoxazole	HPPD inhibitor	[ ]	[ ]	CBI-Deleted
Mesotrione	Triketone		[ ]		CBI-Deleted
Tembotrione	Triketone		[ ]		CBI-Deleted, CBI-Deleted
Topramezone	Triketone		[ ]		CBI-Deleted
Bicyclopyrone	Triketone		[ ]		CBI-Deleted

Table VIII-3. Herbicide Applications in Maize in 2018 in the U.S.[1] (continued)

Herbicide	Chemical Family	Site-of-Action[2] (SOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per SOA	
2,4-D	Phenoxy	Synthetic Auxin	[ ]	[ ]	CBI-Deleted
Clopyralid	Carboxylic acid		[ ]		CBI-Deleted
Dicamba	Benzoic acid		[ ]		CBI-Deleted, CBI-Deleted
Fluroxpyr	Caryridine Carboxylic acid		[ ]		CBI-Deleted
Flumetsulam	Imidazolinone	ALS inhibitor	[ ]	[ ]	CBI-Deleted
Halosulfuron	Sulfonylurea		[ ]		CBI-Deleted
Nicosulfuron	Sulfonylurea		[ ]		CBI-Deleted
Primisulfuron	Sulfonylurea		[ ]		CBI-Deleted
Prosulfuron	Sulfonylurea		[ ]		CBI-Deleted, CBI-Deleted
Rimsulfuron	Sulfonylurea		[ ]		CBI-Deleted
Thifensulfuron	Sulfonylurea		[ ]		CBI-Deleted
Thiencarbazone	Triazolones		[ ]		CBI-Deleted
Tribenuron	Sulfonylurea		[ ]		CBI-Deleted

Table VIII-3. Herbicide Applications in Maize in 2018 in the U.S.[1] (continued)

Herbicide	Chemical Family	Site-of-Action[2] (SOA)	Percent of Maize Acres Treated	Percent of Maize Acres Treated per SOA	
Diflufenzopyr	Semicarbazone	Auxin transport	[ ]	[ ]	CBI-Deleted, CBI-Deleted
Fluthiacet	Thiadiazole	PPO inhibitor	[ ]	[ ]	CBI-Deleted
Carfentrazone	Aryl triazone		[ ]		CBI-Deleted CBI-Deleted
Saflufenacil	Pyrimidinedione		[ ]		CBI-Deleted
Flumioxazin	N-phenylphthalimide		[ ]		CBI-Deleted
Paraquat	Bipyridylium	Photosystem-I-electron diverter	[ ]	[ ]	CBI-Deleted, CBI-Deleted
Glufosinate	Phosphinic acid	Glutamine Synthase Inhibitor	[ ]	[ ]	CBI-Deleted, CBI-Deleted
Pendimethalin	Dinitroanaline	Microtubule inhibitor	[ ]	[ ]	CBI-Deleted, CBI-Deleted

[1][ ] CBI-Deleted

[2](WSSA, 2018)

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

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

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

Herbicide/Application	CT[3]	Common Grass Weeds[1,2]											
		BY	BS	CG	FP	FTg	FTy	GG	SC	JGr	JGs	IR	NSy
		Postemergence											
2,4-D	2	-	0	-	-	-	-	1	-	0	0	0	-
2,4-D/atrazine	2	-	NA	-	-	6	6	NA	-	NA	NA	NA	-
Atrazine	1	7	7	-	-	8	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	6	3	-	0	5	0	-
Fluroxypyr	1	-	NA	-	-	-	-	NA	-	NA	NA	NA	-
Flumiclorac	2	-	NA	-	-	-	-	NA	-	NA	NA	NA	-
Fluroxypyr/clopyralid	1	-	NA	-	-	-	-	NA	-	NA	NA	NA	-
Fluthiacet	2	-	NA	-	-	-	-	NA	-	NA	NA	NA	-
Glufosinate	0*	7	8	8	8	8+	7	5	8	7	8	6	-
Glyphosate	0*	8	9	8	8	9	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	9	NA	9	8	9	6	6
Nicosulfuron/rimsulfuron	1/2	8	NA	-	8	9	9	NA	9	NA	NA	NA	-
Primisulfuron	2	-	NA	-	8	7	7	NA	9	NA	NA	NA	6
Primisulfuron/dicamba	2	-	NA	-	7	6	6	NA	9	NA	NA	NA	-
Prosulfuron/primisulfuron	2	-	NA	-	7	6	6	NA	9	NA	NA	NA	-
Rimsulfuron/ thifensulfuron	1	7	NA	-	7	7	7	NA	7	NA	NA	NA	-
Rimsulfuron/mesotrione	1	7	7	-	7	7	7	8	7	7	9	4	-
Tembotrione	0	8	8	6	-	7	9	7	8	5	6	NA	-
Thiencarbazone/tembotrione	1	8	8	8	8	8+	9	NA	8	5	7	NA	-
Topramezone	0	7	6	7+	6	7+	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, JGs and IR are from the 2015 Weed Control Guidelines for Mississippi, Mississippi State University (Mississippi State University, 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, NA= data not available in (Mississippi State University, 2015). 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, FTg = giant foxtail, FTy= 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. Source: Loux et al., 2014.

*Rating based on glufosinate to Liberty Link® maize and glyphosate applied to Roundup Ready® Corn 2 maize.

Table VIII-5. Broadleaf Weed 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	8+	NA	NA	-	-	8
Acetochlor/atrazine	9	8	9	8	9	8	6	9	9	8	6	9	8	9
Acetochlor/flumetsulam/clopyralid	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	8	NA	NA	-	-	8
Flumetsulam	8	7	7	-	9	-	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	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	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	-	3	9	8	7	NA	-	7	8
Pyroxasulfone/fluthiacet	8		7	-	8	-	3	9	8	7	NA	-	7	8
Pyroxasulfone/fluthiacet/atr	9	7	9	6	9	7	7	9	9	8	8	9	8	9
Rimsulfuron/ thifensulfuron	-	-	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	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	-	0	7	9	0	0	-	-	8

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

Herbicide/Application	Common Broadleaf Weeds[1,2]													
	BN	CB	CR	GR	LQ	MG	HS	PA	PW	PS	SP	SW	VL	WH
						Postemergence								
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	-	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	-	-	-
Dicamba	8	9	9	9	8	9	9	8	8	8	8	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	9	NA	NA	-	9	7
Fluroxypyr/clopyralid	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+	-

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

	Common Broadleaf Weeds[1,2]													
Herbicide/Application	BN	CB	CR	GR	LQ	MG	HS	PA	PW	PS	SP	SW	VL	WH
Rimsulfuron/ thifensulfuron	-	6	6	-	7	-	NA	NA	8	NA	NA	6	7	-
Rimsulfuron/mesotrione	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, PA, PS, and SP are from the 2015 Weed Control Guidelines for Mississippi, Mississippi State University (Mississippi State University, 2015). Weed control ratings for weeds, except HS, 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, PA, PS, and SP are: 9-10 = excellent, 7-8 = good, 4-6 = fair, 0-3 = none to slight, NA= data not available in (Mississippi State University, 2015). 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.

*Rating based on glufosinate to Liberty Link® maize and 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 sites-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 (*Bassia scoparia*) and prickly lettuce (*Lactuca serriola*) (Heap, 2019). Additionally, a population of lambsquarters (*Chenopodium album*) has been confirmed as resistant to dicamba in New Zealand, common hempnettle (*Galeopsis tetrahit*), kochia and wild mustard (*Sinapis arvensis*) in Canada, smooth pigweed (*Amaranthus hybridus*) in Argentina, wild mustard in Turkey and bachelor's button/cornflower *(Centaurea cyanus)* in Poland have been confirmed as resistant, for a total of 7 species worldwide with confirmed resistance to dicamba (Heap, 2019). Currently in the U.S., six grass species (of which three are common weeds in maize; Table VIII-6) and 11 broadleaf species (of which eight are common weeds in maize; Table VIII-6) have been confirmed to have resistance to glyphosate (Heap, 2019). Dicamba provides good to excellent control of all eight of the common broadleaf species resistant to glyphosate. These broadleaf weed species, except kochia biotypes in Kansas state, have not been reported to have populations that are resistant to both glyphosate and dicamba in the U.S. (Heap, 2019). There are a total of 3 weed species worldwide with biotypes that have resistance to glufosinate. The first species resistant to glufosinate in the U.S. was recently confirmed in a glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) population (Avila-Garcia and Mallory-Smith, 2011). Additionally, Italian ryegrass, perennial ryegrass (*L. perenne*) populations in New Zealand (Ghanizadeh et al., 2015), goosegrass (*Eleusine indica*) from Malaysia (Seng et al., 2010) and rigid ryegrass (*L. rigidum*) in Greece (Travlos et al., 2018) have been confirmed resistant to glufosinate. For quizalofop herbicide, johnsongrass (*Sorghum halepense*), Italian ryegrass, giant foxtail (*Setaria faberi*), cheatgrass (*Bromus tectorum*) and common wild oat (*Avena fatua*) have been reported to be resistant in the US (Heap, 2019); and for 2,4-D herbicide, a total of 5 broadleaf weeds (waterhemp, Palmer amaranth, prickly lettuce, wild carrot (*Daucus carota*) and buckhorn plantain (*Plantago lanceolata*) have been confirmed to be resistant in the US (Heap, 2019). A discussion regarding the usefulness of MON 87429 in management of herbicide resistant weeds can be found in Section VIII.F.4. The potential for development of weeds resistant to dicamba, glufosinate, quizalofop, 2,4-D and glyphosate resistance can be found in Appendix K.

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

	Site-of-action											
Weed Species[2]	**ACCase Inhibitors**	**ALS Inhibitors**	**Long Cbain Fatty Acid 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[3]	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		X
Goosegrass				X	X	X				X	X	
Johnsongrass	X	X		X	X							
Shattercane		X										
Yellow nutsedge		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	X			
Lambsquarters		X				X						
Palmer amaranth		X	X	X	X	X	X		X		X	
Prickly sida		X										
Pigweed spp. (waterhemp, redroot, smooth, Powell,)		X	X		X	X	X	X	X		X	
Russian thistle		X			X							
Smartweed spp. (Pennsylvania, ladysthumb)						X						
Sunflower		X			X							
Velvetleaf						X						

[1] (Heap, 2019). [2]Weed species and herbicide sites-of-action listed are only those common in maize.
[3]Indicates confirmed resistance in the U.S.

VIII.F.3. Impact of MON 87429 Maize Introduction on Weed Management Practices

Monsanto has developed a new herbicide-tolerant MON 87429 maize, that can provide growers additional options for an effective and sustainable weed management system. MON 87429 maize offers dicamba, glufosinate, quizalofop and 2,4-D-tolerance, and enables RHS hybrid seed production. For commercial use, MON 87429 maize will be combined with deregulated glyphosate tolerance traits, such NK603, via traditional breeding techniques to provide full-plant tolerance to glyphosate. The combination of herbicide-tolerance traits will allow the preplant and postemergence use of dicamba, glufosinate, quizalofop, 2,4-D, and glyphosate herbicides in an integrated weed management program to control a broad spectrum of grass and broadleaf weed species (Ganie and Jhala, 2017; Johnson et al., 2010). Dicamba, glufosinate and 2,4-D will provide additional options to control hard to control broadleaf weeds in addition to glyphosate (e.g., hemp sesbania, morningglory species, and prickly sida) and these herbicides (dicamba, glufosinate and 2,4-D) also offer an effective control option for glyphosate-resistant broadleaf weed species, namely marestail, common ragweed, giant ragweed, Palmer amaranth and waterhemp when used according to the label (Ganie and Jhala, 2017; Johnson et al., 2010). These herbicides will also offer an effective control option for broadleaf species resistant to acetolactate synthetase (ALS) and protoporphyrinogen oxidase (PPO) chemistries. Similarly, quizalofop and glufosinate herbicides, in addition to glyphosate provide options to control grass weeds effectively, when used according to the label. With the introduction of MON 87429 maize, 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 87429 maize will be stacked with deregulated glyphosate tolerance traits, such as NK603, to provide full plant tolerance to glyphosate, 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 (Monsanto, 2017).

Dicamba is currently authorized by U.S. EPA for preplant and/or early postemergence application in conventional maize. In support of Monsanto's third generation herbicide tolerant product MON 87419 maize, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first (U.S. EPA, 2019). The combined (pre- and post-emergence) maximum annual application rate of dicamba in MON 87419 maize would be 2.0 lbs. a.e. dicamba per acre per year, increased from the current maximum annual application rate of 0.75 lbs. a.e. in conventional maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. Growers will be able to apply dicamba alone or tank mixed with glyphosate or in sequence with glyphosate or glufosinate on MON 87429 maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. 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 (Bayer CropScience, 2016). Glufosinate use in MON 87429 maize will not change from current labeled uses of glufosinate. Growers will be able to apply glufosinate alone and in sequence with dicamba, 2,4-D or quizalofop for preplant or postemergence in-crop applications on MON 87429 maize.

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) (Bayer CropScience, 2016).

Quizalofop herbicide can cause severe crop injury in conventional maize. U.S. EPA has authorized the use of quizalofop on herbicide-tolerant maize for over-the-top application from V2 through V6 stages of maize development for managing grass weeds (DuPont, 2018). Quizalofop use in MON 87429 maize will not change from current labeled uses of quizalofop on herbicide-tolerant maize.

2,4-D has been used for many decades as preplant and postemergence herbicide for control of broadleaf weeds in maize fields and many other weed control applications including other crops, pastures and rangeland. 2,4-D with relative selectivity for broadleaf plants, can cause injury to conventional maize depending on the growth stage and method of application. U.S. EPA has authorized the use of 2,4-D with choline salt for over-the-top application on herbicide-tolerant maize (Dow, 2017) up to the V8 stage of maize development while keeping the total maximum seasonal rate per acre same as its use in conventional maize. 2,4-D use in MON 87429 maize is not anticipated to change from current labeled uses of 2,4-D on herbicide-tolerant maize.

The MON 87429 event confers glyphosate tolerance in specific plant tissues (i.e., not in tassels) and will be used to facilitate the production of hybrid seed. MON 87429 is not intended to be offered for commercial use as a stand-alone product, but will be combined, through traditional breeding methods, with other deregulated events that confer full-plant glyphosate tolerance (e.g., NK603). The general weed management 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 increase the likelihood that multiple effective herbicide sites-of-action are used in maize and to provide protections against additional resistance development to existing maize herbicides. The variability in herbicide recommendations will be dictated by variations in weed spectrum, tillage systems, and environment across these regions and is consistent with Monsanto and academics recommendations for a comprehensive weed resistance management program. These recommendations shown in Table VIII-7 are a high-end estimate of anticipated herbicide use associated with MON 87429 maize combined with glyphosate-tolerant maize.

Table VIII-7. Anticipated Weed Management Recommendations for MON 87429 Maize 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[5]**	**Hard to Control Weeds[2]**	**GR[3] Weeds and Hard to Control Weeds[5]**
Preemergence (burndown, at planting)	Residual	Residual + Dicamba or 2,4-D	Glyphosate + Residual	Glyphosate + Residual + Dicamba or 2,4-D
Postemergence[4]	Glyphosate or glufosinate +[4] Dicamba or 2,4-D	Glyphosate or Glufosinate +[4] Dicamba or 2,4-D and/or Quizalofop	Glyphosate or Glufosinate + residual +[4] Dicamba or 2,4-D	Glyphosate or Glufosinate +[4] Dicamba or 2,4-D and/or Quizalofop

[1] The anticipated use patterns represent a high-end estimate for potential dicamba, 2,4-D, glufosinate and/or quizalofop use associated with MON 87429 combined with glyphosate-tolerant maize. Actual weed control practices by growers will vary depending on the specific weed spectrum, agronomic situation of the individual maize field and herbicide label recommendations.

[2] Hard to control weeds namely, morningglory species, hemp sesbania, prickly sida¸ and wild buckwheat.

[3] GR = glyphosate resistant.

[4] + = premix, tank mix or sequential application of herbicides.

[5] Dicamba and 2,4-D have broadleaf selectivity and quizalofop has grass selectivity. Follow label recommendations and best management practices for herbicide rotations. Recommendations for all fields will assume GR weeds are present.

As a result of introducing MON 87429, there are no major anticipated changes in the production of hybrid maize seed with the exception of the intended non-viable pollen phenotype resulting from glyphosate applications during reproductive development. Glyphosate can also be used for weed control in MON 87429 inbred seed increases or hybrid seed production (may result in non viable pollen phenotype). Other agricultural management practices for the production of hybrid maize seed and for the cultivation of commercial maize would also be no different for MON 87429 than for conventional maize hybrids.

Upon stacking of MON 87429 maize with deregulated glyphosate tolerance traits, such as NK603, growers will have the ability to continue use of established maize production practices including tillage systems; the same planting and harvesting machinery; established practices for 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 87429 Maize 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. Crops engineered with tolerance to established herbicides combined with tolerance to glyphosate may provide more diverse weed management options (Pallett, 2018). The combination of dicamba, glufosinate, quizalofop and 2,4-D herbicide-tolerance traits with glyphosate-tolerant maize, will facilitate the utilization of multiple herbicide sites-of-action in a grower's weed control system, and thereby reduce the potential for further resistance development to glyphosate, dicamba, glufosinate, quizalofop and 2,4-D herbicides as well as other important maize herbicides. Research conducted by Monsanto showed the value of multiple preplant herbicides (including soil active herbicides) followed by an early postemergence application of a mixture of herbicides with multiple sites-of-action, including a soil active herbicide, against broadleaf weeds that included species in the genus *Amaranthus* (Bayer CropScience, 2019b). Such a program would optimize the likelihood of using two or more effective sites-of-action against the targeted weeds. In areas with glyphosate-resistant and hard to control broadleaf weed populations, dicamba or 2,4-D may be applied pre-planting as a tank mix with preemergence herbicides and/or applied post-emergence in addition to glufosinate in MON 87429 maize. For control of glyphosate-resistant grass weed species, quizalofop provides a post-emergence option and may also be applied in sequence with glufosinate. The herbicide tolerance traits in MON 87249 maize also increase the potential to target late-emerging weed cohorts and weeds resistant to multiple herbicides with multiple herbicide sites-of-action.

Stewardship of dicamba, glufosinate, quizalofop and 2,4-D herbicides to preserve their usefulness to growers is an important aspect of Monsanto's stewardship commitment, as is discussed in Appendix K. Specifically, Monsanto has implemented and will continue to develop and proactively provide recommended weed resistance management practices[9], 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) (Monsanto, 2019) will set forth the requirements and best practices for the cultivation of MON 87429 maize including recommendations on weed resistance management practices. Growers purchasing products containing MON 87429 maize are required by Monsanto's Technology Stewardship Agreement (TSA) to read and follow the TUG. For weed management during the production of hybrid seeds using MON 87429 as the female inbred line, seed producers are encouraged to follow good management practices for herbicide resistance management to minimize the potential for further selection of herbicide-resistant weeds. Furthermore, Monsanto and its affiliates are committed to actively evaluating herbicide performance and weed efficacy on a continuing basis, and developing additional mitigation plans as necessary to manage resistance development for glyphosate, dicamba, glufosinate, quizalofop and 2,4-D.

[9] Weed resistance management guidelines available at http://www.iwm.bayer.com/ and http://www.roundupreadyplus.com/.

VIII.F.5. Weed Management Conclusion

Combining MON 87429 maize with deregulated glyphosate tolerance traits, such as NK603, will allow the use of dicamba, glufosinate, quizalofop, 2,4-D 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 distinct sites-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. With MON 87429 maize, growers will have options to use and rotate and/or use combinations of herbicides with effective herbicide sites-of-action for in-crop control of glyphosate's hard to control and resistant broadleaf and grass weeds that are present in U.S. maize production.

Furthermore, the integration of MON 87429 maize, 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; established 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 not anticipated that the commercialization of MON 87429 maize in the U.S. will have significant impacts on current maize agronomic practices, beyond the intended benefits of more effective and durable management of common and troublesome weeds, including herbicide-resistant weeds and for consistently producing high-quality, high-purity hybrid maize seed.

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. Table VIII-8 provides an assessment of the common rotational crops following maize at the U.S. country level. Approximately 30% of the U.S. maize acres were rotated back to maize and 57% are rotated to soybean the following year in 2013 (USDA-APHIS Petition #15-113-01p Table VIII-2 pg. 153). Wheat and cotton are other significant rotational crops with approximately 5% and 2%. In addition, based on the integrated pest management annual surveys of a subset of maize growers who planted maize with corn rootworm (CRW) trait in the U.S. in high CRW pressure states of Iowa, Illinois, Indiana, Minnesota, Nebraska and South Dakota, the percentage of continuous maize planting has largely remained the same between 2016, 2017 and 2018 (31%, 27% and 29%, respectively) (ABSTC, 2017; 2018; 2019).

Introduction of MON 87429 maize is not expected to limit crop rotation practices and there would be no significant changes anticipated in post-maize planting due to the availability of MON 87429 maize since (1) the use of dicamba, glufosinate, quizalofop, 2,4-D and glyphosate herbicides does not limit crop rotation choices due to their limited herbicidal persistence and carry-over potential attributes when applied according to the label; and, (2) application use patterns for glufosinate, quizalofop, 2,4-D or glyphosate herbicides for MON 87429 maize will be the same as already approved rates in current biotechnology-derived herbicide tolerant products available to growers and pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of Monsanto's third-generation herbicide tolerance product, MON 87419 maize (U.S. EPA, 2019).

Crop rotation is a valuable tool in managing herbicide resistance. In addition, the use of herbicide mixtures have been shown to be effective in delaying development of herbicide resistance (Beckie and Harker, 2017; Evans et al., 2016). The herbicide tolerance traits in MON 87429 combined with a deregulated glyphosate-tolerance trait support integrated weed management programs that include herbicides and/or herbicide mixtures with diverse sites-of-action, both within a single maize growing season and between maize and rotational crops, to delay the development of herbicide resistance in weeds. Due to natural tolerances or introduced traits, common rotational crops for maize (e.g., soybeans, cotton) may also tolerate dicamba, glufosinate, 2,4-D, quizalofop, and/or glyphosate. However, given the number of sites-of-action for herbicides that may be applied in MON 87429 maize and rotational crops (including those that may be applied for the conventional crops), an herbicide program including sites-of-action that differ between the crops is expected to be feasible.

Table VIII-8. Rotational Crops in the U.S. Following Maize Production

United States Total Maize Acres[1,5]	**Rotational Crops Following Maize**	**Rotational Crop Acres[2,5]**	**% Rotational Crop of Total Maize[2]**
95,365	Maize	28,291	29.7
	Soybean	54,451	57.1
	Wheat	4,527	4.7
	Cotton	1,870	2.0
	Alfalfa[3]	1,303	1.4
	Other Hay	1,118	1.2
	Other crops[4]	3807	3.9
		Total: 95,365	

This table was developed by compiling the data from all four regional summaries.
[1] Maize acreage based on 2013 planting data (USDA-NASS, 2014).
[2]Rotational Crop Acres source: USDA Petition #15-113-01p Table VIII-2 pg. 153
[3] Newly seeded alfalfa.
[4] Other crops: combined acreage of rotated crops following maize that are individually less than 1000,000 acres. It includes sorghum, oats, sugar beets, sunflower, barley, peanut, dry beans, potatoes, tobacco, millet, rice, safflower and vegetables (chili peppers, cantaloupe, watermelon, tomatoes, onions, snap beans, sweet corn, cabbage, lima beans, cucumbers, bell peppers, squash, green peas, carrots).
[5] All acreages are expressed as 1000s of acres. Totals may not be exact due to rounding.

VIII.H. Maize Volunteer Management

Volunteer maize is defined as a maize plant that germinates and emerges unintentionally in a subsequent crop. Volunteer maize commonly occurs in rotational crops in the season following cultivation of maize. Factors reducing the numbers of volunteers in the following crop include the harvesting of maize for silage before grain physiological maturity (approximately 6.1 million U.S. maize acres in 2018 (USDA-NASS, 2019c) and warm, moist climates such as those in the Southeast, where many seeds will germinate in the fall but not survive 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 practices.

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 (if significant volunteers have already emerged) and in-crop cultivation are very effective management tools. In addition, Gramoxone® (paraquat) is a preplant option to control volunteer maize, including MON 87429 (Appendix L, Table L-1) alone or with Tricor® (metribuzin), before planting another maize crop (Ikley et al., 2017; Steckel et al., 2009). Similarly, use of Gramoxone® (paraquat) tank mixed with residual herbicides such as metribuzin or linuron is recommended for preplant control of volunteer maize in soybean rotation (Bayer CropScience, 2019a). Several postemergence herbicides also are available to control volunteer maize (conventional or herbicide-tolerant maize) in each of the major maize rotational crops. Because MON 87429 maize is tolerant to the ACCase 'fop' herbicides, quizalofop and other fop herbicides will not be effective to remove volunteer MON 87429 maize. Nonetheless, other ACCase herbicides such as cyclohexanedione or "dims" (e.g., clethodim, sethoxydim) are still effective in control of MON 87429 (Appendix L, Table L-1) as volunteer maize in broadleaf crops. Table VIII-9 provides a summary of labeled selective postemergence herbicides for the effective control of volunteer maize, including MON 87429 maize, in specific rotational crops and include Poast® (sethoxydim), and Select® 2EC (clethodim). These herbicides are labeled for use in 11 vegetable rotation crops and 10 field crops that include soybean, cotton, sugar beet and alfalfa. Imazamox (Raptor®) is an option for postemergence control of volunteer maize in soybean, alfalfa, dry beans, peas, lima bean, snap bean, clover, and edamame (BASF, 2015). In cases of farm sites with grass weed biotypes resistant to quizalofop and/or "dims", and to delay weed resistance development to ACCase herbicides, there are other preplant and postemergence herbicide options available to control grass weeds and/or volunteer maize (see Appendix L, Table L-1). For volunteer control of maize including MON 87429, in wheat crop, there are

® Select is a trademark of Valent U.S.A. Corporation; ® Powerflex, Goldsky and Perfectmatch are the trademarks of Dow AgroSciences Corporation; ® Gramoxone is a trademark of Syngenta Corporation; ® Tricor is a trademark of United Phosphorus, Inc. ® Raptor is a trademark of BASF.

additional postemergence herbicide options available such as Powerflex® (pyroxsulam), GoldSky® (florasulam+ fluroxypyr+ pyroxsulam), Perfectmatch® (clopyralid+ floroxypyr+ pyroxsulam), etc (NDSU, 2018). Additionally, there are also many non-chemical methods of weed control (mechanical removal, tilling, etc.) that are effective in removing unwanted maize, including MON 87429.

MON 87429 combined with deregulated glyphosate tolerance traits, such as NK603, is not expected to notably impact the management of maize volunteer plants based on the availability of various herbicidal and cultivation methods for controlling volunteer MON 87429 maize as well as the demonstrated lack of agronomic and phenotypic differences, including germination and dormancy characteristics, between MON 87429 and conventional maize (see Section VII.C.1).

Table VIII-9. Herbicides Labeled for Control of Volunteer Maize in Labeled Rotational Crops[1]

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

[1]Source: (BASF, 2019; Valent, 2006)

VIII.I. Stewardship of MON 87429 Maize

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[10] (BIO, 2012). 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, 2019) 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 component of fulfilling this stewardship commitment, Monsanto will seek biotechnology regulatory approvals for MON 87429 maize in all important maize import countries with a functioning regulatory system 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, 2018), Biotechnology Innovation Organization (BIO) Policy on Product Launches (BIO, 2012). Monsanto continues to monitor other countries that import significant quantities 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 is also committed following industry best practices on seed quality assurance and control to ensure the purity and integrity of MON 87429 maize hybrid seed. As with all of Monsanto's products, before commercializing MON 87429 maize in any country, a MON 87429 maize detection method will be available via a third-party vendor to maize producers, processors, and buyers regardless of whether they have purchased MON 87429 or not.

Stewardship of glyphosate, dicamba, glufosinate, quizalofop and 2,4-D herbicides to preserve their usefulness to growers is also an important consideration, especially in light of recent U.S. EPA guidance on herbicide resistance management, labeling, education, training and stewardship that is intended to provide growers and registrants strategies to delay herbicide resistance development and prolong efficacy of herbicides (U.S. EPA, 2017b; c). Detailed information regarding dicamba, glufosinate, quizalofop, 2,4-D and glyphosate weed resistance and the usefulness of the herbicide-tolerant MON 87429 maize trait in combination with glyphosate-tolerant traits in maize to address herbicide-resistance issues is presented in Section VIII.F and Appendix K.

[10] www.excellencethroughstewardship.org/

VIII.J. Summary and Conclusion: Impact of the Introduction of MON 87429 Maize on Agricultural Practices

MON 87429 maize has been developed to facilitate greater choices for growers implementing effective weed management including tough-to-control and herbicide resistant broadleaf weeds. The ability to use dicamba, glufosinate, quizalofop and 2,4-D herbicides with multiple sites-of-action can be part of an effective weed management system for maize production in the U.S. Glufosinate, quizalofop, 2,4-D, and glyphosate herbicides are already labeled for use in maize (Liberty®: EPA Reg No. 264-660, Enlist One™: EPA Reg No 62719-695, Assure® II: EPA Reg No. 352-541, Durango® DMA®: EPA Reg No. 62719-556). Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of Monsanto's third generation herbicide tolerant product MON 87419 maize. For MON 87419 maize, Monsanto requested that EPA allow the 0.5 lb a.e./ac postemergence application window for dicamba to be extended from V5 to V8 growth stage or 36-inch height of maize, whichever occurs first (U.S. EPA, 2019). The combined (pre- and post-emergence) maximum annual application rate of dicamba in MON 87419 maize would be 2.0 lbs. a.e. dicamba per acre per year, increased from the current maximum annual application rate of 0.75 lbs. a.e. in conventional maize. Pending approval of the expanded label at EPA, the dicamba use pattern for MON 87429 would be no different than that of MON 87419. Therefore, the introduction of MON 87429 is not expected to have adverse impacts on current agronomic, cultivation and management practices for commercial grain or hybrid maize seed production. No significant changes are anticipated in crop rotations, tillage practices, planting practices, fertility management, weed and disease management, and volunteer management from the introduction of MON 87429 maize.

IX. PLANT PEST ASSESSMENT

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 87429 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 is unlikely to pose a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

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

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not expected to pose a 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) potential impacts to NTOs; 6) abiotic stressor, 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 87429 is unlikely to pose a plant pest risk, and therefore should no longer be subject to regulation under 7 CFR § 340.

IX.B. Plant Pest Assessment of MON 87429 and Expressed Protein

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the DMO, PAT, CP4 EPSPS and FT_T proteins expressed in MON 87429 used to evaluate the food, feed, and environmental safety of MON 87429.

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

IX.B.1.1. Genetic Insert

As described in Section III, MON 87429 was developed by *Agrobacterium*-mediated transformation of maize embryos using plasmid vector PV-ZMHT519224. Characterization of the DNA insert in MON 87429 was conducted using a combination of sequencing, PCR, and bioinformatics methods. The results of this characterization demonstrate that MON 87429 contains one copy of the intended transfer DNA containing the *dmo*, *pat*, *ft_t* and *cp4 epsps* 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 87429. Additionally, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 87429 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 87429 upon DNA integration.

IX.B.1.2. Mode-of-Action

MON 87429 contains the *dmo* gene from *Stenotrophomonas maltophilia* that expresses a dicamba mono-oxygenase (DMO) protein to confer tolerance to dicamba herbicide, the *pat* gene from *Streptomyces viridochromogenes* that expresses the phosphinothricin N-acetyltransferase (PAT) protein to confer tolerance to glufosinate herbicide, the *ft_t* gene derived from *Sphingobium herbicidovorans* that expresses a FOPs and 2,4-D dioxygenase (FT_T) protein to confer tolerance to quizalofop and 2,4-D herbicides, and the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4 that expresses the CP4 EPSPS protein and contains an siRNA Target Sequence in the 3' UTR, to confer tissue-specific glyphosate tolerance, to facilitate the production of hybrid maize seed.

DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound 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, 2002a). FT_T protein catalyzes a dioxygenase reaction, in the presence of alpha-ketoglutarate and oxygen, metabolizing quizalofop into quizalofop-phenol and pyruvate and metabolizing 2,4-D into 2,4-dichlorophenol (2,4-DCP) and glyoxylic acid. The safety of 2,4-D, quizalofop, and their metabolites have been assessed by US EPA which concluded that there is a reasonable certainty that no harm will result to the general population, or to infants and children from aggregate exposure to 2,4-D (U.S. EPA, 2017a) or quizalofop (U.S. EPA, 2018), residues or their metabolites. In plants, the endogenous EPSPS protein, an enzyme involved in the shikimate pathway for the biosynthesis of aromatic amino acids, is inhibited by the herbicide glyphosate resulting in cell death (Franz et al., 1997). MON 87429 produces the same CP4 EPSPS protein that is produced in commercial Roundup Ready® crop products which is insensitive to glyphosate and therefore confers tolerance to glyphosate. As described in Section IV.G expression of CP4 EPSPS in MON 87429 plants is driven by the CaMV 35S promoter, a known constitutive promoter (Holtorf et al., 1995; Terada and Shimamoto, 1990). In maize and other monocot plants, CaMV 35S has been shown to drive weak gene expression in pollen tissue (Hamilton et al., 1992; Heck et al., 2005). Additionally, MON 87429 maize utilizes an endogenous maize regulatory element to target CP4 EPSPS mRNA for degradation specifically in tassel tissues, resulting in reduced CP4 EPSPS protein expression in pollen. Appropriately timed glyphosate applications produce a non-viable pollen phenotype and allow for desirable cross pollinations to be made in maize without using traditional methods to control self-pollination in female inbred parents. A focused study on the MON 87429 siRNA Target Sequence demonstrated the absence of unintended effects on endogenous gene regulation. These data taken together with data demonstrating a lack of change in pollen morphology or viability between MON 87429 and control (Section VII) suggest the siRNA Target Sequence for tissue-selective CP4 EPSPS expression does not result in unintended effect on gene regulation in male tissue.

IX.B.1.3. Protein Safety and Expression Levels

The safety and expression of the DMO, PAT, CP4 EPSPS and FT_T proteins are detailed in Section V. MON 87429 DMO, PAT, CP4 EPSPS and FT_T protein levels in forage, root, leaf and grain of MON 87429 were determined using a multiplexed immunoassay. To further demonstrate the MON 87429 RHS trait mode-of-action (MOA), CP4 EPSPS expression in pollen tissue was determined to illustrate the differential expression between vegetative and pollen tissue. The mean DMO protein level in MON 87429 across all sites was highest in leaf at 35 µg/g dw and lowest in root at 2.3 µg/g dw. The mean PAT protein level in MON 87429 across all sites was highest in leaf at 5.8 µg/g dw and lowest in grain at 0.84 µg/g dw. The mean CP4 EPSPS protein level in MON 87429 across all sites was the highest in leaf at 54 µg/g dw and lowest in grain at 0.63 µg/g dw. As intended the mean CP4 EPSPS protein level in MON 87429 pollen across all sites was below the limit of quantitation (0.11 µg/g dw). The mean FT_T protein level in MON 87429 across all sites was the highest in leaf at 440 µg/g dw and lowest in root at 41 µg/g dw.

MON 87429 DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound 3,6-dichlorosalicylic acid (DCSA) and formaldehyde. DMO proteins have been previously characterized and the safety of these proteins have been well established. Therefore, the consumption of the DMO protein from MON 87429 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 87429, have been previously characterized and the safety of the PAT protein has been extensively assessed and in 1997 a tolerance exemption was issued for the PAT protein by U.S. EPA (40 CFR § 180.1151). Therefore, the consumption of the PAT protein from MON 87429 or its progeny is considered safe for humans and animals.

The CP4 EPSPS is key enzyme involved in aromatic amino acid biosynthesis and catalyzes the reaction where the enolpyruvyl group from phosphoenol pyruvate (PEP) is transferred to the 5-hydroxyl of shikimate-3-phosphate to form 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate. CP4 EPSPS proteins, including the CP4 EPSPS protein from MON 87429, have been previously characterized and the safety of the CP4 EPSPS protein has been extensively assessed. U.S. EPA has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (U.S. EPA, 1996b). Therefore, the consumption of the CP4 EPSPS protein from MON 87429 or its progeny is considered safe for humans and animals.

The FT_T protein catalyzes a dioxygenase reaction in the presence of αKG and oxygen to metabolize quizalofop, a FOP herbicide, into the herbicidally-inactive quizalofop phenol and pyruvate. The FT_T protein also catalyzes the dioxygenase reaction that degrades 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin herbicide, into herbicidally-inactive 2,4-dichlorophenol (2,4-DCP) and glyoxylic acid in the presence of alpha-ketoglutarate and oxygen. The physicochemical characteristics of the MON 87429-produced FT_T protein were determined and equivalence between MON 87429-produced FT_T and *E. coli*-produced FT_T proteins was demonstrated. Therefore *E. coli*-produced FT_T is an appropriate test substance for

the protein safety studies (heat susceptibility and digestibility). Expression studies demonstrated that FT_T is expressed at low levels in grain. An assessment of the allergenic and toxic potential of the FT_T protein found that the FT_T protein does not pose a significant allergenic risk and was not similar to know toxins. In addition, the donor organism for the MON 87429 FT_T coding sequence is from a genus, *Sphingobium,* that is ubiquitous in the environment and is not commonly known for allergenicity and human or animal pathogenicity. The FT_T 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 FT_T protein from MON 87429 or its progeny is considered safe for humans and animals. Taken together, the results of these analyses support a determination that MON 87429 is no more likely to pose a plant pest risk than conventional maize.

IX.B.2. Compositional Characteristics

For MON 87429, the introduced proteins, DMO, PAT, CP4 EPSPS and FT_T, confer herbicide tolerance and lack catalytic activity that is intended to or expected to affect the plant's metabolism. Given the nature of these introduced traits and the overall lack of meaningful unintended compositional characteristics observed for biotechnology-derived products characterized to date (Herman and Price, 2013; Venkatesh et al., 2015), compositional changes that would affect the levels of components in MON 87429 maize were not expected. Monsanto is currently in consultation with the Food and Drug Administration following their policy, "Foods Derived from New Plant Varieties," on the food and feed safety of MON 87429 maize (Submitted 05-Feb-2019). Composition data for 25 components including major nutrients in grain (protein, amino acids, total fat, linoleic acid, carbohydrates, acid detergent fiber, neutral detergent fiber and ash), major nutrients in forage (protein, total fat, carbohydrates, acid detergent fiber, neutral detergent fiber and ash) and anti-nutrients in grain (phytic acid and raffinose) were submitted to FDA as part of the voluntary food/feed safety and nutritional assessment for MON 87429 maize. The results of the compositional assessment found that MON 87429 did not meaningfully alter component levels in maize and confirms the compositional equivalence of MON 87429 to conventional maize.

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-, glufosinate-, quizalofop- and 2,4-D-tolerance and RHS traits altered the plant pest potential of MON 87429 compared to the conventional control (Section VII). Phenotypic, agronomic, and environmental interaction characteristics of MON 87429 were evaluated and compared to those of the conventional control. As described previously, these assessments included seed germination and dormancy characteristics and pollen characteristics in the laboratory as well as phenotypic and agronomic characteristics and plant responses to abiotic stressors, diseases, and arthropod pests in the field. Results from all phenotypic, agronomic, and environmental interaction assessments demonstrated that MON 87429 does not possess increased weedy characteristics or increased susceptibility or tolerance to specific abiotic stressors, diseases, or arthropod pests compared to the conventional control. Taken together, the assessments support a determination that MON 87429 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 87429 and the conventional control. The results of this assessment, including the lack of significant differences and particularly the lack of increased hard seed, support the conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

IX.B.3.2. Phenotypic and Agronomic Assessment

Evaluations of phenotypic and agronomic characteristics in the field are useful for assessing characteristics typically associated with weediness, such as lodging and seed loss. The characteristics early stand count, days to flowering, plant height, days to maturity, lodging, seed loss, final stand count, moisture, seed weight, and yield were assessed. The lack of observed differences between MON 87429 and the conventional control for phenotypic and agronomic characteristics across sites supports the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

IX.B.3.3. Response to Abiotic Stressors, Diseases, and Arthropod Pests

In an assessment of plant response to abiotic stressors, diseases, and arthropod pests, no differences were observed between MON 87429 and the conventional control for any of the 96 observations for each stressor category across eight sites (Section VII.C.2.2.). The lack of observed differences between MON 87429 and the conventional control for plant responses to abiotic stressors, diseases, and arthropod pests in multiple field environments supports the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased plant pest risk of MON 87429 compared to conventional maize.

IX.B.3.4. Pollen Viability and Morphology

Evaluations of pollen viability and morphology 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 87429 and the conventional control for percentage viable pollen or pollen diameter. Furthermore, no visual differences in general pollen morphology were observed between MON 87429 and the conventional control. The lack of observed differences between MON 87429 and the conventional control for pollen characteristics support the overall conclusion that the introduction of the dicamba-, glufosinate-, quizalofop-, and 2,4-D-tolerance and RHS traits does not result in increased weediness or plant pest risk of MON 87429 compared to conventional maize.

IX.B.3.5. Interactions with Non-Target Organisms Including Those Beneficial to Agriculture or Threatened and Endangered Species

The potential for MON 87429 to influence non-target organisms (NTOs) was evaluated using a combination of biochemical information and experimental data. The modes-of-action are well-established and lack toxic effects; experimental evidence reviewed in the current or previously submitted Petitions indicates that the DMO, PAT, CP4 EPSPS and FT_T proteins are safe; and no safety concerns have occurred during the history of environmental exposure to DMO, PAT, CP4 EPSPS and the alpha-ketoglutarate-dependent dioxygenase family of proteins (the class of enzymes to which FT_T belongs). Additionally, molecular characterization did not suggest potential for unintended effects, no evidence was found for unintended changes to plant metabolism, compositional characteristics were substantially equivalent for MON 87429 and the conventional control, and the germination and dormancy, phenotypic and agronomic, and pollen characteristics were not indicative of increased weediness or plant pest risk for MON 87429 compared to conventional maize. Since MON 87429 does not possess pesticidal activity, all organisms that interact with MON 87429 are considered to be NTOs. Environmental interactions data showed a lack of differences in plant responses to a subset of NTOs (specific diseases and arthropod pests). Taken together, these results suggest that MON 87429 is unlikely to adversely affect NTOs, including those beneficial to agriculture, 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 states where maize is grown have no plants listed as threatened or endangered, or that are proposed for listing, that are sexually compatible with maize. Because MON 87429 has been shown to be agronomically and phenotypically equivalent to conventional maize without increased weediness potential, the planting of MON 87429 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 for a threatened or endangered species (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 (2014a; b), 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 87429 DMO, PAT, CP4 EPSPS and FT_T proteins, and the compositional, agronomic and phenotypic equivalence of MON 87429 to conventional maize, support a conclusion that no effects to listed threatened or endangered species, including no biologically significant changes to habitat or diet, are expected due to the planting of MON 87429.

IX.C. Weediness Potential of MON 87429

Although grown extensively throughout the world, maize is not considered a threat to invade natural or agricultural ecosystems. Maize does not establish self-sustaining populations outside of cultivation (Crawley et al., 2001; OECD, 2003; Raybould et al., 2012). This lack of weediness may reflect its poor competitive ability (Olson and Sander, 1988), lack of seed dormancy, and barriers to seed dispersal, as maize cobs retain seed and are covered in a husk (Wilkes, 1972). A number of other characteristics common in weeds, such as rapid flowering following emergence, are lacking in maize (Keeler, 1989). Traits often associated with weediness are typically not selected for during domestication and subsequent breeding and selection. Similarly, the history of maize breeding and production in the U.S. does not indicate there are any changes in the characteristics of maize that would increase the weediness of the crop. Even if kernels of maize are distributed within a field or along transportation routes from the fields to storage or processing facilities, self-sustaining volunteer maize populations are not found growing in fields, fence rows, ditches, or road sides.

In comparative studies between MON 87429 and a conventional control, germination and dormancy, phenotypic and agronomic, environmental interaction, and pollen characteristics were evaluated (Section VII) for changes that would impact the plant pest potential, and in particular, plant weediness potential. Results of these evaluations show that there is no biologically meaningful difference between MON 87429 and the conventional control for characteristics (percentage viable hard seed, lodging, and seed loss) typically associated with weediness. Furthermore, field observations of MON 87429 and the conventional control indicated no differences in plant responses to abiotic stressors, diseases, or arthropod pests. Collectively, these findings support the conclusion that MON 87429 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 87429 to other cultivated maize and the potential for introgression of the MON 87429 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 87429 seed or grain have been conducted under USDA notification according to these standards. Since no meaningful differences were observed for MON 87429 compared to conventional maize in nutritional value, composition, or in pest/weed potential in field evaluations, no adverse effects are expected from gene flow from commercial production of MON 87429 to other maize.

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

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
~1	28.6	Three- year study with one site per year. Outcrossing was detected by yellow seeds in the white-seeded pollen recipient. Values are averaged over years.	USA	(Jones and Brooks, 1950)
25	14.2			
75	5.8			
126	2.3			
201	1.2			
302	0.5			
402	0.2			
503	0.2			
1	19.0	Three-year study with two to three sites per year. Outcrossing was detected by yellow seeds in the white-seeded pollen recipient Values are averaged over site-years for the downwind direction.	Canada	(Ma et al., 2004)
5	2.6			
10	2.0			
14	0.6			
19	0.4			
24	0.3			
28	0.5			
33	0.3			
36	0.1			
24–32	0.69	Two-year study with two sites per year. Outcrossing was detected by purple seeds in the yellow-seeded pollen recipient. Values are the greatest observed between years for the site where outcrossing was most prevalent.	USA	(Halsey et al., 2005)
60–62	0.23			
123–125	0.08			
244–254	0.02			
486–500	0.005			
743–745	0.002			
1	29.9	Two-year study with one site per year. Outcrossing was detected by yellow seeds in the non-transgenic white-seeded pollen recipient and confirmed by tests for a glyphosate tolerance gene in the yellow seeds. Values are the greatest between years as averaged across all directions from the pollen source.	USA	(Goggi et al., 2006)
10	2.5			
35	0.4			
100	0.05			
150	0.03			
200	0.03			
250	0.03			

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

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
0	12.65	One-year study with one site. Outcrossing was detected by yellow seeds in the white-seeded pollen recipient. Values are the greatest reported at listed distances from the edge of two sampling areas, one adjacent to and one 10 m from the pollen source. The values are half of those observed to reflect flow of a hemizygous gene.	Spain	(Pla et al., 2006)
2	8.81			
5	2.33			
10	3.69			
20	1.05			
40	0.74			
80	0.65			
0	0.74	Three-year study with a total of 55 sites. Outcrossing was quantified on ground samples of nontransgenic pollen recipient grain using a PCR assay for the *pat* herbicide tolerance gene. Values are means for percentage GM DNA for distances with >30 samples.	UK	(Weekes et al., 2007)
2	0.66			
5	0.35			
10	0.27			
20	0.16			
25	0.14			
50	0.12			
100	0.10			
150	0.12			

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

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
1	44	Two-year study with one site per year. Outcrossing was detected by herbicide tolerance and PCR tests for an herbicide tolerance transgene in progeny of the non-transgenic pollen recipient. Values are the greatest between years as averaged across all directions from the pollen source.	China	(Zhang et al., 2011)
5	16			
10	10			
15	5			
30	1			
60	1			
100	0			
150	0			
200	0			
300	0			
1	12.9	Three-year study with a total of eight sites. Outcrossing was detected by yellow seeds in the white-seeded pollen recipient. Values are averaged over all directions and sites.	Mexico	(Baltazar et al., 2015)
2	4.6			
4	2.7			
8	1.4			
12	1.0			
16	0.8			
20	0.5			
25	0.5			

IX.D.2. Hybridization with 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 species or subspecies of teosinte. Although teosinte is not native to the U.S., a compatible subspecies of annual teosinte (*Zea mays* subsp. *mexicana*) is reported to have feral populations in Florida, Alabama, and Maryland (USDA-NRCS, 2019).

Hybrids of maize and teosinte are not expected in the U.S. In a study of maize and *Zea mays* subsp. *mexicana*, very few ovules (approximately 1-2%) produced seed after hand pollination with pollen from the other subspecies (Baltazar et al., 2005). Natural hybridization rates for maize and *Zea mays* subsp. *mexicana* have also been reported to be low (≤0.2% of progeny) (Ellstrand et al., 2007). A genetic barrier to hybridization of maize and teosinte has been described (Evans and Kermicle, 2001) and if present may limit hybridization rates of maize and *Zea mays* subsp. *mexicana*. In addition, differences in developmental and morphological factors (Baltazar et al., 2005), potential differences in flowering time, and limited geographical distribution of teosinte make natural crosses and gene introgression from maize into teosinte unlikely in the U.S.

IX.D.3. Hybridization with *Tripsacum*

Tripsacum is a genus with 15 recognized species (Zuloaga et al., 2003). There are three species of *Tripsacum* that are native to the U.S.: *T. floridanum* (Florida gamagrass), *T. lanceolatum* (Mexican gamagrass), and *T. dactyloides* (Eastern gamagrass) (USDA-NRCS, 2019). Two additional species have introduced populations: *T. latifolium* (wideleaf gamagrass) and *T. fasciculatum* (Guatemalan gamagrass) (USDA-NRCS, 2019).

Tripsacum floridanum (Florida gamagrass) naturally grows in the extreme southern Florida counties of Miami-Dade, Collier, Martin, and Monroe (USDA-NRCS, 2019; Wunderlin et al., 2019). Florida gamagrass has been described as rare and occurring in "low, rocky pinelands" (Blakey et al., 2007) and is categorized as a threatened species in Florida (USDA-NRCS, 2019). *Tripsacum lanceolatum* (Mexican gamagrass) has been reported in Arizona and New Mexico (USDA-NRCS, 2019) and is found on "stream banks or moist cliffs" (de Wet and Harlan, 1978). *Tripsacum dactyloides* (eastern gamagrass) is found primarily throughout the eastern U.S. It is categorized as endangered in Massachusetts and Pennsylvania, threatened in New York, and special concern in Rhode Island (USDA-NRCS, 2019). *Tripsacum fasciculatum* (Guatemalan gamagrass) and *T. latifolium* (wideleaf gamagrass) and are present as introduced populations in Puerto Rico (USDA-NRCS, 2019).

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 exhibit male sterility that is not resolved by several backcrosses to maize (Russell and Hallauer, 1980). Empirical data showed that "no evidence of gene flow from transgenic maize to eastern gamagrass in nature was observed even

though the two species have grown in close proximity for years and have had ample opportunities for outcrossing" (Lee et al., 2017). 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 evidence of hybrid existence 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 87429 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 unaware of any reports regarding the unaided transfer of genetic material from maize species to other sexually-incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk associated with horizontal gene flow from MON 87429 due to the presence of the dicamba, glufosinate, quizalofop and 2,4-D-tolerance and RHS traits are not expected. The consequence of horizontal gene flow of the MON 87429 traits into other plants that are sexually-incompatible is negligible since, as data presented in this petition confirm, the genes and traits confer no increased plant pest potential to maize. Thus, in the highly unlikely event that horizontal gene transfer was to occur, the presence of the MON 87429 traits 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 87429 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, pat, ft_t and cp4 epsps* coding sequence that provides tolerance to dicamba, glufosinate, quizalofop and 2,4-D, and glyphosate herbicides, MON 87429 is similar to other conventional maize hybrids.

The data presented demonstrate that MON 87429 is similar to conventional maize in its phenotypic and agronomic characteristics, and has levels of susceptibility to abiotic stressors, diseases, and arthropod pests comparable to other conventional maize. Based on this assessment, the introduction of MON 87429 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

Several biotechnology-derived maize products have been deregulated or are under consideration for deregulation. Once deregulated, MON 87429 may be bred with these deregulated biotechnology-derived maize products, as well as with conventional maize, creating new improved hybrids. APHIS has determined that none of the individual biotechnology-derived maize products it has previously deregulated displays increased plant pest characteristics. APHIS has also concluded that any progeny derived from crosses of these deregulated

biotechnology-derived maize products with conventional or previously deregulated biotechnology-derived 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 or potential environmental impacts not observed in the single event biotech product, is based upon several facts. Namely: 1) stability of the genetic inserts is confirmed in each approved biotech-derived maize product across multiple generations (See Section IV.E for MON 87429 data); 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 the well-established process of conventional breeding that has been safely used for thousands of years to generate new varieties (Cellini et al., 2004; NRC, 2004; 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; U.S. FDA, 2001; WHO, 1995); and 5) practical applications in the field have shown that two unrelated biotechnology traits combined together by conventional breeding do not display new characteristics or properties distinct from those present in the single event biotech products (Brookes and Barfoot, 2012; James, 2010; Lemaux, 2008; Pilacinski et al., 2011; Sankula, 2006).

Therefore, based on the considerations above and the conclusion that MON 87429 is no more likely to pose a plant pest risk than conventional maize it can be concluded that any progeny derived from crosses between MON 87429 and conventional maize or other maize with deregulated biotechnology-derived events are no more likely to pose a plant risk than conventional maize.

IX.G. Summary of Plant Pest Assessments

A plant pest, as defined in the Plant Protection Act, is the living stage of any of the following that can directly or indirectly injure, damage, or cause disease in any plant or plant product: (A) a protozoan; (B) a nonhuman animal; (C) a parasitic plant; (D) a bacterium; (E) a fungus; (F) a virus or viroid; (G) an infectious agent or other pathogens; or (H) any article similar to or allied with any of the articles specified in the preceding subparagraphs (7 U.S.C. § 7702(14)). Characterization data presented in Sections III through VII of this petition confirm that MON 87429, with the exception of the dicamba, glufosinate, quizalofop and 2,4-D-tolerance and RHS traits, is not meaningfully different from conventional maize in terms of plant pest potential. Monsanto is not aware of any other study results or observations associated with MON 87429 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 87429 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 *pat*, *dmo*, *ft_t* and *cp4 epsps* cassette; 2) characterization and safety of the expressed products; 3) compositional equivalence of MON 87429 grain and forage compared to a conventional control; 4) phenotypic, agronomic, and environmental interactions characteristics demonstrating no increased plant pest potential compared to conventional maize; 5) negligible risk to NTOs; 6) familiarity with maize

as a cultivated crop and 7) no greater likelihood to impact agronomic practices, cultivation practices, or the management of weeds, diseases and pests, 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 87429 is unlikely to pose a plant pest risk. Therefore, Monsanto Company requests a determination from USDA-APHIS that MON 87429 and any progeny derived from crosses between MON 87429 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 87429 indicating that there would be adverse consequences from its introduction. MON 87429 produces the DMO, PAT, CP4 EPSPS and FT_T proteins. The safety of the MON 87429 DMO, PAT and CP4 EPSPS proteins, which have been fully characterized, has been thoroughly assessed in this and previous submissions. The safety of the MON 87429 FT_T protein, which has been fully characterized, has been thoroughly assessed in this submission. As demonstrated by field results and laboratory tests, the only phenotypic differences between MON 87429 and conventional maize are dicamba, glufosinate, quizalofop and 2,4-D tolerance and tissue-specific glyphosate tolerance to facilitate the production of hybrid maize seed.

The data and information presented in this request demonstrate that MON 87429 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 *pat, dmo, ft_t* and *cp4 epsps* expression cassettes that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations.

Analysis of key nutrients of MON 87429 demonstrate that MON 87429 is compositionally equivalent to conventional maize. The phenotypic evaluations, including an assessment of seed germination and dormancy characteristics, phenotypic and agronomic characteristics, pollen characteristics, environmental interactions also indicated MON 87429 is unchanged compared to conventional maize. There is no indication that MON 87429 would have an adverse impact on beneficial or non-target organisms (NTOs), 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 87429 are negligible.

The introduction of MON 87429 will not adversely impact cultivation practices or the management of weeds, diseases, and pests 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 tolerance traits while providing the desired agronomic practice(s).

REFERENCES

ABSTC. 2017. 2016 Integrated Pest Management (IPM) Stewardship Program for Corn Rootworm Resistance Management. Agricultural Biotechnology Stewardship Technical Committee MRID No. 50170801:10.

ABSTC. 2018. 2017 Integrated Pest Management (IPM) Stewardship Program for Corn Rootworm Resistance Management. Agricultural Biotechnology Stewardship Technical Committee MRID No. 50510201:9.

ABSTC. 2019. 2018 Integrated Pest Management (IPM) Stewardship Program for Corn Rootworm Resistance Management. Agricultural Biotechnology Stewardship Technical Committee MRID No. 50924301:10.

Adrian-Romero, M., G. Blunden, B.G. Carpenter and E. Tyihák. 1999. HPLC quantification of formaldehyde, as formaldemethone, in plants and plant-like organisms. Chromatographia 50:160-166.

Alibhai, M.F. and W.C. Stallings. 2001. Closing down on glyphosate inhibition - With a new structure for drug discovery. Proceedings of the National Academy of Sciences of the United States of America 98:2944-2946.

Anderson, J.E., J.M. Michno, T.J. Kono, A.O. Stec, B.W. Campbell, S.J. Curtin and R.M. Stupar. 2016. Genomic variation and DNA repair associated with soybean transgenesis: A comparison to cultivars and mutagenized plants. BMC Biotechnology 16:41.

Anderson, M.S. and M.C. de Vicente. 2010. Maize, corn (*Zea mays* L.). Pages 255-291 in Gene Flow Between Crops and Their Wild Relatives John Hopkins University Press, Baltimore, Maryland.

Anderson, W.P. 1996. Weed ecology. Pages 27-38 in Weed Science: Principles and Applications. Third Edition. West Publishing Company, St. Paul, Minnesota.

AOSA. 2009. Seedling evaluation. Rules for Testing Seeds. Volume 4. Association of Official Seed Analysts, Ithaca, New York.

AOSA. 2017a. AOSA Rules for testing seeds. Volume 1: Principles and procedures. Association of Official Seed Analysts, Washington, D.C.

AOSA. 2017b. AOSA Rules for testing seeds. Volume 4: Seedling evaluation. Association of Official Seed Analysts, Washington, D.C.

Aref, S. and D.R. Pike. 1998. Midwest farmers' perceptions of crop pest infestation. Agronomy Journal 90:819-825.

Avila-Garcia, W.V. and C. Mallory-Smith. 2011. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. Weed Science 59:305-309.

Baltazar, B.M., L. Castro Espinoza, A. Espinoza Banda, J.M. de la Fuente Martinez, J.A. Garzon Tiznado, J. Gonzalez Garcia, M.A. Gutierrez, J.L. Guzman Rodriguez, O. Heredia Diaz, M.J. Horak, J.I. Madueno Martinez, A.W. Schapaugh, D. Stojsin, H.R. Uribe Montes and F. Zavala Garcia. 2015. Pollen-mediated gene flow in maize: Implications for isolation requirements and coexistence in Mexico, the center of origin of maize. PLoS One 10:e0131549.

Baltazar, B.M., J. de Jesús Sánchez-Gonzalez, L. de la Cruz-Larios and J.B. Schoper. 2005. Pollination between maize and teosinte: An important determinant of gene flow in Mexico. Theoretical and Applied Genetics 110:519-526.

Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology 2:335-350.

Barry, G.F., G.M. Kishore, S.R. Padgette and W.C. Stallings. 2001. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 6,248,876, U.S. Patent Office, Washington, D.C.

BASF. 2015. Raptor herbicide label. BASF Corporation, Research Triangle Park, North Carolina.

BASF. 2019. Poast herbicide label. BASF Corporation, Triangle Park, North Carolina.

Bayer CropScience. 2016. Liberty® herbicide label. Bayer CropScience LP, Research Triangle Park, North Carolina.

Bayer CropScience. 2019a. Agronomy Spotlight. Managing volunteer corn in soybeans. Bayer CropScience, St. Louis, Missouri.

Bayer CropScience. 2019b. Spray Early with Confidence Program. Roundup Ready Xtend Crop System. Bayer CropScience, St. Louis, Missouri.

Beckie, H. and N. Harker. 2017. Our top 10 herbicide-resistant weed management practices. Pest Managment Science 73:1045-1052.

Behrens, M.R., N. Mutlu, S. Chakraborty, R. Dumitru, W.Z. Jiang, B.J. LaVallee, P.L. Herman, T.E. Clemente and D.P. Weeks. 2007. Dicamba resistance: Enlarging and preserving biotechnology-based weed management strategies. Science 316:1185-1188.

BIO. 2012. Product launch stewardship. Biotechnology Innovation Organization, Washington, D.C. www.bio.org (Accessed September 16, 2019).

Bisson, J., J. McAlpine, J. Graham and G.F. Pauli. 2016. NAPRALERT, from an historical information silo to a linked resource able to address the new challenges in Natural Products Chemistry and Pharmacognosy. CEUR Workshop Proceedings 1747.

Blakey, C.A., D. Costich, V. Sokolov and M.N. Islam-Faridi. 2007. *Tripsacum* genetics: From observations along a river to molecular genomics. Maydica 52:81-99.

Borges, F. and R.A. Martienssen. 2015. The expanding world of small RNAs in plants. Molecular Cell Biology 16:727-741.

Bosnic, A.C. and C.J. Swanton. 1997. Influence of barnyardgrass (*Echinochloa crus-galli*) time of emergence and density on corn (*Zea mays*). Weed Science 45:276-282.

Brodersen, P. and O. Voinnet. 2006. The diversity of RNA silencing pathways in plants. Trends in Genetics 22:268-280.

Brookes, G. and P. Barfoot. 2012. GM crops: Global socio-economic and environmental impacts 1996-2010. PG Economics Ltd, Dorchester, United Kingdom.

Bugg, T.D.H. 2003. Dioxygenase enzymes: Catalytic mechanisms and chemical models. Tetrahedron 59:7075-7101.

Capehart, T., O. Liefert and D. Olson. 2018. Reduced corn and sorghum grind dampens disapperance. United States Department of Agricuture Washington, D.C.

Carey, J.B. and J.J. Kells. 1995. Timing of total postemergence herbicide applications to maximize weed control and corn (*Zea mays*) yield. Weed Technology 9:356-361.

Carolis, E.d. and V.d. Luca. 1994. 2-Oxoglutarate-dependent dioxygenase and related enzymes: Biochemical characterization. Phytochemistry 36:1093-1107.

Carthew, R. and E. Sontheimer. 2009. Origins and mechanisms of miRNAs and siRNAs. Cell 136:642-655.

Cellini, F., A. Chesson, I. Colquhoun, A. Constable, H.V. Davies, K.H. Engel, A.M.R. Gatehouse, S. Kärenlampi, E.J. Kok, J.-J. Leguay, S. Lehesranta, H.P.J.M. Noteborn, J. Pedersen and M. Smith. 2004. Unintended effects and their detection in genetically modified crops. Food and Chemical Toxicology 42:1089-1125.

Chakraborty, S., M. Behrens, P.L. Herman, A.F. Arendsen, W.R. Hagen, D.L. Carlson, X.-Z. Wang and D.P. Weeks. 2005. A three-component dicamba *O*-demethylase from *Pseudomonas maltophilia*, strain DI-6: Purification and characterization. Archives of Biochemistry and Biophysics 437:20-28.

Chaudhary, D.K., S.-W. Jeong and J. Kim. 2017. *Sphingobium naphthae* sp. nov., with the ability to degrade aliphatic hydrocarbons, isolated from oil-contaminated soil. International Journal of Systematic and Evolutionary Microbiology 67:2986-2993.

Christ, B., R. Hochstrasser, L. Guyer, R. Francisco, S. Aubry, S. Hortensteiner and J.K. Weng. 2017. Non-specific activities of the major herbicide-resistance gene *BAR*. Nature Plants 3:937-945.

Clark, S.E. and G.K. Lamppa. 1992. Processing of the precursors for the light-harvesting chlorophyll-binding proteins of photosystem II and photosystem I during import and in an organelle-free assay. Plant Physiology 98:595-601.

CLI. 2005. Position paper: Regulation of plant biotechnology products containing two or more traits combined by conventional plant breeding. CropLife International, Brussels, Belgium.

Codex Alimentarius. 2009. Foods derived from modern biotechnology. Volume Second Edition. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Cornejo, M.-J., D. Luth, K.M. Blankenship, O.D. Anderson and A.E. Blechl. 1993. Activity of a maize ubiquitin promoter in transgenic rice. Plant Molecular Biology 23:567-581.

Crawley, M.J., S.L. Brown, R.S. Hails, D.D. Kohn and M. Rees. 2001. Transgenic crops in natural habitats. Nature 409:682-683.

D'Ordine, R.L., T.J. Rydel, M.J. Storek, E.J. Sturman, F. Moshiri, R.K. Bartlett, G.R. Brown, R.J. Eilers, C. Dart, Y. Qi, S. Flasinski and S.J. Franklin. 2009. Dicamba monooxygenase: Structural insights into a dynamic Rieske oxygenase that catalyzes an exocyclic monooxygenation. Journal of Molecular Biology 392:481-497.

de Wet, J.M.J. and J.R. Harlan. 1978. Tripsacum and the origin of maize. Pages 129-141 in Maize Breeding and Genetics. D.B. Walden (ed.). University of Illinois, Urbana.

della-Cioppa, G., S.C. Bauer, B.K. Klein, D.M. Shah, R.T. Fraley and G.M. Kishore. 1986. Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants *in vitro*. Proceedings of the National Academy of Sciences of the United States of America 83:6873-6877.

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H.M. Goodman. 1982. Nopaline synthase: Transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1:561-573.

Devine, M., S.O. Duke and C. Fedtke. 1993. Herbicide translocation. Pages 67-90 in Physiology of Herbicide Action. PTR Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Dow. 2017. Enlist One hebicide label. Dow AgroSciences, Indianapolis, Indiana.

Duke, S.O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. Pest Management Science 61:211-218.

Dumitru, R., W.Z. Jiang, D.P. Weeks and M.A. Wilson. 2009. Crystal structure of dicamba monooxygenase: A Rieske nonheme oxygenase that catalyzes oxidative demethylation. Journal of Molecular Biology 392:498-510.

DuPont. 2018. Assure II herbicide label. E.I. du Pont de Nemours and Company, Wilmington, Delaware.

Duvick, D.N. 1999. Heterosis: Feeding people and protecting natural resources. Pages 19-29 in The Genetics and Exploitation of Heterosis in Crops. J.G. Coors and S. Pandey (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Ellstrand, N.C., L.C. Garner, S. Hegde, R. Guadagnuolo and L. Blancas. 2007. Spontaneous hybridization between maize and teosinte. Journal of Heredity 98:183-187.

Enya, J., H. Shinohara, S. Yoshida, T. Tsukiboshi, H. Negishi, K. Suyama and S. Tsushima. 2006. Culturable leaf-associated bacteria on tomato plants and their potential as biological controls agents. Microbial Ecology 53:524-536.

ETS. 2018. Guide for stewardship of biotechnology-derived plant products. Excellence Through Stewardship, Washington, D.C.

Evans, J.A., P.J. Tranel, A.G. Hager, B. Schutte, C. Wu, L.A. Chatham and A.S. Davis. 2016. Managing the evolution of herbicide resistance. Pest Management Science 72:74-80.

Evans, M.M.S. and J.L. Kermicle. 2001. *Teosinte crossing barrier1*, a locus governing hybridization of teosinte with maize. Theoretical and Applied Genetics 103:259-265.

FAO-WHO. 1996. Joint FAO/WHO expert consultation on biotechnology and food safety. Paper 61. Food and Agriculture Organization of the United Nations, World Health Organization, Rome, Italy.

FAO-WHO. 2011a. Summary report: Acceptable daily intakes, acute reference doses, short-term and long-term dietary intakes, recommended maximum residue limits and supervised trials median residue values recorded by the 2011 meeting. Food and Agriculture Organization of the United Nations, World Health Organization, Geneva, Switzerland.

FAO-WHO. 2011b. Pesticide residues in food 2010: Joint FAO/WHO meeting on pesticide residues. FAO Plant Production and Protection Paper 200. Food and Agriculture Organization of the United Nations, World Health Organization, Rome, Italy.

FAOSTAT. 2019. Food and Agricultural Organization statistical database. Food and Agricultural Organization of the United Nations, Rome, Italy. http://www.fao.org/faostat/en/#data/QC (Accessed October 09, 2019).

Farnham, D.E., G.O. Benson and R.B. Pearce. 2003. Corn perspective and culture. Pages 1-33 in Corn: Chemistry and Technology. Second Edition. P.J. White and L.A. Johnson (eds.). American Association of Cereal Chemists, St. Paul, Minnesota.

Fausey, J.C., J.J. Kells, S.M. Swinton and K.A. Renner. 1997. Giant foxtail (*Setaria faberi*) interference in nonirrigated corn (*Zea mays*). Weed Science 45:256-260.

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver and C.C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806-811.

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn*7* gene encoding an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase. Nucleic Acids Research 13:7095-7106.

Franz, J.E., M.K. Mao and J.A. Sikorski. 1997. Glyphosate's molecular mode of action. Pages 521-535 in Glyphosate: A Unique Global Herbicide. American Chemical Society, Washington, D.C.

Ganie, Z.A. and A.J. Jhala. 2017. Interaction of 2,4-D or dicamba with glufosinate for control of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in glufosinate-resistant maize (*Zea mays* L.). Frontier in Plant Science 8:1207.

Ghanizadeh, H., K. Harrington and T. James. 2015. Glyphosate-resistant *Lolium multiflorum* and *Lolium perenne* populations from New Zealand are also resistant to glufosinate and amitrole. Crop Protection 78:1-4.

Gianessi, L.P., C.S. Silvers, S. Sankula and J.E. Carpenter. 2002. Plant biotechnology: Current and potential impact for improving pest management in U.S. agriculture. An analysis of 40 case studies - herbicide tolerant field corn. National Center for Food & Agricultural Policy, Washington, D.C.

Goggi, A.S., P. Caragea, H. Lopez-Sanchez, M. Westgate, R. Arritt and C. Clark. 2006. Statistical analysis of outcrossing between adjacent maize grain production fields. Field Crops Research 99:147-157.

Gorski, S.A., J. Vogel and J.A. Doudna. 2017. RNA-based recognition and targeting: sowing seeds of specificty. Molecular Cell Biology 18:215-225.

Gowda, M., M. Worku, S.K. Nair and B.M. Prasanna. 2017. Quality Assurance/Quality Control (QA/QC) in Maize Breeding and Seed Production: Theory and Practice. CIMMYT: Nairobi:1-22.

Griffin, S.L., J.A. Godbey, T.J. Oman, S.K. Embrey, K. A., K. Kuppannan, B.W. Barnett, G. Lin, N.V.J. Harpham, A.N. Juba, B.W. Schafer and R.M. Cicchillo. 2013. Characterization of aryloxyalkanoate dioxygenase-12, a nonheme Fe(II)/α-ketoglutarate-dependent dioxygenase, expressed in transgenic soybean and *pseudomonas fluorescens*. Journal of Agrcultural and Food Chemistry 61:6589-6596.

Gruys, K.J., M.C. Walker and J.A. Sikorski. 1992. Substrate synergism and the steady-state kinetic reaction mechanism for EPSP synthase from *Escherichia coli*. Biochemistry 31:5534-5544.

Hall, M.R., C.J. Swanton and G.W. Anderson. 1992. The critical period of weed control in grain corn (*Zea mays*). Weed Science 40:441-447.

Halsey, M.E., K.M. Remund, C.A. Davis, M. Qualls, P.J. Eppard and S.A. Berberich. 2005. Isolation of maize from pollen-mediated gene flow by time and distance. Crop Science 45:2172-2185.

Hamilton, D.A., M. Roy, J. Rueda, R.K. Sindhu, J. Stanford and J.P. Mascarenhas. 1992. Dissection of a pollen-specific promoter from maize by transient transformation assay. Plant Molecular Biology 18:211-218.

Harayama, S., M. Kok and E.L. Neidle. 1992. Functional and evolutionary relationships among diverse oxygenases. Annual Review of Microbiology 46:565-601.

Harrigan, G.G., D. Lundry, S. Drury, K. Berman, S.G. Riordan, M.A. Nemeth, W.P. Ridley and K.C. Glenn. 2010. Natural variation in crop composition and the impact of transgenesis. Nature Biotechnology 28:402-404.

Harrigan, G.G., W.P. Ridley, K.D. Miller, R. Sorbet, S.G. Riordan, M.A. Nemeth, W. Reeves and T.A. Pester. 2009. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. Journal of Agricultural and Food Chemistry 57:9754-9763.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126:728-740.

Haslam, E. 1993. Introduction, commentary and overview. Pages 1-16 in Shikimic Acid: Metabolism and Metabolites. John Wiley & Sons, Inc., Chichester, England.

Hausinger, R.P. 2004. Fe(II)/α-Ketoglutarate-dependent hydroxylases and related enzymes. Critical Reviews in Biochemistry and Molecular Biology 39:21-68.

Heap, I. 2019. International survey of herbicide resistant weeds. Corvallis, Oregon. http://www.weedscience.org/in.asp (Accessed August 26, 2019).

Heck, G.R., C.L. Armstrong, J.D. Astwood, C.F. Behr, J.T. Bookout, S.M. Brown, T.A. Cavato, D.L. DeBoer, M.Y. Deng, C. George, J.R. Hillyard, C.M. Hironaka, A.R. Howe, E.H. Jakse, B.E. Ledesma, T.C. Lee, R.P. Lirette, M.L. Mangano, J.N. Mutz, Y. Qi, R.E. Rodriguez, S.R. Sidhu, A. Silvanovich, M.A. Stoecker, R.A. Yingling and J. You. 2005. Development and characterization of a CP4 EPSPS-based, glyphosate-tolerant corn event. Crop Science 45:329-339.

Herman, P.L., M. Behrens, S. Chakraborty, B.M. Chrastil, J. Barycki and D.P. Weeks. 2005. A three-component dicamba *O*-demethylase from *Pseudomonas maltophilia*, strain DI-6: Gene isolation, characterization, and heterologous expression. Journal of Biological Chemistry 280:24759-24767.

Herman, R.A. and W.D. Price. 2013. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. Journal of Agricultural and Food Chemistry 61:11695-11701.

Hérouet, C., D.J. Esdaile, B.A. Mallyon, E. Debruyne, A. Schulz, T. Currier, K. Hendrickx, R.-J. van der Klis and D. Rouan. 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regulatory Toxicology and Pharmacology 41:134-149.

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. The Plant Cell 7:907-919.

Hoff, M., D.-Y. Son, M. Gubesch, K. Ahn, S.-I. Lee, S. Vieths, R.E. Goodman, B.K. Ballmer-Weber and G.A. Bannon. 2007. Serum testing of genetically modified soybeans with special emphasis on potential allergenicity of the heterologous protein CP4 EPSPS. Molecular Nutrition & Food Research 51:946-955.

Holtorf, S., K. Apel and H. Bohlmann. 1995. Comparison of different constitutive and inducible promoters for the overexpression of transgenes in *Arabidopsis thaliana*. Plant Molecular Biology 29:637-646.

Hunt, A.G. 1994. Messenger RNA 3' end formation in plants. Annual Review of Plant Physiology and Plant Molecular Biology 45:47-60.

Ikley, J., T. Legleiter and B. Johnson. 2017. Methods to control "volunteer" corn in a corn replant situation. Purdue University. Indiana State, Indiana.

ILSI-CERA. 2010. A review of the environmental safety of the CP4 EPSPS protein. International Life Sciences Institute, Center for Environmental Risk Assessment, Washington, D.C.

ILSI-CERA. 2011. A review of the environmental safety of the PAT protein. International Life Sciences Institute, Center for Environmental Risk Assessment, Washington, D.C. http://cera-gmc.org/docs/cera_publications/pub_05_2011.pdf.

ISF. 2005. Genetically modified crops and plant breeding. International Seed Foundation, Nyon, Switzerland.

James, C. 2010. Global status of commercialized biotech/GM crops: 2010. Brief 42. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, New York.

Jhala, A.J., S.Z. Knezevic, Z.A. Ganie and M. Singh. 2014. Integrated weed management in maize. Recent Advances in Weed Management. B. Chauhan and G. Mahajan (eds.). Springer, New York, N.Y.

Johnson, B., B. Young, J. Matthews, P. Marquardt, C. Slack, K. Bradley, A. York, S. Culpepper, A. Hager, K. Al-Khatib, L. Steckel, M. Moechnig, M. Loux, M. Bernards and R. Smeda. 2010. Weed control in dicamba-resistant soybeans. Plant Management Network, St. Paul, Minnesota. http://www.plantmanagementnetwork.org/sub/cm/research/2010/dicamba/ (Accessed September 20, 2010).

Jones-Rhoades, M.W., D.P. Bartel and B. Bartel. 2006. MicroRNAs and their regulatory roles in plants. Annual Review of Plant Biology 57:19-53.

Jones, M.D. and J.S. Brooks. 1950. Effectiveness of distance and border rows in preventing outcrossing in corn. Oklahoma Agricultural Experiment Station Technical Bulletin No. T-38 (ed.). Oklahoma State Univeristy, Stillwater, Oklahoma.

Keeler, K.H. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Kistler, L., S.Y. Maezumi, J.G. de Souza, N.A.S. Przelomska, F.M. Costa, O. Smih, H. Loiselle, J. Ramos-Madrigal, N. Wales, E.R. Ribeiro, R.R. Morrison, C. Grimaldo, A.P. Prous, B. Arriaza, M.T.P. Gilbert, F.O. Freitas and R.G. Allaby. 2018. Multiproxy evidence higlights a complex evolutionary legacy of maize in South America. Science 362:1309-1313.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210:437-442.

Knake, E.L., J.F. Miller, O.E. Strand and J.D. Doll. 1990. Annual grass weeds in corn. National Corn Handbook. Michigan State University, East Lansing, Michigan.

Knake, E.L. and F.W. Slife. 1965. Giant foxtail seeded at various times in corn and soybeans. Weeds 13:331-334.

Knezevic, S.Z. and A. Datta. 2015. The critical period for weed control: Revisiting data analysis. Weed Science 63:188-202.

Kovalic, D., C. Garnaat, L. Guo, Y. Yan, J. Groat, A. Silvanovich, L. Ralston, M. Huang, Q. Tian, A. Christian, N. Cheikh, J. Hjelle, S. Padgette and G. Bannon. 2012. The use of next generation sequencing and junction sequence analysis bioinformatics to achieve molecular characterization of crops improved through modern biotechnology. The Plant Genome 5:149-163.

Kundu, S. 2012. Distribution and prediction of catalytic domains in 2-oxoglutarate dependent dioxygenases. Kundu BMC Research Notes 5:410.

CBI-Deleted

[ ]

Lamppa, G.K., G. Morelli and N.-H. Chua. 1985. Structure and developmental regulation of a wheat gene encoding the major chlorophyll a/b-binding polypeptide. Molecular and Cellular Biology 5:1370-1378.

Lee, M.-S., E.K. Anderson, D. Stojsin, M.A. McPherson, B. Baltazar, M.J. Horak, J.M. de la Fuente, K. Wu, J.H. Crowley, A.L. Rayburn and D.K. Lee. 2017. Assessment of the potential for gene flow from transgenic maize (*Zea Mays* L.) to eastern gamagrass (*Tripsacum dactyloide*s L.). Transgenic Research 26:501-514.

Lemaux, P.G. 2008. Genetically engineered plants and foods: A scientist's analysis of the issues (Part I). Annual Review of Plant Biology 59:771-812.

Lingenfelter, D.D. and N.L. Hartwig. 2007. Introduction to weeds and herbicides. Penn State University Agricultural Research and Cooperative Extension, University Park, Pennsylvania.

Loux, M.M., D. Doohan, A.F. Dobbels, W.G. Johnson and T.R. Legleiter. 2014. 2014 Ohio and Indiana weed control guide. The Ohio State University and Purdue Extension, Columbus, Ohio.

Ma, B.L., K.D. Subedi and L.M. Reid. 2004. Extent of cross-fertilization in maize by pollen from neighboring transgenic hybrids. Crop Science 44:1273-1282.

Ma, J., D.S. Skibbe, J. Fernandes and V. Walbot. 2008. Male reproductive development: Gene expression profiling of maize anther and pollen ontogeny. Genome Biology 9:R181.181-R181.117.

Maeda, H. and N. Dudareva. 2012. The shikimate pathway and aromatic amino Acid biosynthesis in plants. Annu Rev Plant Biol 63:73-105.

Massinga, R.A., R.S. Currie and T.P. Trooien. 2003. Water use and light interception under Palmer amaranth (*Amaranthus palmeri*) and corn competition. Weed Science 51:523-531.

Matsuoka, Y., Y. Vigouroux, M.M. Goodman, J. Sanchez G., E. Buckler and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the National Academy of Sciences of the United States of America 99:6080-6084.

McElroy, D., W. Zhang, J. Cao and R. Wu. 1990. Isolation of an efficient actin promoter for use in rice transformation. The Plant Cell 2:163-171.

Meinnel, T. and C. Giglione. 2008. Tools for analyzing and predicting N-terminal protein modifications. Proteomics 8:626-649.

Meyers, B.C., S.V. Tingey and M. Morgate. 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Research 11:1660-1676.

Mississippi State University. 2015. 2015 weed control guidelines for Mississippi. Mississippi State University Extension Service, Mississippi State, Mississippi.

Monsanto. 2017. Roundup Power Max II herbicide label. Monsanto Company, St. Louis, Missouri.

Monsanto. 2019. U.S. technology use guide. St. Louis, Missouri.

Morris, M.L. 1998. Overview of the world maize economy. Pages 13-34 in Maize Seed Industries in Devoloping Countries. M.L. Morris (ed.). Lynne Rienner Publishers, Inc., Boulder, Colorado.

Müller, T.A., M.I. Zavodszky, M. Feig, L.A. Kuhn and R.P. Hausinger. 2006. Structural basis for the enantiospecificities of R- and S-specific phenoxypropionate/α-ketoglutarate dioxygenases. Protein Science 15:1356-1368.

NDSU. 2018. Chemical weed control for field crops. North Dakota State University. North Dakota.

NRC. 2004. New approaches for identifying unintended changes in food composition. Pages 73-102 in Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. Institute of Medicine and National Research Council, National Academies Press, Washington, D.C.

Odell, J.T., F. Nagy and N.-H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313:810-812.

OECD. 1993. Safety considerations for biotechnology: Scale-up of crop plants. Organisation for Economic Co-operation and Development, Paris, France. www.biosafety.be/CU/BSL_Ressources/PDF/M00034525.pdf (Accessed January 9, 2009).

OECD. 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide. ENV/JM/MONO(99)13. Series on

Harmonization of Regulatory Oversight in Biotechnology No.11. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2002a. Module II: Herbicide biochemistry, herbicide metabolism and the residues in glufosinate-ammonium (Phosphinothricin)-tolentant transgenic plants. ENV/JM/MONO(2002)14. Series on Harmonization of Regulatory Oversight in Biotechnology No. 25. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2002b. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients and secondary plant metabolites. OECD ENV/JM/MONO (2002)25. Organisation of Economic Co-Operation and Development, Paris, France.

OECD. 2003. Consensus document on biology of *Zea mays* subs. *mays* (maize), ENV/JM/MONO(2003)11. Organization for Economic Co-operation and Develpoment, Washington, D.C.

OGTR. 2008. The biology of *Zea mays* L. ssp *mays* (maize or corn). Version 1, September 2008, Australian Government, Department of Health and Ageing, Office of the Gene Technology Regulator, Canberra, Australia.

Olson, R.A. and D.H. Sander. 1988. Corn production. Pages 639-686 in Corn and Corn Improvement. 3rd Edition. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996. New weed control opportunities: Development of soybeans with a Roundup Ready™ gene. Pages 53-84 in Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. S.O. Duke (ed.). CRC Press, Inc., Boca Raton, Florida.

Pallett, K. 2018. Engineered crop tolerance to glyphosate and its impact on the use of the herbicide. Outlooks on Pest Management 29:277-281.

Pilacinski, W., A. Crawford, R. Downey, B. Harvey, S. Huber, P. Hunst, L.K. Lahman, S. MacIntosh, M. Pohl, C. Rickard, L. Tagliani and N. Weber. 2011. Plants with genetically modified events combined by conventional breeding: An assessment of the need for additional regulatory data. Food and Chemical Toxicology 49:1-7.

Piperno, D.R., A.J. Ranere, I. Holst, J. Iriarte and R. Dickau. 2009. Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Cental Balsas River Valley, Mexico. PNAS 106:5019-5024.

Pla, M., J.-L. La Paz, G. Peñas, N. García, M. Palaudelmàs, T. Esteve, J. Messeguer and E. Melé. 2006. Assessment of real-time PCR based methods for quantification of pollen-mediated gene flow from GM to conventional maize in a field study. Transgenic Research 15:219-228.

Prado, J.R., G. Segers, T. Voelker, D. Carson, R. Dobert, J. Phillips, K. Cook, C. Cornejo, J. Monken, L. Grapes, T. Reynolds and S. Martino-Catt. 2014. Genetically engineered crops: From idea to product. Annual Review of Plant Biology 65:769-790.

Raybould, A., L.S. Higgins, M.J. Horak, R.J. Layton, N.P. Storer, J.M. de la Fuente and R.A. Herman. 2012. Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize. Transgenic Research 21:655-664.

Rijavec, T., A. Lapanje, M. Dermastia and M. Rupnik. 2007. Isolation of bacterial endophytes from germinated maize kernels. Canadian Journal of Microbiology 53:802-808.

Ritchie, S.W., J.J. Hanway and G.O. Benson. 1997. How a corn plant develops. Iowa State University of Science and Technology, Cooperative Extension Service, Ames, Iowa.

Russell, W.A. and A.R. Hallauer. 1980. Corn. Pages 299-312 in Hybridization of Crop Plants. W.R. Fehr and H.H. Hadley (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Madison, Wisconsin.

Salomon, S. and H. Puchta. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal 17:6086-6095.

Sankula, S. 2006. Quantification of the impacts on US agriculture of biotechnology-derived crops planted in 2005. National Center for Food and Agricultural Policy, Washington, D.C.

SanMiguel, P., A. Tikhonov, Y.-K. Jin, N. Motchoulskaia, D. Zakharov, A. Melake-Berhan, P.S. Springer, K.J. Edwards, M. Lee, Z. Avramova and J.L. Bennetzen. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274:765-768.

Seng, C.T., L. Van Lun, C.T. San and I. Sahid. 2010. Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Biology and Management 10:229-233.

Sidorov, V. and D. Duncan. 2009. *Agrobacterium*-mediated maize transformation: Immature embryos versus callus. Pages 47-58 in Methods in Molecular Biology: Transgenic Maize - Methods and Protocols. M.P. Scott (ed.). Humana Press, Inc, Totowa, New Jersey.

Sikorski, J.A. and K.J. Gruys. 1997. Understanding glyphosate's molecular mode of action with epsp synthase: Evidence favoring an allosteric inhibitor model. Accounts of Chemical Research 30:2-8.

Singer, J. and P. Bauer. 2009. Soil management practices: Crop rotations for row crops. Iowa State University Extension, Ames, Iowa. http://soilquality.org/practices/row_crop_rotations.html (Accessed March 11, 2013).

Soltani, N., J.A. Dille, I.C. Burke, W.J. Everman, M.J. VanGessel, V.M. Davis and P.H. Sikkema. 2016. Potential corn yield losses from weeds in North America. Weed Technology 30:979-984.

Stalker, D.M., C.M. Thomas and D.R. Helinski. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Molecular and General Genetics 181:8-12.

Steckel, L.E., M.A. Thompson and R.M. Hayes. 2009. Herbicide options for controlling glyphosate-tolerant corn in a corn replant situation. Weed Technology 23:243-246.

Steinrücken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochemical and Biophysical Research Communications 94:1207-1212.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symposia on Quantitative Biology 43:77-90.

Takeuchi, M., K. Hamana and A. Hiraishi. 2001. Proposal of the genus *Sphingomanas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. International Journal of Systematic and Evolutionary Microbiology 51:1405-1417.

Tapia, L.S., T.T. Bauman, R.G. Harvey, J.J. Kells, G. Kapusta, M.M. Loux, W.E. Lueschen, M.D.K. Owen, L.H. Hageman and S.D. Strachan. 1997. Postemergence herbicide application timing effects on annual grass and corn (*Zea mays*) grain yield. Weed Science 45:138-143.

Tashkov, W. 1996. Determination of formaldehyde in foods, biological media and technological materials by headspace gas chromatography. Chromatographia 43:625-627.

Terada, R. and K. Shimamoto. 1990. Expression CaMV35S-GUS gene in transgenic rice plants. Molecular and General Genetics 220:389-392.

Thomas, P., S. Kumari, G.K. Swarna and T.K. Gowda. 2007. Papaya shoot tip associated endophytic bacteria isolated from in vitro cultures and host-endophyte interaction in vitro and in vivo. Canadian Journal of Microbiology 53:380-390.

Thompson, C.J., N.R. Movva, R. Tizard, R. Crameri, J.E. Davies, M. Lauwereys and J. Botterman. 1987. Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO Journal 6:2519-2523.

Timmons, F.L. 2005. A history of weed control in the United States and Canada. Weed Science 53:748-761.

Travlos, I.S., N. Cheimona, R. De Prado, A.J. Jhala, D. Chachalis and E. Tani. 2018. First case of glufosinate-resistant rigid ryegrass (*Lolium rigidum* Gaud.). Greece. Agronomy 8:35.

Tzin, V., S. Malitsky, M.M. Ben Zvi, M. Bedair, L. Sumner, A. Aharoni and G. Galili. 2012. Expression of a bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase of the shikimate pathway in Arabidopsis elucidates potential metabolic bottlenecks between primary and secondary metabolism. New Phytol 194:430-439.

U.S. EPA. 1996a. Plant pesticide inert ingredient CP4 enolpyruvylshikimate-3-D and the genetic material necessary for its production in all plants. Federal Register 61:40338-40340.

U.S. EPA. 1996b. Residue chemistry test guidelines - Background. OPPTS 860.1000. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

U.S. EPA. 1997. Phosphinothricin acetyltransferase and the genetic material necessary for its production in all plants; Exemption from the requirement of a tolerance on all raw agricultural commodities. Federal Register 62:17717-17720.

U.S. EPA. 2009. Reregistration eligibility decision for dicamba and associated salts. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

U.S. EPA. 2014a. Memorandum: Addendum to 2,4-D choline salt section 3 risk assessment: Refined endangered species assessment for proposed new uses on herbicide-tolerant corn and soybean for AR, KS, LA, MN, MS, MO, NE, ND, OK, TN, September 26, 2014. DP Barcode 421678, 422714. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2014b. Memorandum: Addendum to 2,4-D choline salt section 3 risk assessment: Refined endangered species assessment for proposed new uses on herbicide-tolerant corn and soybean: February 12, 2014. DP Barcode D 418022. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2017a. Federal Register, 82 FR 9523-9529, 2,4-D; Pesticide Tolerances. U.S. Enviornmental Protection Agency, Washington, D.C.

U.S. EPA. 2017b. PRN 2017-1: Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2017c. PRN 2017-2: Guidance for Herbicide-Resistance Management, Labeling, Education, Training, and Stewardship. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2018. Federal Register, 83 FR 7111-7115, Quizalofop ethyl; Pesticide Tolerances. Washington, D.C.

U.S. EPA. 2019. Federal Register, 84 FR 9781-9782; Dicamba New Uses. U.S. Environmental Protection Agency, Washington, D.C.

U.S. FDA. 1992. Statement of policy: Foods derived from new plant varieties. Federal Register 57: 22984-23005.

U.S. FDA. 1995a. AgrEvo BNF No. 29: Corn: Tolerance to the herbicide glufosinate-ammonium. T14, T25. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-ZM002-1 (Accessed April 8, 2015).

U.S. FDA. 1995b. AgrEvo BNF No. 23: Oilseed rape: Tolerance to the herbicide glufosinate-ammonium. HCN92. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-BN007-1 (Accessed April 8, 2015).

U.S. FDA. 1996. Dekalb Genetics BNF No. 28: Corn: Tolerance to the herbicide glufosinate-ammonium. DLL25. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=DKB-89790-5 (Accessed April 8, 2015).

U.S. FDA. 1997. AgrEvo BNF No. 46: Canola: Tolerance to the herbicide glufosinate-ammonium. T45. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-BN008-2 (Accessed April 8, 2015).

U.S. FDA. 1998a. AgrEvo BNF No. 38: Sugar beet: Tolerance to the herbicide glufosinate-ammonium. T120-7. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-BV001-3 (Accessed April 8, 2015).

U.S. FDA. 1998b. AgrEvo BNF No. 55: Soybean: Tolerance to the herbicide glufosinate-ammonium. A2704-12, A5547-127. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-GM006-4 (Accessed April 8, 2015).

U.S. FDA. 1999. Aventis BNF No. 63: Rice: Tolerance to the herbicide glufosinate-ammonium. LLRICE E06, LLRICE E62. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-OS002-5 (Accessed April 8, 2015).

U.S. FDA. 2001. Premarket notice concerning bioengineered foods. Federal Register 66:4706-4738.

U.S. FDA. 2002. Bayer CropScience BNF No. 86: Cotton: Tolerance to the herbicide glufosinate-ammonium. LLCotton25. U.S. Food and Drug Administration, Washington, D.C. http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=Biocon&id=ACS-GH001-3 (Accessed April 8, 2015).

USDA-APHIS. 1986. Coordinated framework for regulation of biotechnology. Federal Register 51:23302.

USDA-APHIS. 2014. Dow AgroSciences petitions (09-233-01p, 09-349-01p, and 11-234-01p) for determinations of nonregulated status for 2,4-D-Resistant Corn and Soybean varieties: Final environmental impact statement - August 2014. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.

USDA-ARS. 2015. Germplasm Resources Information Network. U.S. Department of Agriculture, Agricultural Research Service. https://www.ars-grin.gov/.

USDA-ERS. 2018a. Feed grains database: custom queries. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. https://data.ers.usda.gov/FEED-GRAINS-custom-query.aspx (Accessed March 1, 2018).

USDA-ERS. 2018b. Recent trends in GE adoption. United States Department of Agriculture, Economic Research Service, Washington, D.C. https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx (Accessed October 24, 2018).

USDA-FAS. 2018. World agricultural production. Circular Series WAP 12-18. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C. https://www.fas.usda.gov/data/world-agricultural-production (Accessed December 21, 2018).

USDA-FAS. 2019. World agricultural production. Circular Series World Agricultural Production 2-19. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C.

USDA-NASS. 2014. Crop production 2013 summary, January 2014. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018a. Data and statistics – Statistics by subject. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. https://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS (Accessed October 24, 2018).

USDA-NASS. 2018b. Data and statistics – Charts and Maps. United States Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. https://www.nass.usda.gov/Charts_and_Maps/Field_Crops/index.php (Accessed October 24, 2018).

USDA-NASS. 2019a. Crop Values 2018 Summary, April 2019. United States Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2019b. Surveys - Chemical Use. U.S. Department of Agriculture, National Agricultural Statictics Service, Washington, D.C. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/ (Accessed October 24, 2019).

USDA-NASS. 2019c. Crop Production 2018 Summary. United States Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NRCS. 2019. The PLANTS database. National Plant Data Team, Greensoro, N.C. http://plants.usda.gov (Accessed January 25, 2019).

Valent. 2006. Select 2EC herbicide label. Valent USA Corporation, Walnut Creek, California.

Van Wychen. 2015. 2015 Baseline survey of the most common and troublesome weeds in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wssa/weed/surveys/.

Venkatesh, T.V., M.L. Breeze, K. Liu, G.G. Harrigan and A.H. Culler. 2014. Compositional analysis of grain and forage from MON 87427, an inducible male sterile and tissue selective glyphosate-tolerant maize product for hybrid seed production. Journal of Agricultural and Food Chemistry 62:1964-1973.

Venkatesh, T.V., G.G. Harrigan, T. Perez and S. Flint-Garcia. 2015. Compositional assessments of key maize populations: B73 hybrids of the Nested Association Mapping founder lines and diverse landrace inbred lines. Journal of Agricultural and Food Chemistry 63:5282-5295.

Wang, C., K.C. Glenn, C. Kessenich, E. Bell, L.A. Burzio, M.S. Koch, B. Li and A. Silvanovich. 2016. Safety assessment of dicamba mono-oxygenases that confer dicamba tolerance to various crops. Regulatory Toxicology and Pharmacology 81:171-182.

Wang, X.-Z., B. Li, P.L. Herman and D.P. Weeks. 1997. A three-component enzyme system catalyzes the O demethylation of the herbicide dicamba in *Pseudomonas maltophilia* DI-6. Applied and Environmental Microbiology 63:1623-1626.

Webster, T.M., M. Patterson and J. Everest. 2012. Weed survey - Southern states 2012: Grass crops subsection (Corn; Grain Sorghum, Hay, Pastures, and Rangelands; Rice; Small Grains; Sugarcane; Turf; Wheat). 2012 Proceedings, Southern Weed Science Society 65:267-288.

Weekes, R., T. Allnutt, C. Boffey, S. Morgan, M. Bilton, R. Daniels and C. Henry. 2007. A study of crop-to-crop gene flow using farm scale sites of fodder maize (*Zea mays* L.) in the UK. Transgenic Research 16:203-211.

Wehrmann, A., A.V. Vliet, C. Opsomer, J. Botterman and A. Schulz. 1996. The similarities of *bar* and *pat* gene products make them equally applicable for plant engineers. Nature Biotechnology 14:1274-1278.

Whitelaw, C.A., W.B. Barbazuk, G. Pertea, A.P. Chan, F. Cheung, Y. Lee, L. Zheng, S. van Heeringen, S. Karamycheva, J.L. Bennetzen, P. SanMiguel, N. Lakey, J. Bedall, Y. Yuan, M.A. Budiman, A. Resnick, S. Van Aken, T. Utterback, S. Riedmuller, M. Williams, T. Feldblyum, K. Schubert, R. Beachy, C.M. Fraser and J. Quackenbush. 2003. Enrichment of gene-coding sequences in maize by genome filtration. Science 3002:2118-2120.

WHO. 1995. Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. World Health Organization, Geneva, Switzerland.

Wilkes, H.G. 1972. Maize and its wild relatives. Science 177:1071-1077.

Wishart, D.S. 2010. Human metabolome database. www.hmdb.ca (Accessed June 2, 2010).

Wishart, D.S., C. Knox, A.C. Guo, R. Eisner, N. Young, B. Gautam, D.D. Hau, N. Psychogios, E. Dong, S. Bouatra, R. Mandal, I. Sinelnikov, J. Xia, L. Jia, J.A. Cruz, E. Lim, C.A. Sobsey, S. Shrivastava, P. Huang, P. Liu, L. Fang, J. Peng, R. Fradette, D. Cheng, D. Tzur, M. Clements, A. Lewis, A. De Souza, A. Zuniga, M. Dawe, Y. Xiong, D. Clive, R. Greiner, A. Nazyrova, R. Shaykhutdinov, L. Li, H.J. Vogel and I. Forsythe. 2009. HMDB: A knowledgebase for the human metabolome. Nucleic Acids Research 37:D603-D610.

Wohlleben, W., W. Arnold, I. Broer, D. Hillemann, E. Strauch and A. Pühler. 1988. Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. Gene 70:25-37.

WSSA. 2018. WSSA herbicide site-of-action (SOA) classification list. Weed Science Society of America, Westminster, CO. http://wssa.net/wssa/weed/herbicides/.

Wunderlin, R., B. Hansen, A. Franck and F.B. Essig. 2019. Atlas of Florida Plants. Institute for Systematic Botany, Tampa, Florida. http://florida.plantatlas.usf.edu/Plant.aspx?id=2396 (Accessed January 25, 2019).

Wych, R.D. 1988. Production of hybrid seed corn. Pages 565-607 in Corn and Corn Improvement. Third Edition. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Yang, H., Y. Qi, M.E. Goley, J. Huang, S. Ivashuta, Y. Zhang, O.C. Sparks, J. Ma, B.M. van Scoyoc, A.L. Caruano-Yzermans, J. King-Sitzes, X. Li, A. Pan, M.A. Stoecker, B.E. Wiggins and M.J. Varagona. 2018. Endogenous tassel-specific small RNAs-mediated RNA interference enables a novel glyphosate-inducible male sterility system for commercial production of hybrid seed in *Zea mays* L. PLoS One 13:e0202921.

Yousef, G.G. and J.A. Juvik. 2001. Crop breeding, genetics & cytology: Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Science 41:645-655.

Zambryski, P., A. Depicker, K. Kruger and H.M. Goodman. 1982. Tumor induction by *Agrobacterium tumefaciens*: Analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1:361-370.

Zhang, K., Y. Li and L. Lian. 2011. Pollen-mediated transgene flow in maize grown in the Haung-haui-hai region in China. Journal of Agricultural Science 149:205-216.

Zhang, L., J.-M. Chia, S. Kumari, J.C. Stein, Z. Liu, A. Narechania, C.A. Maher, K. Guill, M.D. McMullen and D. Ware. 2009. A genome-wide characterization of microRNA genes in maize. PLoS Genetics 5:e1000716.

Zipper, C., K. Nickel, W. Angst and H.-P.E. Kohler. 1996. Complete mictobial degradation of both enantionmers of the chiral herbicide mecoprop [(RS)-2-(4-Chloro-2-Methylphenoxy) propionic Acid] in an enantioselective manner by *Sphingomonas herbicidovorans* sp. nov. Applied and Enviornmental Microbiology 62:4318-4322.

Zuloaga, F.O., O. Morrone, G. Davidse, T.S. Filgueiras, P.M. Peterson, R.J. Soreng and E.J. Judziewicz. 2003. Catalogue of New World grasses (Poaceae) : III, subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae. National Museum of Natural History, Department of Systematic Biology - Botany, Washington, D.C.

APPENDICES

Appendix A: USDA Notifications and Permits

Field trials of MON 87429 have been conducted in the U.S. since 2014. 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 87429 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2017-2018 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 87429 and Status of Trials Planted under These Notifications

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State(s)	Sites
2014	14-080-103n	4/20/2014	Submitted	HI	1
				PR	1
	14-120-113rm	9/2/2014	Submitted	HI	1
2015	14-120-113rm	9/2/2014	Submitted	PR	1
	14-302-112rm	3/1/2015	Submitted	HI	1
				PR	1
	14-309-102rm	3/15/2015	Submitted	IL	5
				IN	1
				KS	5
				NE	4
	14-309-108rm	3/15/2015	Submitted	IA	9
				IL	2
				NE	1

Table A-1. USDA Notifications and Permits Approved for MON 87429 and Status of Trials Planted under These Notifications (continued)

2015	15-042-103n	3/13/2015	Submitted	IA	1
	15-120-112rm	9/1/2015	Submitted	HI	1
2016	15-120-112rm	9/1/2015	Submitted	HI	1
				PR	1
	15-289-112rm	3/2/2016	Submitted	HI	2
	15-306-101rm	3/15/2016	Submitted	IA	6
				IL	2
				KS	4
	15-306-107rm	3/15/2016	Submitted	IA	3
				IL	6
				NE	3
	16-047-104n	3/17/2016	Submitted	IA	1
	16-104-104n	5/11/2016	Submitted	NE	1
	16-116-104n	5/25/2016	Submitted	HI	1
				PR	1
	16-117-104rm	9/1/2016	Submitted	HI	1
				PR	1
	16-117-111rm	9/1/2016	Submitted	HI	1
	16-117-112rm	9/1/2016	Submitted	HI	1
	16-299-102n	11/24/2016	Submitted	HI	1
2017	16-117-111rm	9/1/2016	Submitted	PR	1
	16-182-104rm	12/1/2016	Submitted	HI	1
	16-299-102n	11/24/2016	Submitted	HI	2

Table A-1. USDA Notifications and Permits Approved for MON 87429 and Status of Trials Planted under These Notifications (continued)

2017	16-302-110rm	3/1/2017	Submitted	HI	1
	16-302-111rm	3/1/2017	Submitted	HI	1
	16-315-101rm	3/15/2017	Submitted	IA	5
				IL	10
	16-315-105rm	3/15/2017	Submitted	NE	3
	17-037-101n	3/9/2017	Submitted	CA	1
	17-052-103n	3/23/2017	Submitted	IA	2
				IN	1
				MO	1
				NC	1
				NE	1
				OH	1
				PA	1
	17-053-103n	3/18/2017	Submitted	IA	2
				IL	3
				NE	1
	17-053-105n	3/24/2017	Submitted	IA	1
				IL	2
	17-065-103n	4/5/2017	Submitted	IL	1
	17-100-104n	6/1/2017	Submitted	HI	2
	17-115-111rm	9/1/2017	Submitted	HI	1
2018	17-214-101rm	12/1/2017	Submitted	HI	2

Table A-1. USDA Notifications and Permits Approved for MON 87429 and Status of Trials Planted under These Notifications (continued)

2018	17-304-101n	11/30/2017	Submitted	HI	3
	17-304-101rm	3/1/2018	In Progress	HI	1
	17-320-101rm	3/15/2018	In Progress	IA	3
				IL	5
				NE	2
	17-320-102rm	3/15/2018	In Progress	IL	2
				NE	1
	18-031-104rm	5/25/2018	In Progress	HI	1
	18-060-107n	3/31/2018	In Progress	IL	2
	18-065-105n	4/5/2018	In Progress	CA	1
				IA	2
				IL	2
				KS	1
				NE	1
				TX	1
				WI	1
	18-085-101n	4/25/2018	In Progress	HI	2
	18-122-103rm	9/1/2018	In Progress	HI	1
	18-122-104rm	9/1/2018	In Progress	HI	1
	18-122-105rm	9/1/2018	In Progress	HI	1
	18-122-106rm	9/1/2018	In Progress	HI	2
	18-283-103n	11/7/2018	In Progress	IL	1
	18-290-101n	11/12/2018	In Progress	IA	1
Total					**158**

Appendix B: Overview, Materials, Methods and Supplementary Results for Molecular Analyses of MON 87429

B.1. Test Substance

The test substance in this study was MON 87429. Five breeding generations of MON 87429 were used to assess the stability of the T-DNA insert. Genomic DNA for use in this study was extracted from seed tissue listed in the table below.

MON 87429 Breeding Generation	Seed ID
R3	11464932
R3F1	11464934
R4	11464931
R4F1	11465707
R5	11465738

B.2. Control Substance

The control substance is the conventional crop variety which is the similar genetic background as the test substances. Genomic DNA was extracted from seed as listed in the table below.

Control Substance	Seed ID	Breeding Generations
LH244	11464930	R3, R4, R5
LH244 × HCL617	11464933	R3F1
LH244 × L7126Z	11465706	R4F1

B.3. Reference Substance

The reference substance was plasmid vector PV-ZMHT519224, which was used to develop MON 87429. Whole plasmid DNA and its sequence served as a positive control for sequencing and bioinformatic analyses. The identity of the reference plasmid was confirmed by sequencing within the study. Documentation of the confirmation of the plasmid vector identity was archived with the raw data. Appropriate molecular weight 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.4. Characterization of Test, Control, and Reference Substances

The Study Director reviewed the chain-of-custody forms to confirm the identity of the test and control substances prior to the use of these materials in the study. Further confirmation of test and control substances identity was determined by sequencing in the study. Test, control and reference DNA and sequencing library substances were considered stable during storage if they yield interpretable signals in sequencing experiments, or did not appear visibly degraded on the stained gels (DNA) or sufficient cluster amplification was observed during flow cell generation (sequencing libraries).

B.5. Genomic DNA Isolation

For sequencing library construction and PCR reactions, genomic DNA was isolated from seed tissues of the test and control substances. First the seeds were decontaminated by vigorously agitating them by hand for 30 seconds with 0.05% (v/v) Tween-20, followed by a tap water rinse. The seeds were then vigorously agitated with 0.5% (w/v) NaOCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The seeds were then vigorously agitated with 1% (v/v) HCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The 1% (v/v) HCl rinse was repeated one time, and then the seeds were rinsed with distilled water and air dried. 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 ground seed tissue. The samples were incubated at 60°C-70°C for 20-25 minutes with intermittent mixing. The samples were cooled to room temperature and subjected to multiple rounds of chloroform:isoamyl alcohol (24:1) extraction. An additional round of extraction with 10% CTAB solution (10% (w/v) CTAB and 0.7 M NaCl) and chloroform:isoamyl alcohol (24:1) was performed. Genomic DNA was precipitated by adding ~3× volumes of CTAB precipitation buffer (1% (w/v) CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA (pH 8.0) to the samples, followed by resuspension in high salt TE buffer (10 mM Tris HCl (pH 8.0), 1 mM EDTA (pH 8.0), 1 M NaCl). Genomic DNA was precipitated again with 3 M sodium acetate (pH 5.2) and 100% (v/v) ethanol, 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.6. DNA Quantification

PV-ZMHT519224 DNA and extracted genomic DNA were quantified using a Nanodrop™ Spectrophotometer (Thermo Scientific).

B.7. Agarose Gel Electrophoresis

After quantification, the extracted DNA for NGS library construction was run on a 1% (w/v) agarose gel to check the quality.

B.8. Shearing of DNA

Approximately 1 µg of DNA from the test, control and reference substances were sheared by sonication. The DNA was diluted to ~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.

B.9. Paired End Library Preparation

Paired end genomic DNA libraries were prepared for the test, control, and reference substances using the KAPA Hyper Prep kit (Kapa Biosystems) and a Sage Science BluePippin DNA Size Selection system (Sage Science Inc.) was used to size select the DNA fragments.

First, the 3' and 5' overhangs of the DNA fragments generated by the shearing process were converted into blunt ends and adenylated by following the manufacturer's KAPA Hyper Prep kit instructions (Kapa Biosystems).

Next, adaptors were ligated to the end repaired and A-tailing reaction products by following the manufacturer's KAPA Hyper Prep kit instructions. Following adaptor ligation, an AMPure XP (Beckman Coulter) cleanup was performed on the libraries which were then resuspended in 25 µl of Buffer EB prior to PCR amplification of the libraries. A five cycle PCR amplification of the libraries was carried out following the manufacturer's KAPA Hyper Prep kit instructions. A second AMPure XP cleanup was performed on the libraries which were then resuspended in 22.5 µl of Qiagen Elution Buffer (EB) and stored at -20°C.

The libraries were run on the Sage Science BluePippin Size Selection system using 1.5% agarose gel cassettes and following the manufacturer's instructions. After elution of the desired size range (~500 bp) of DNA fragments, the DNA sample in the elution chamber of the cassette was removed and a 1:1 bead cleanup was performed using the 1x KAPA Pure Beads according to the KAPA Hyper Prep kit instructions.

After bead cleanup, an aliquot of the libraries was analyzed using a Fragment Analyzer QC (Advanced Analytical Technologies, Inc.) according to the manufacturer's instructions. Additional, size selection was conducted using Aline DNA Size Selector-I beads to remove fragments under ~400 bp. An aliquot of the Aline DNA size selected libraries was analyzed using a Fragment Analyzer QC according to the manufacturer's instructions. All purified library DNA was stored in a -20°C freezer.

B.10. Next-Generation Sequencing

The library samples described above were sequenced by Monsanto's Sequencing Technologies using Illumina NextSeq technology that produced short sequence reads (~150 bp long). Sufficient numbers of these sequence fragments were obtained to comprehensively cover the entire genomes of the test samples and the conventional control (Kovalic et al., 2012). Furthermore, a transformation plasmid spike was sequenced to >75× to assess method sensitivity through modeling of $1/10^{th}$ and one full genome equivalent plasmid spike.

B.11. Read Mapping and Junction Identification

High-throughput sequence reads were captured by aligning to the PV-ZMHT519224 transformation plasmid sequence using the read alignment software Bowtie (V2.2.3) (Langmead and Salzberg, 2012) in order to collect all reads that were sourced from the plasmid as well as reads with sequences representing integration points. Captured reads were subsequently mapped using the FASTA (V36.3.6) local alignment program (Pearson, 2000). All software versions were documented in the archived data package and the software versions which were used in this study have been archived.

B.12. Mapping and Junction Detection

Captured reads from both test and control samples were mapped to the complete PV-ZMHT519224 transformation plasmid sequence in order to detect junction sequences using the FASTA (V36.3.6) local alignment program. Reads with partial matches to the transformation plasmid of at least 30 bases and 96.6% or greater identity were also collected as potential junction sequences (Kovalic et al., 2012).

B.12.1. Effective Sequencing Depth Determination

A single copy locus from the native plant genome (*Zea Mays*) *pyruvate decarboxylase* (*pdc3,* GenBank accession AF370006.2) was used to determine the actual sequence depth coverage. All reads with at least 30 bases matching and 96.6% identity were considered as reads sourced from this locus. Read alignments were further filtered to identify the best alignment as judged by the numerically lowest FASTA alignment *E*-score. Furthermore, final sequencing depths were calculated using a reads best aligning position when multiple alignments were returned. In instances where a read yielded two alignments with identical *E*-scores, both were retained.

Table B-1. Sequencing (NGS) Conducted for the Control and Test Substance

Sample	Total Nucleotide Number (Gb)	Effective Median Depth of Coverage (×-fold)
LH244	286.44	121×
LH244 × HCL617	267.68	106×
LH244 × L7126Z	215.17	86×
MON 87429 (R3)	265.77	108×
MON 87429 (R3F1)	244.53	98×
MON 87429 (R4)	292.95	123×
MON 87429 (R4F1)	269.65	102×
MON 87429 (R5)	254.53	102×

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.12.2. Positive Control

To produce the positive control sample for sequencing, a plasmid DNA library was created as described in Appendix Sections B.8 to B.10 and spiked into a LH244 conventional control library at approximately 0.03%. The collected data were scaled to represent a single genome equivalent dataset and a $1/10^{th}$ genome equivalent dataset.

Table B-2. Summary of NGS Data for the Conventional Control DNA Sample Spiked with PV-ZMHT519224

	0.1 Genome Equivalent (14× coverage)	1 Genome Equivalent (123× coverage)
Extent of coverage[1] of PV-ZMHT519224	100%	100%
Percent identity of coverage[2] of PV-ZMHT519224	100%	100%

[1]Extent of coverage is calculated as the percent of the PV-ZMHT519224 plasmid that is observed to have coverage by the captured sequence reads.
[2]Percent identity of coverage is calculated as the per position consensus relative to reference of mapped PV-ZMHT519224 bases from the randomly sampled reads.

B.13. PCR and DNA Sequence Analyses to Examine the Insert and Flanking Sequences in MON 87429

Overlapping PCR products, denoted as Product A and Product B were generated that span the insert and adjacent 5' and 3' flanking DNA sequences in MON 87429. 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 87429, as well as that of the DNA flanking the 5′ and 3′ ends of the insert, as depicted in Figure IV-7.

The PCR analyses for both Product A and Product B were conducted using approximately 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, 0.2 mM of each dNTP, and 1.25 units/reaction of PrimeSTAR GXL Polymerase (TaKaRa Bio Inc.).

The PCR amplification of both Product A and Product B was performed under the following cycling conditions: 25 cycles at 98°C for 10 seconds; 60°C for 15 seconds; 68°C for 9 minutes.

Aliquots of each PCR product were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were the expected sizes. Each PCR product was purified with Agencourt AMPure XP (Beckman Coulter Life Sciences). Approximately 200 ng of purified amplicon was submitted to Monsanto's Genome Sequencing Center for library preparation and MiSeq sequencing.

A consensus sequence was generated by compiling captured reads from MiSeq sequencing of the overlapping PCR products. This consensus sequence was aligned to the PV-ZMHT519224 sequence to determine the integrity and organization of the integrated DNA and the 5' and 3' insert to flank DNA junctions in MON 87429.

B.14. PCR and DNA Sequence Analyses to Examine the Integrity of the DNA Insertion Site in MON 87429

To examine the MON 87429 T-DNA insertion site in LH244 conventional maize, PCR and sequence analyses were performed on genomic DNA from the LH244 conventional control as depicted in Figure IV-7.

The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87429. A forward primer specific to the DNA sequence flanking the 5' end of the insert was paired with a reverse complement primer specific to the DNA sequence flanking the 3' end of the insert.

The PCR reactions were conducted using approximately 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, 0.2 mM of each dNTP, and 1.25 units/reaction of PrimeSTAR GXL Polymerase. The PCR amplification was performed under the following cycling conditions: 30 cycles at 98°C for 10 seconds; 60°C for 15 seconds; 68°C for 30 seconds.

Aliquots of each PCR product were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were the expected sizes. Each PCR product was purified with Agencourt AMPure XP (Beckman Coulter Life Sciences). Approximately 200 ng of purified amplicon was submitted to Monsanto's Genome Sequencing Center for library preparation and MiSeq sequencing.

A consensus sequence was generated by compiling sequences from MiSeq sequencing reactions of the verified PCR product. This consensus sequence was aligned to the 5' and 3' sequences flanking the MON 87429 insert to determine the integrity and any rearrangement of the insertion site.

References for Appendix B

Kovalic, D., C. Garnaat, L. Guo, Y. Yan, J. Groat, A. Silvanovich, L. Ralston, M. Huang, Q. Tian, A. Christian, N. Cheikh, J. Hjelle, S. Padgette and G. Bannon. 2012. The use of next generation sequencing and junction sequence analysis bioinformatics to achieve molecular characterization of crops improved through modern biotechnology. The Plant Genome 5:149-163.

Langmead, B. and S.L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357-359.

Pearson, W.R. 2000. Flexible sequence similarity searching with the FASTA3 program package. Methods in Molecular Biology 132:185-219.

Appendix C: MON 87429 RHS Mechanism of Action

C.1. Lack of Secondary siRNA from CP4 EPSPS mRNA Suppression Materials and Methods

C.1.1. Low molecular weight RNA blotting

Total RNA from each sample, a 1kb RNA Ladder (Thermo Fisher Scientific) combined with the 25-nt CP4 EPSPS RNA oligos, or the size markers were mixed with the RNA loading Buffer (Ambion, Grand Island, NY), denatured at 95°C for 5 min followed by 4°C for 5 min, loaded to wells of a Criterion Precast 15% polyacrylamide TBE-Urea gel, separated by electrophoresis in a Criterion Cell, and transferred onto positively charged nylon membrane via electroblotting with a Criterion Blotter (Bio-Rad, Hercules, CA) following the manufacture's instruction.

Using T-DNA templates and three sets of the gene-specific primers (Table C-1), three fragments covering the entire CP4 EPSPS transcript except the siRNA target sequence (to avoid cross hybridization with the endogenous mts-siRNAs) (Figure C-1) were obtained by PCR amplifications using Ex Taq DNA polymerase (Takara Bio Inc.) and purified with the QIAquick Gel Extraction Kit (Qiagen). DIG-labeled probes CP4 EPSPS 5', CP4 EPSPS 3', and OsGRP3 3'UTR were prepared from the PCR products with a PCR DIG Probe Synthesis Kit (Roche, Basel, Switzerland). A 25-nt RNA oligo with a sequence (GGGUGCUGACAUCGAGGUUAUCAAU) identical to a portion of the CP4 EPSPS mRNA for use as a positive control and two DIG-labeled 20- and 25-nt RNA oligos for use as size markers were synthesized. Hybridizations were then conducted at 38°C with rotation in DIG Easy Hyb (Sigma). Post-hybridization washes were done as recommended by the manufacture, and DIG detection was performed using reagents and Lumi-Film X-ray films from Roche. In addition, the RNA blot was rinsed in RNase-free water briefly, incubated in the Stripping Buffer (0.2% SDS and 0.1× SSC) for 45 min at 70°C twice to remove the DIG-labeled probes, equilibrated in 2× SSC, and then reprobed with a synthetic DIG-labeled 21-nt DNA oligo (DIG-CAGAGCTCCCTTCAATCCAAA) designed for detection of zm-miR159b as a control.

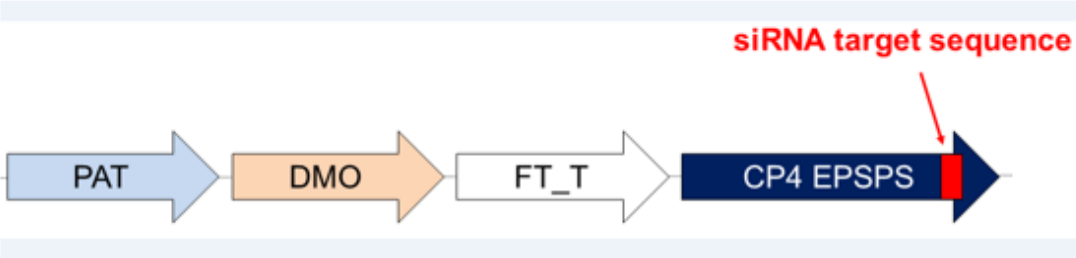


Figure C-1. Transgenic elements in the T-DNA for generating plants containing MON 87429

PAT: phosphinothricin N-acetyltransferase; DMO: dicamba mono-oxygenase; FT_T: FOPs and 2,4-D tolerance enzyme variant T (α-ketoglutarate-dependent dioxygenase); CP4 EPSPS: *Agrobacterium sp.* strain CP4 5-enolpyruvylshikimate-3-phosphate synthase; siRNA TS: small interfering RNA Target Sequence. Not drawn to scale.

C.2. Expression of Endogenous Genes with Sequence Homology to the siRNA Target Sequence Materials and Methods

Total RNA was used for expression analyses of endogenous genes by a TaqMan or a SYBR Green assay (Table C-1). One-step real-time PCR was performed in singleplex with three replicates for each RNA sample. Maize elongation factor 1a (EF1a) was used as a normalizer. Relative expression levels were determined as described in Yang et al 2009.

Table C-1. Primers and probes used in this study

Primer ID	Sequence	Tm	Expected product
For expression analyses of endogenous genes			
Forward Primer 1	AAACCTACGGGAAAGTTGCG	61.33	105 bp
FAM Probe 1	6FAM-ACAAGAAATACCCCTGCTCCAACCTG-MGBNFQ	68.10	
Reverse Primer 1	TCTCGCATAATTTGTAGTTCAGCTG	61.83	
Forward Primer 2	TGGATGAATGKAATCTGAACAAGCT	62.13	73 bp
Reverse Primer 2	CGGGGCAMTTGAACAATCCTA	62.97	
For normalization of expression			
EF1a Forward Primer	GCTAGCTTTACCTCCCAGGTCATC	63.55	64 bp
EF1a VIC Probe	VIC-TCATGAACCACCCTGGC-MGBNFQ	73.29	
EF1a Reverse Primer	GGGCATAGCCATTGCCAATC	64.68	

Table C-1. Primers and probes used in this study (continued)

For preparation of probes for Northern hybridization			
CP4 EPSPS 5' Probe F	AGCAGCATCCACGAGCTTAT	59.98	709 bp
CP4 EPSPS 5' Probe R	ATGGGTTCAATCACGGTTGT	60.08	
CP4 EPSPS 3' Probe F	GCGCTAATCTAACGGTCGAA	60.33	737 bp
CP4 EPSPS 3' Probe R	TCAAGCGGCCTTAGTATCAGA	59.96	
OsGRP3 3'UTR Probe F	CATCGTGGCCAGTTATCCTT	59.93	554 bp
OsGRP3 3'UTR Probe R	TGCAAAATGGAAATGCTGTG	60.63	

References for Appendix C

Yang H, Schmuke JJ, Flagg LM, Roberts JK, Allen EM, Ivashuta S, et al. A novel real-time polymerase chain reaction method for high throughput quantification of small regulatory RNAs. Plant Biotechnol J. 2009; 7: 621–630. pmid:1961

Appendix D: Characterization of FT_T Protein Produced in MON 87429

D.1. Characterization of the FT_T Protein

D.1.1. FT_T Protein Identity and Equivalence

The safety assessment of crops derived through biotechnology includes characterization of the physicochemical and functional properties and confirmation of the safety of the introduced protein(s). For the safety data generated using the *E. coli*-produced FT_T protein to be applied to the MON 87429-produced FT_T protein (plant-produced FT_T), the equivalence of the plant- and *E. coli*-produced proteins must first be demonstrated. To assess the equivalence between the MON 87429-produced FT_T and *E. coli*-produced FT_T proteins, a small quantity of the MON 87429-produced FT_T protein was purified from MON 87429 grain. The MON 87429-produced FT_T protein was characterized and the equivalence of the physicochemical characteristics and functional activity between the MON 87429-produced and *E. coli*-produced FT_T proteins was assessed using a panel of analytical tests; as shown in Table D-1. Taken together, these data provide a detailed characterization of the MON 87429-produced FT_T protein and establish the equivalence of the MON 87429-produced FT_T and *E. coli*-produced FT_T proteins. Based on this established equivalence, conclusions derived from digestibility, heat susceptibility and oral acute toxicology studies conducted with *E. coli*-produced FT_T protein are applicable to MON 87429-produced FT_T protein.

Table D-1. Summary of MON 87429 FT_T Protein Identity and Equivalence

Analytical Test	Assessment	Analytical Test Outcome
1. N-terminal sequence	Identity	• The expected N-terminal sequence for MON 87429-produced FT_T and was observed by Nano LC-MS/MS[1]
2. Nano LC-MS/MS[1]	Identity	• Nano LC-MS/MS[1] analysis of trypsin digested peptides from for MON 87429-produced FT_T protein yielded peptide masses consistent with expected peptide masses from the theoretical trypsin digest of the amino acid sequence
3. Western blot analysis	Identity and Equivalence	• MON 87429-produced FT_T protein identity was confirmed using a western blot probed with antibodies specific for FT_T protein • Immunoreactive properties of the MON 87429-produced FT_T and the *E. coli*-produced FT_T proteins were shown to be equivalent
4. Apparent molecular weight (MW)	Equivalence	• Electrophoretic mobility and apparent molecular weight of the MON 87429-produced FT_T and the *E. coli*-produced FT_T proteins were shown to be equivalent
5. Glycosylation analysis	Equivalence	• Glycosylation status of MON 87429-produced FT_T and *E. coli*-produced FT_T proteins were shown to be equivalent
6. FT_T enzymatic activity	Equivalence	• Functional activity of the MON 87429-produced FT_T and the *E. coli*-produced FT_T proteins were shown to be equivalent

[1] Nano LC-MS/MS = Nanoscale liquid chromatography-tandem mass spectrometry

The details of the materials and methods for the panel of analytical tests used to evaluate and compare the properties of the MON 87429-produced FT_T and *E. coli*-produced FT_T proteins are described at the end of Appendix D. A summary of the data obtained to support a conclusion of protein equivalence is below.

D.1.2. Results of the N-Terminal Sequencing Analysis

The expected N-terminal sequence for the FT_T protein deduced from the *ft_t* gene present in maize of MON 87429 was confirmed by Nano LC-MS/MS. The experimentally determined sequence corresponds to the deduced FT_T protein beginning at the initial alanine position (Figure D-1). The alanine is derived from the chloroplast transit peptide, MDH (Section V.D.1). Alternative cleavage of CTP from FT_T in *planta* by a general stromal processing peptidase is common (Richter and Lamppa, 1998). The N-terminal sequencing results for MON 87429-produced FT_T protein were consistent with the sequencing results for the *E. coli*-produced FT_T protein (Figure D-1). The expression plasmid designed to express the mature MON 87429 FT_T protein in *E. coli* included an N-terminal methionine residue that was likely cleaved by methionine aminopeptidase and other aminopeptidases. Therefore, the *E. coli*-produced FT_T sequence begins at position 2 of the expected sequence deduced from the *E. coli* expression plasmid, which corresponds to position 1 of the mature FT_T protein present in MON 87429. The cleavage of the N-terminal methionine from proteins *in vivo* by methionine aminopeptidase is common in many organisms (Bradshaw et al., 1998). Hence, the sequence information confirms the identity of the FT_T protein isolated from the grain of MON 87429.

Amino Acids Residue # from the N-terminus	→	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
E. coli-produced FT_T sequence	→	A	M	H	A	A	L	T	P	L	T	N	K	Y	R	F
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Expected FT_T Sequence	→	A	M	H	A	A	L	T	P	L	T	N	K	Y	R	F
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
MON 87429-produced FT_T Experimental Sequence	→	A	M	H	A	A	L	T	P	L	T	N	K	Y	R	F

Figure D-1. N-Terminal Sequence of the MON 87429-Produced FT_T Protein

The experimental sequence obtained from the MON 87429-produced FT_T was compared to the expected sequence of the mature protein deduced from the *ft_t* gene, a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa*) gene, present in MON 87429. The *E. coli*-produced FT_T sequence begins at position 2 of the expected sequence deduced from the *E. coli* expression plasmid, which corresponds to position 1 of the mature FT_T protein present in MON 87429. The single letter International Union of Pure and Applied Chemistry- International Union of Biochemistry (IUPAC-IUB) amino acid code is A, alanine; M, methionine; H, histidine; L, leucine; T, threonine; P, proline; N, asparagine; K, lysine; Y, tyrosine; R, arginine; F, phelyalanine.

D.1.3. Results of Mass Fingerprint Analysis

Peptide mass fingerprint analysis is a standard technique used for confirming the identity of proteins. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In general, protein identification made by peptide mapping is considered to be reliable if $\geq 40\%$ of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments (Biron et al., 2006; Krause et al., 1999). The identity of the MON 87429-produced FT_T protein was confirmed by Nano LC-MS/MS analysis of peptide fragments produced by the trypsin digestion of the MON 87429-produced FT_T protein.

There were 34 unique peptides identified that corresponded to the masses expected to be produced by trypsin digestion of the MON 87429-produced FT_T protein (Table D-2). The identified masses were used to assemble a coverage map of the entire FT_T protein (Figure D-2A). The experimentally determined coverage of the FT_T protein was 97% (Figure D-2A 288 out of 296 amino acids). This analysis further confirms the identity of MON 87429-produced FT_T protein.

There were 20 unique peptides identified that corresponded to the masses expected to be produced by trypsin digestion of the *E. coli*-produced FT_T protein (Table D-3) by MALDI-TOF MS analysis during the protein characterization. The identified masses were used to assemble a coverage map of the entire FT_T protein (Figure D-2B). The experimentally determined coverage of the *E. coli*-produced FT_T protein was 65% (Figure D-2B, 194 out of 297 amino acids). This analysis further confirms the identity of *E. coli*-produced FT_T protein.

Table D-2. Summary of the Tryptic Masses Identified for the MON 87429-Produced FT_T Using Nano LC-MS/MS

[1]Experimental Mass[2]	Calculated Mass[3]	Diff[4]	Fragment[5]	Sequence[6]
1266.6764	1266.6754	0.001	1 – 12	AMHA...LTNK
1308.6891	1308.686	0.0031	1 - 12	*AMHA…LTNK
2531.3715	2531.3748	-0.0033	13 - 35	YRFI...VDLR
2212.2081	2212.2104	-0.0023	15 - 35	FIDV...VDLR
6659.3313	6659.3289	0.0024	15 - 73	FIDV...AFSR
4465.1336	4465.1291	0.0045	36 - 73	EPLD...AFSR
1336.7871	1336.7867	0.0004	74 - 85	RFGP...PILK
1180.6852	1180.6856	-0.0004	75 - 85	FGPV...PILK
2583.3786	2583.3771	0.0015	75 - 97	FGPV...QMIR
1420.7026	1420.702	0.0006	86 - 97	SIEG...QMIR
947.4422	947.442	0.0002	98 - 105	REANESSR
791.3409	791.3409	0	99 - 105	EANESSR
2560.2049	2560.2057	-0.0008	106 - 128	FIGD...VVMR
4500.1576	4500.1505	0.0071	129 - 170	AIEV...SATK
5922.8591	5922.8549	0.0042	129 - 182	AIEV...TNWR
1440.7149	1440.715	-0.0001	171 - 182	VFGS...TNWR
2204.1021	2204.1015	0.0006	171 - 189	VFGS...TSVK
781.3974	781.397	0.0004	183 - 189	FSNTSVK
3362.7007	3362.7042	-0.0035	183 - 213	FSNT...VTGR
976.4284	976.4284	0	190 - 198	VMDV...AGDR
2599.317	2599.3177	-0.0007	190 - 213	VMDV...VTGR
1640.8988	1640.8999	-0.0011	199 - 213	ETVH...VTGR
1796.9998	1797.001	-0.0012	199 - 214	ETVH...TGRR
1601.7407	1601.7442	-0.0035	214 - 225	RALY...YCQK
1445.6441	1445.6431	0.001	215 - 225	ALYC...YCQK
1078.4969	1078.4965	0.0004	226 - 235	IQGM...AESK
1448.7651	1448.7663	-0.0012	236 - 247	SLLQ...HATK

[1] All imported values were rounded to 4 decimal places.
[2] Only experimental masses that matched calculated masses with the highest scores are listed.
[3] The calculated mass is the relative molecular mass calculated from the matched peptide sequence.
[4] The calculated difference = (experimental mass – calculated mass).
[5] Position refers to amino acid residues within the predicted MON 87429-produced FT_T sequence as depicted in Figure D-1.
[6] For peptide matches greater than nine amino acids in length the first 4 residues and last 4 residues are shown separated by dots (…).
[*] acetylation and oxidation observed.

Table D-2. Summary of the Tryptic Masses Identified for the MON 87429-Produced FT_T Using Nano LC-MS/MS (Continued)

[1]Experimental Mass[2]	Calculated Mass[3]	Diff[4]	Fragment[5]	Sequence[6]
2275.1126	2275.1096	0.003	236 - 253	SLLQ...FTCR
844.3548	844.3538	0.001	248 - 253	FDFTCR
1913.9253	1913.924	0.0013	258 - 272	KDQV...TMHR
1785.8299	1785.8291	0.0008	259 - 272	DQVL...TMHR
819.4129	819.4127	0.0002	273 - 280	AVPDYAGK
1122.5821	1122.5822	-0.0001	273 - 282	AVPD...GKFR
1030.5409	1030.5407	0.0002	288 - 296	TTVA...KPSR

[1] All imported values were rounded to 4 decimal places.
[2] Only experimental masses that matched calculated masses with the highest scores are listed.
[3] The calculated mass is the relative molecular mass calculated from the matched peptide sequence.
[4] The calculated difference = (experimental mass – calculated mass).
[5] Position refers to amino acid residues within the predicted MON 87429-produced FT_T sequence as depicted in Figure D-1.
[6] For peptide matches greater than nine amino acids in length the first 4 residues and last 4 residues are shown separated by dots (…).
[*] acetylation and oxidation observed.

Table D-3. Summary of the Tryptic Masses Identified for *E. coli*-produced FT_T Using MALDI-TOF MS[1]

Experimental Mass[2]	Calculated Mass[3]	Diff.[4]	Fragment[5]	Enzyme	Sequence[6]
1266.5457	1266.6754	-0.1297	2 - 13	Trypsin	AMHA…LTNK
2211.9397	2212.2104	-0.2706	16 - 36	Trypsin	FIDV…VDLR
1336.6551	1336.7867	-0.1316	75 - 86	Trypsin	RFGP...PILK
1180.5685	1180.6856	-0.1171	76 - 86	Trypsin	FGPV...PILK
1420.5619	1420.7020	-0.0792	87 - 98	Trypsin	SIEG...QMIR
791.2727	791.3409	-0.0682	99 - 106	Trypsin	REANESSR
2559.8802	2560.2057	-0.3255	107 - 129	Trypsin	FIGD…VVMR
1440.5745	1440.7150	-0.1405	172 - 183	Trypsin	VFGS...TNWR
781.3226	781.3970	-0.0744	184 - 190	Trypsin	FSNTSVK
2598.9771	2599.3177	-0.3406	191 - 214	Trypsin	VMDV…VTGR
1640.7249	1640.8999	-0.1749	200 - 214	Trypsin	ETVH…VTGR
1603.5482	1603.7123	-0.1640	215 - 226	Trypsin	RALY…YCQK
1447.4541	1447.6112	-0.1570	216 - 226	Trypsin	ALYC…YCQK
1448.6154	1448.7663	-0.1509	237 - 248	Trypsin	SLLQ…HATK
2275.8086	2276.0936	-0.2850	237 - 254	Trypsin	SLLQ…FTCR
845.2653	845.3378	0.0725	249 - 254	Trypsin	FDFTCR
1786.6216	1786.8131	-0.1915	260 - 273	Trypsin	DQVL…TMHR
819.3327	819.4127	-0.0799	274 - 281	Trypsin	AVPDYAGK
1030.4390	1030.5407	-0.1017	288 - 297	Trypsin	TTVAGDKPSR

[1] All imported values were rounded to 4 decimal places.
[2] Only experimental masses that matched calculated FT_T trypsin digested masses are listed in the table.
[3] The calculated mass is the exact molecular mass calculated from the matched peptide sequence.
[4] The calculated difference = experimental mass - calculated mass.
[5] Position refers to amino acid residues within the predicted *E. coli*-produced FT_T sequence.
[6] For peptide matches greater than nine amino acids in length, the first 4 residues and last 4 residues are show separated by three dots (...).

(A)

001 AMHAALTPLT NKYRFIDVQP LTGVLGAEIT GVDLREPLDD STWNEILDAF

051 HTYQVIYFPG QAITNEQHIA FSRRFGPVDP VPILKSIEGY PEVQMIRREA

101 NESSRFIGDD WHTDSTFLDA PPAAVVMRAI EVPEYGGDTG FLSMYSAWET

151 LSPTMQATIE GLNVVHSATK VFGSLYQATN WRFSNTSVKV MDVDAGDRET

201 VHPLVVTHPV TGRRALYCNQ VYCQKIQGMT DAESKSLLQF LYEHATKFDF

251 TCRVRWKKDQ VLVWDNLCTM HRAVPDYAGK FRYLTRTTVA GDKPSR

(B)

002 MAMHAALTPL TNKYRFIDVQ PLTGVLGAEI TGVDLREPLD DSTWNEILDA

052 FHTYQVIYFP GQAITNEQHI AFSRRFGPVD PVPILKSIEG YPEVQMIRRE

102 ANESSRFIGD DWHTDSTFLD APPAAVVMRA IEVPEYGGDT GFLSMYSAWE

152 TLSPTMQATI EGLNVVHSAT KVFGSLYQAT NWRFSNTSVK VMDVDAGDRE

202 TVHPLVVTHP VTGRRALYCN QVYCQKIQGM TDAESKSLLQ FLYEHATKFD

252 FTCRVRWKKD QVLVWDNLCT MHRAVPDYAG KFRYLTRTTV AGDKPSR

Figure D-2. Peptide Map of the MON 87429-Produced FT_T and *E. coli*-Produced FT_T

(A) The amino acid sequence of the MON 87429-produced FT_T protein was deduced from the *ft_t* gene, a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa*) gene, present in MON 87429. Boxed regions correspond to peptides that were identified from the MON 87429-produced FT_T protein sample using Nano LC-MS/MS. In total, 97% coverage (288 out of 296 amino acids) of the expected protein sequence was covered by the identified peptides.

(B) The amino acid sequence of the *E. coli*-produced FT_T protein was deduced from the *ft_t* gene, a modified version of the R-2,4-dichlorophenoxypropionate dioxygenase (*Rdpa*) gene, that is contained on the expression plasmid. Boxed regions correspond to peptides that were identified from the *E. coli*-produced FT_T protein sample using MALDI-TOF MS. In total, 65% coverage (194 out of 297 amino acids) of the expected protein sequence was covered by the identified peptides.

D.1.4. Results of Western Blot Analysis of the FT_T Protein Isolated from the Grain of MON 87429 and Immunoreactivity Comparison to *E. coli*-produced FT_T Protein

Western blot analysis was conducted using mouse anti-FT_T monoclonal antibody to provide additional confirmation of the identity of the FT_T protein isolated from the grain of MON 87429 and to assess the equivalence of the immunoreactivity of the MON 87429-produced and *E. coli*-produced FT_T proteins. The results showed that immunoreactive bands with the same electrophoretic mobility were present in all lanes loaded with the MON 87429-produced and *E. coli*-produced FT_T proteins (Figure D-3). For each amount loaded, comparable signal intensity was observed between the MON 87429-produced and *E. coli*-produced FT_T protein bands. As expected, the signal intensity increased with increasing load amounts of the MON 87429-produced and *E. coli*-produced FT_T proteins, thus, supporting the identity of the MON 87429-produced FT_T protein.

To compare the immunoreactivity of the MON 87429-produced and the *E. coli*-produced FT_T proteins, densitometric analysis was conducted on the bands that migrated at the expected apparent molecular weight (MW) for FT_T proteins (~35 kDa). The signal intensity (reported in OD × mm^2) of the band of interest in lanes loaded with MON 87429-produced and the *E. coli*-produced FT_T proteins was measured (Table D-4). Because the mean signal intensity of the MON 87429-produced FT_T protein band was within 35% of the mean signal of the *E. coli*-produced FT_T protein, the MON 87429-produced FT_T and *E. coli*-produced FT_T proteins were determined to have equivalent immunoreactivity.

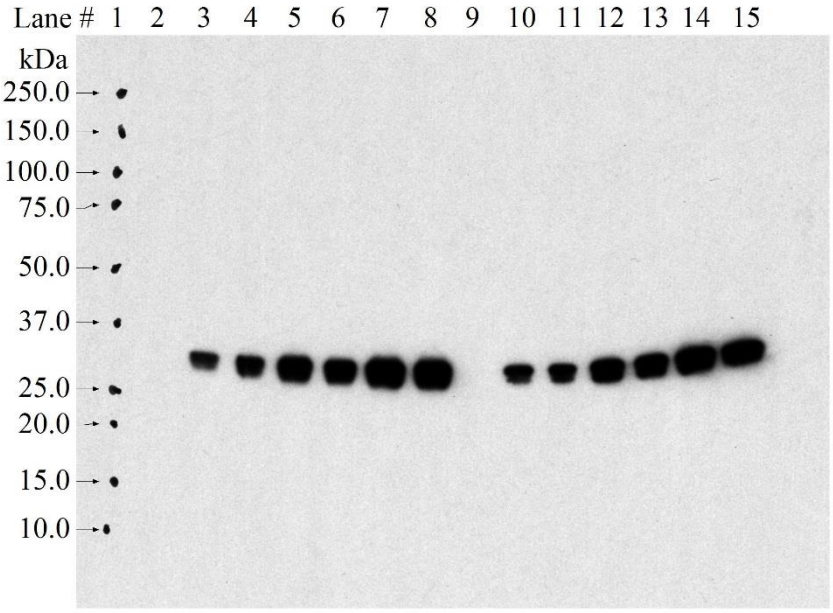


Figure D-3. Western Blot Analysis and Immunoreactivity of MON 87429-Produced and *E. coli*-Produced FT_T Proteins

Aliquots of the MON 87429-produced FT_T protein and the *E. coli*-produced FT_T protein were subjected to SDS-PAGE and electrotransferred to a PVDF membrane. Proteins were detected using mouse anti-FT_T monoclonal antibody and then horse anti-mouse polyclonal antibody conjugated with peroxidase. Immunoreactive bands were visualized using an ECL system. The approximate MW (kDa) of the standards are shown on the left. The 45 second exposure is shown. Lane designations are as follows:

Lane	Sample	Amount (ng)
1	Precision Plus Protein™ Standards	-
2	Blank	-
3	MON 87429-produced FT_T	5
4	MON 87429-produced FT_T	5
5	MON 87429-produced FT_T	10
6	MON 87429-produced FT_T	10
7	MON 87429-produced FT_T	20
8	MON 87429-produced FT_T	20
9	Blank	-
10	*E. coli*-produced FT_T	5
11	*E. coli*-produced FT_T	5
12	*E. coli*-produced FT_T	10
13	*E. coli*-produced FT_T	10
14	*E. coli*-produced FT_T	20
15	*E. coli*-produced FT_T	20

Table D-4. Immunoreactivity of the MON 87429-Produced and *E. coli*-Produced FT_T Proteins

Mean Signal Intensity from MON 87429-Produced FT_T[1] (OD x mm^2)	Mean Signal Intensity from E. coli-Produced FT_T[1] (OD x mm^2)	Acceptance Limits[2] (OD x mm^2)
94,017.0	97,454.4	63,345.4 – 131,563.4

[1] Each value represents the mean of six values (n = 6).
[2] The acceptance limits are for the MON 87429-produced FT_T protein and are based on the interval between -35% (97,454.4 x 0.65 = 63,345.4) and +35 % (97,454.4 x 1.35 = 131,563.4) of the mean of the *E. coli*-produced FT_T signal intensity across all loads.

D.1.5. Results of the FT_T Protein Molecular Weights and Purity Analysis

For apparent MW and purity determination, the MON 87429-produced FT_T and the *E. coli*-produced FT_T proteins were subjected to SDS-PAGE. Following electrophoresis, the gel was stained with Brilliant Blue G-Colloidal stain and analyzed by densitometry. The MON 87429-produced FT_T protein (Figure D-4, lanes 3-8) migrated with the same mobility on the gel as the *E. coli*-produced FT_T protein (Figure D-4, lane 2) and the apparent MW was calculated to be 36.0 kDa (Table D-5). Because the experimentally determined apparent MW of the MON 87429-produced FT_T protein was within the acceptance limits for equivalence (Table D-6), the MON 87429-produced FT_T and *E. coli*-produced FT_T proteins were determined to have equivalent apparent molecular weights.

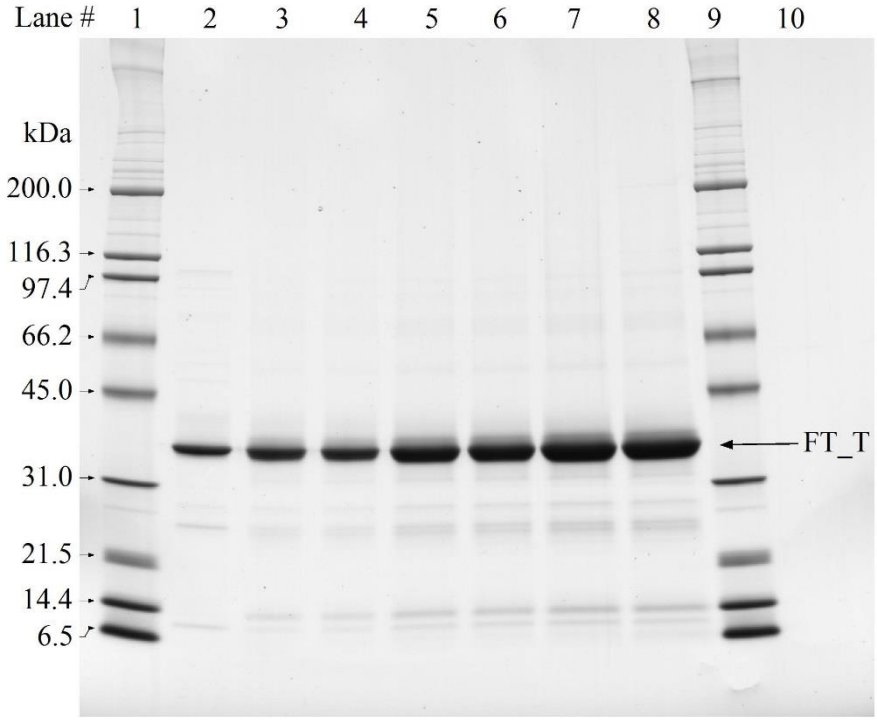


Figure D-4. Purity and Apparent Molecular Weight Analysis of the MON 87429-Produced FT_T Protein

Aliquots of the MON 87429-produced and the *E. coli*-produced FT_T 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 designations are as follows:

Lane	Sample	Amount (µg)
1	Broad Range MW Standard	5.0
2	*E. coli*-produced FT_T	1.0
3	MON 87429-produced FT_T	1.0
4	MON 87429-produced FT_T	1.0
5	MON 87429-produced FT_T	2.0
6	MON 87429-produced FT_T	2.0
7	MON 87429-produced FT_T	3.0
8	MON 87429-produced FT_T	3.0
9	Broad Range MW Standard	5.0
10	Blank	

Table D-5. Apparent Molecular Weight and Purity Analysis of the MON 87429-Produced FT_T Protein

	Apparent MW[1] (kDa)	Purity[2] (%)
Average (n=6)	36.0	91

[1]Final MW was rounded to one decimal place.
[2]Average % purity was rounded to the nearest whole number.

Table D-6. Apparent Molecular Weight Comparison Between the MON 87429-Produced FT_T and *E. coli*-Produced FT_T Proteins

Apparent MW of MON 87429-Produced FT_T Protein (kDa)	Apparent MW of *E. coli*-Produced FT_T Protein (kDa)	Acceptance Limits[1] (kDa)
36.0	35.5	34.1 – 36.8

[1] Data obtained from the *E. coli*-produced FT_T protein was used to generate a prediction interval for setting the acceptance limits (Table D-8).

D.1.6. FT_T Glycosylation Equivalence

Some eukaryotic proteins are post-translationally modified by the addition of carbohydrate moieties (Rademacher et al., 1988). To test whether the FT_T protein was glycosylated when expressed in the maize grain of MON 87429, the MON 87429-produced FT_T protein was analyzed using an ECL™ glycoprotein detection method. Transferrin, a glycosylated protein, was used as a positive control in the assay. To assess equivalence of the MON 87429-produced and *E. coli*-produced FT_T proteins, the *E. coli*-produced FT_T 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 D-5, panel A). In contrast, no glycosylation signal was observed in the lanes containing the *E. coli*-produced FT_T protein or MON 87429-produced FT_T protein (Figure D-5, panel A).

To confirm that MON 87429-produced FT_T and *E. coli*-produced FT_T 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 87429-produced and *E. coli*-produced FT_T proteins were detected (Figure D-5, panel B). These data indicate that the glycosylation status of MON 87429-produced FT_T protein is equivalent to that of the *E. coli*-produced FT_T protein and that neither is glycosylated.

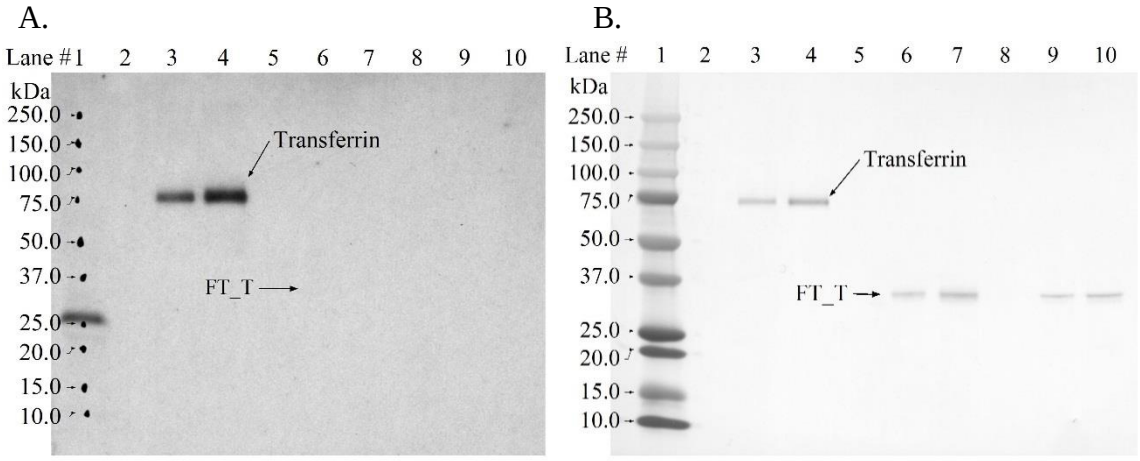


Figure D-5. Glycosylation Analysis of the MON 87429-Produced and *E. coli*-Produced FT_T Proteins

Aliquots of the transferrin (positive control), *E. coli*-produced FT_T and MON 87429-produced FT_T were subjected to SDS-PAGE and electrotransferred to a PVDF membrane. The MWs (kDa) correspond to the Precision Plus Dual Color Protein™ Standards. The arrows show the expected migration of the MON 87429-produced and *E. coli*-produced FT_T proteins and transferrin. (A) Where present, the labeled carbohydrate moieties were detected by addition of streptavidin conjugated to HRP followed by a luminol-based detection using ECL reagents and exposure to Hyperfilm®. The 30 second 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	Blank	-
3	Transferrin (positive control)	100
4	Transferrin (positive control)	200
5	Blank	-
6	MON 87429-produced FT_T	100
7	MON 87429-produced FT_T	200
8	Blank	-
9	*E. coli*-produced MON 87429 FT_T	100
10	*E. coli*-produced MON 87429 FT_T	200

D.1.7. FT_T Functional Activity

The functional activities of the MON 87429-produced and *E. coli*-produced FT_T proteins were determined using a colorimetric assay that measures the FT_T catalyzed degradation of 2,4-D to 2,4-DCP. In this assay, protein-specific activity is expressed as units per microgram of protein (U/mg), where a unit (U) is 1 nmole of 2,4-D to 2,4-DCP per min at 25°C. The MON 87429-produced and *E. coli*-produced FT_T proteins were considered to have equivalent functional activity if the specific activity of both were within the preset acceptance limits of 183 to 974 U/mg (the prediction interval calculated from a data set of historically determined FT_T protein activity; see Table D-9).

The experimentally determined specific activity for the MON 87429-produced and *E. coli*-produced FT_T proteins are presented in Table D-7. The specific activities of MON 87429-produced and *E. coli*-produced FT_T proteins were 723 U/mg and 720 U/mg of FT_T protein, respectively. Because the specific activities of MON 87429-produced and *E. coli*-produced FT_T proteins fall within the preset acceptance limits (Table D-7), the MON 87429-produced FT_T protein was considered to have equivalent functional activity to that of the *E. coli*-produced FT_T protein.

Table D-7. Functional Activity of MON 87429-Produced and *E. Coli*-Produced FT_T Protein

MON 87429-Produced FT_T [1] (U/mg)	*E. coli*-Produced FT_T [1] (U/mg)	Acceptance Limits[2] (U/mg)
723	720	183.4 – 974.0

[1] Value refers to mean calculated based on samples spectrophotometrically read in triplicate plate wells.
[2] Data obtained from two *E. coli*-produced FT_T lots were used to generate a prediction interval for setting the acceptance limits (Appendix D, Table D-9)

D.1.8. FT_T Protein Identity and Equivalence Conclusion

The MON 87429-produced FT_T protein was purified from MON 87429 grain, was characterized, and a comparison of the physicochemical and functional properties between the MON 87429-produced and the *E. coli*-produced FT_T proteins was conducted following a panel of analytical tests: 1) N-terminal sequence analysis established the same identity for the MON 87429-produced and *E. coli*-produced FT_T proteins; 2) Nano LC-MS/MS analysis yielded peptide masses consistent with the expected peptide masses from the theoretical trypsin digest of the *ft_t* gene product present in MON 87429; 3) the MON 87429-produced and the *E. coli*-produced FT_T proteins were both detected on a western blot probed with antibodies specific for FT_T protein and the immunoreactive properties of both proteins was shown to be equivalent; 4) the electrophoretic mobility and apparent molecular weight of the MON 87429-produced and *E. coli*-produced FT_T proteins were shown to be equivalent; 5) the glycosylation status of MON 87429-produced and *E. coli*-produced FT_T proteins was determined to be equivalent; and 6) the functional activity of the MON 87429-produced and *E. coli*-produced FT_T was demonstrated to be equivalent. These results demonstrate that the MON 87429-produced FT_T protein and the *E. coli*-produced FT_T protein are equivalent. This demonstration of protein equivalence confirms that the *E. coli*-produced FT_T protein is appropriate for use in the evaluation of the safety of the MON 87429-produced FT_T protein.

D.2. Materials and Methods for Characterization of FT_T Protein Produced in MON 87429

D.2.1. Materials

The test substance is the MON 87429-produced FT_T protein (lot 11478812) purified from grain of MON 87429 (lot 11464934). The MON 87429-produced FT_T protein was stored in a -80 ºC freezer in a buffer containing 50 mM Tris, pH 8, 200 mM NaCl.

The reference substance is the *E. coli*-produced FT_T protein (lot 11478164). The FT_T reference substance was generated from cell paste produced by fermentation of E. coli containing pMON374248 expression plasmid. The DNA sequence encoding FT_T protein contained in the expression plasmid was confirmed both prior to and following fermentation.

D.2.2. Protein Purification

The plant-produced FT_T protein used in this equivalence study was purified from grain of MON 87429. The purification procedure was not performed under a good laboratory practice (GLP) plan; however, all procedures were documented in an electronic lab notebook (ELN) and, where applicable, standard operating procedures (SOPs) were followed. All purification steps were performed at ~4°C, except where specifically stated. FT_T was purified from an extract of ground grain using immunoaffinity chromatography (IAC). A detailed description of the purification procedure specific for FT_T was archived in the Monsanto Regulatory Archives and a brief purification procedure was described below.

Grain of MON 87429 was ground to fine powder in the presence of dry ice and stored at -80°C until use. A total of ~50 g of ground powder was mixed with 0.5 L of Extraction Buffer (50 mM Tris, pH 8.0, 200 mM NaCl, 2 mM benzamidine-HCl) at room temperature for ~1 hr. The slurry

was centrifuged twice at 13,500 x g for 15 min at ~4°C to be clarified, and about 410 ml supernatant was collected, filtered, and loaded onto a ~1 ml IAC column (Column size: ID = 1 cm, h ≈ 1.5 cm; Resin: MabSelect Protein A resin cross-linked with ~3 mg of anti-FT_T mAb (Monsanto, lot 109529) following the manufacturer's instruction Catalog #: 17-5199-03, GE Healthcare, IL) which was connected to a AKTA FPLC system at 4°C. Before sample load, the resin was equilibrated with Equilibration Buffer (50 mM Tris, pH 8.0, 200 mM NaCl). The sample was loaded on the resin at 500 cm/hr. The resin was subsequently washed 50 column volumes (CV) Equilibration Buffer and 35 CV Wash Buffer (50 mM Tris, pH 8.0, 1 M NaCl). The protein was eluted with 50 CV Elution Buffer (2 M arginine, 50 mM Tris, pH 8.0, 1 M NaCl). As the fractions were collected, they were immediately diluted 1:3 with Equilibrium Buffer. Fractions containing FT_T, identified by SDS-PAGE analyses, were pooled to a final volume of ~200 ml. This pooled sample was concentrated and buffer-exchanged to storage buffer (50 mM Tris, pH 8.0, 200 mM NaCl) using centrifugal filtration devices (Millipore, Cat. #: UFC901024). This FT_T protein, purified from the grain of MON 87429, was aliquoted, assigned lot 11478812, and stored in a -80 °C freezer.

D.2.3. Methods for Characterization

D.2.3.1. N-Terminal Sequencing/Mass Fingerprint Analysis

The test substance was analyzed for N-terminal amino acid sequence and peptide mass fingerprint analysis by Nano LC-MS/MS. An aliquot of the test substance was subjected to acetone precipitation to remove buffer components and re-suspended in 30 µl 40% TFE/100 mM NH_4HCO_3. The sample was reduced by 10 mM DTT at 37ºC for 1 hr, alkylated by incubation in the dark for 30 min at room temperature with 50 mM iodoacetamide (IAM), and then trypsin digested in an enzyme to protein ration of 1:20. at 37ºC. After 15 hr the digestion was quenched with 1µl of formic acid. The digest was extracted with 30 µl of 60% acetonitrile/0.1% FA twice. The sample was dried to completion using vacuum centrifugation and then solubilized in 20 µl of 0.1 % FA for LC-MS/MS analysis.

The LC-MS/MS data acquisition was performed on a Dionex 3000 Ultimate nanoLC system (Dionex) interfaced to an Orbitrap Fusion mass spectrometer (Thermo Scientific), which is equipped with a nano-ESI source. The sample was loaded and separated online using an Acclaim PepMap100 C18 nano column (75 µm- id × 150 mm, 2 µm, 100 Å, Dionex). The separation of the digests was achieved at a 300 nl/min flow rate using an acetonitrile gradient as follows: 0-4 min sustaining 2% solvent B (100% acetonitrile with 0.1% FA), 4-80 min ramping solvent B 2-40%, 80-85 min ramping solvent B 40-90%. The column was washed/equilibrated between injections as follows: 85-90 min ramping maintaining solvent B at 90%, 90-90.1 min decreasing solvent B 90-2%, and 90.1-100 min maintaining solvent B at 2%. Solvent A was 0.1% FA.

The Orbitrap Fusion mass spectrometer was operated with two scan events. The first scan event was a full Fourier transform mass spectrometry scan with a range of m/z from 200 to 1800 and a mass resolution of 120,000 at m/z of 200. The second scan event was a tandem mass spectrometry scan of fragments from collision induced dissociation (CID) of precursor ions from the first scan event with an isolation width of 2.0 m/z.

The LC-MS/MS dataset were analyzed using Mascot (version 2.5, Matrix Science). MS/MS spectra were searched against the theoretical FT_T protein sequence. The mass tolerances of MS1 mass and MS/MS mass were set as 5 ppm and 0.5 Da, respectively. Carboxymethylation of cysteine was set as a fixed modification. Methionine oxidation and N-terminal acetylation were set as variable modifications. A maximum of two missed cleavages for trypsin were allowed. The mass coverage was accepted if it is greater than 40% of the protein sequence.

D.2.3.2. Western Blot Analysis-Immunoreactivity

Western blot analysis was performed to confirm the identity of the MON 87429-produced FT_T protein and to compare the immunoreactivity of the MON 87429-produced and *E. coli*-produced FT_T protein. MON 87429-produced and *E. coli*-produced FT_T proteins were initially diluted to 200 ng/µl (purity-corrected FT_T protein concentration) with Milli-Q water, and finally diluted to 2 ng/µl in 5 × LB and Milli-Q water and then heated to 100°C for 5 min. Three amounts (~5, ~10, and ~20 ng) of the intact test substance (total protein concentration × purity of the intact FT_T protein) and the intact reference substance (total protein concentration × purity of the intact FT_T 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 Dual Color 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 PVDF membrane (Invitrogen).

The Western blotting procedure was performed using an iBind™ Western System apparatus (Life Technologies). The membrane was blocked with 1 × iBind™ Solution (Life Technologies) and incubated with mouse anti-FT_T monoclonal antibody (Monsanto, lot #: 109529) at a dilution of 1:1250 in 1 × iBind™ Solution. The membrane was then washed with 1 × iBind™ Solution and incubated with horse anti-mouse polyclonal antibody conjugated with peroxidase (Vector, Cat #: PI-2000, lot #: X0328) at a dilution of 1:1250 in 1 × iBind™ Solution. After washing 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 Minolta).

Quantification of the bands on the blot was performed on a Bio-Rad GS-900 densitometer with the supplied Image Lab 5.0 Security Edition software using the volume tool. The signal intensities of the immunoreactive bands migrating at the expected position for the FT_T protein were quantified as volume values. The immunoreactivity was reported in OD × mm^2.

D.2.3.3. Apparent Molecular Weight and Purity Determination by SDS-PAGE

MON 87429-produced and *E. coli*-produced FT_T proteins were diluted in 5 × loading buffer (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 Milli-Q water and then heated to 95-105°C for 3-5 min. The MON 87429-produced FT_T protein was loaded in duplicate at ~1.0, ~2.0, and ~3.0 µg, based on total protein concentration, onto a Tris-glycine 4-20% polyacrylamide mini-gel (Invitrogen) in lanes 3, 4, 5, 6, 7 and 8. The *E. coli*-produced FT_T protein was loaded at ~1.0 µg total protein in lane 2. Broad Range Molecular Weight Standards (Bio-Rad) were prepared and

loaded in lanes 1 and 9 on the gel. Following electrophoresis at a constant voltage, proteins were briefly fixed in 40% (v/v) methanol, 7% (v/v) acetic acid and stained for 18 hr with Brilliant Blue G-Colloidal stain (Sigma). Gels were briefly destained in 10% (v/v) acetic acid, 25% (v/v) methanol followed by 7 hr in 25% (v/v) methanol. Analysis of the gel was performed using a Bio-Rad GS-900 densitometer supplied with Image Lab 5.0 Security Edition software. Apparent MW and purity were reported as an average of all 6 lanes containing the MON 87429-produced FT_T protein.

D.2.3.4. Glycosylation Analysis

ECL Glycoprotein Detection method (GE Healthcare) was used for glycoprotein detection. The MON 87429-produced FT_T protein, *E. coli*-produced FT_T protein and a positive control (Transferrin, Sigma) were diluted in 5 × LB and water to a final concentration of 25 µg/ml protein and 1 × LB and then heated to 100.0°C for 5 min. Two amounts (~100 ng and ~200 ng) of the intact MON 87429-produced FT_T protein (purity-corrected), the *E. coli*-produced FT_T protein (purity-corrected), and the positive control were loaded onto a pre-cast Tris-glycine 4-20% polyacrylamide mini-gel (Invitrogen). Pre-stained molecular weight markers (Precision Plus Protein Dual Color 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 Module (GE Healthcare) as directed by the manufacturer. 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 was scanned using Bio-Rad GS-900 densitometer with the supplied Image Lab 5.0 Security Edition software.

D.2.3.5. Functional Activity

In preparation for analysis, the *E. coli*-produced FT_T protein was initially diluted with storage buffer to the same purity-corrected concentration as the MON 87429-produced FT_T protein (0.82 mg FT_T/ml). Prior to functional analysis, both MON 87429-produced and *E. coli*-produced FT_T proteins were diluted with assay reaction buffer (20 mM MOPS, pH 6.8, 50 µM $(NH_4)_2Fe(SO_4)_2$, 50 µM sodium ascorbate, 0.5 mM α-ketoglutarate) to a purity-corrected concentration of ~10 µg/ml. The reactions were performed in assay reaction buffer with 5 µg/ml FT_T at ~25°C for 30 min. The reactions for both proteins were conducted in triplicate and were initiated by the addition of 2,4-D to a final concentration of 0.5 mM. After 30 min incubation, the reactions were quenched with 15 µl stop buffer (50 mM Boric acid, pH 10.0, 50 mM potassium chloride, 1 mM EDTA). A 800 µM 2,4-dichlorolphenol (2,4-DCP) solution was used to prepare a standard curve ranging from 0 to 400 µM 2,4-DCP in assay reaction buffer and then quenched with 15 µl stop buffer. An assay blank (assay reaction buffer only) and negative control (0.5 mM 2,4-D in assay reaction buffer) were also run in triplicate. After quenching the reactions, 15 µl of 0.8% potassium ferricyanide was added to standard, blank, negative control

and sample wells and incubated at ~25°C for 1 min. The absorbance of each reaction and standard was measured at 510 nm with a reference wave length of 700 nm using SpectraMax 384 plus Microplate Reader. The amount of 2,4-DCP in each reaction was determined using the standard curve. The specific activity of FT_T was defined in unit per µg of protein (U/mg), where a unit (U) is 1 nmole of 2,4-D to 2,4-DCP per min at 25°C.

D.2.3.6. Prediction Intervals as Acceptance Criteria

Acceptance criteria (acceptance limits) based on prediction intervals were used to assess the equivalence of the MON 87429-produced and *E. coli*-produced FT_T 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; *i.e.*, prediction intervals are generated based on statistical analysis of existing data.

The source of the data used to generate the prediction intervals for apparent MW and functional activity for FT_T protein equivalence assessment are provided in Table D-8 and Table D-9, respectively. The data used were generated under GLP guidelines and included apparent MW and functional activity estimates for the reference *E. coli*-produced FT_T protein during the initial characterization and for an additional *E. coli*-produced FT_T protein lot during re-characterization (functional assay only).

The two-sided 95% prediction interval (PI) for one future assay was calculated using JMP software (version 9.00, SAS Institute, Inc., Cary, NC) according to the guidance document of Ramírez (2009).

Table D-8. Individual Assay Data and 95% Prediction Interval Generated for the Apparent MW of FT_T Protein for One Future Assay

Assay Number[1]	Apparent MW of FT_T Protein (kDa)[2]
1	35.833
2	35.589
3	36.033
4	35.989
5	34.827
6	35.006
7	34.690
8	35.729
Mean	35.5
Standard Deviation	0.54
95% Prediction Interval	34.1 – 36.8

[1] Assay 1-8 represents the MW data from the initial characterization of *E. coli*-produced FT_T (lot 11478164).

[2] The values in the table represent the mean of six (n=6) data points within each assay. With 95% confidence, the mean of the 6 data points from the next single assay of the population will fall within the stated interval. Mean and prediction interval values rounded to one decimal point.

Table D-9. Individual Assay Data and 95% Prediction Interval Generated for the Functional Activity of the FT_T Protein for One Future Assay

Assay Number[1]	Functional Activity of FT_T Protein[2] (U/mg)
1	742.671
2	756.702
3	842.367
4	775.289
5	748.196
6	761.855
7	554
8	486
9	293
10	343
11	440
12	410
13	471
14	583
15	473
Mean	578.7
Standard Deviation	178.46
95% Prediction Interval	183.4 – 974.0

[1] Assay 1-6 represents the specific activity from the initial characterization of *E. coli*-produced FT_T (lot 11478164). Assays 7-15 represent the specific activity data for re-characterization of a different lot of *E. coli*-produced FT_T (lot 11462796).

[2] The values in the table represent the mean of 3 replicates (n=3) within each assay. With 95% confidence, the mean of the 3 replicates from the next single assay of the population will fall within the stated interval. Mean and prediction interval values rounded to one decimal point.

References for Appendix D

Bradshaw, R.A., W.W. Brickey and K.W. Walker. 1998. N-terminal processing: The methionine aminopeptidase and Na-acetyl transferase families. Trends in Biochemical Sciences 23:263-267.

Biron, D.G., C. Brun, T. Lefevre, C. Lebarbenchon, H.D. Loxdale, F. Chevenet, J.-P. Brizard and F. Thomas. 2006. The pitfalls of proteomics experiments without the correct use of bioinformatics tools. Proteomics 6:5577-5596.

Krause, E., H. Wenschuh and P.R. Jungblut. 1999. The dominance of arginine-containing peptides in MALDI-derived tryptic mass fingerprints of proteins. Analytical Chemistry 71:4160-4165.

Rademacher, T.W., R.B. Parekh and R.A. Dwek. 1988. Glycobiology. Annual Review of Biochemistry 57:785-838.

Ramírez, J.G. 2009. Statistical Intervals: Confidence, Prediction, Enclosure. JMP White Paper (SAS Institute, Inc., Cary, NC). http://www.sas.com/reg/wp/corp/4430.

Richter, S. and G.K. Lamppa. 1998. A chloroplast processing enzyme functions as the general stromal processing peptidase. Proceedings of the National Academy of Science of the United States of America 95:7463-7468.

Appendix E: Specificity of the MON 87429 Dicamba Mono-Oxygenase (DMO) Enzyme Using o-Anisic Acid as a Substrate

E.1. MON 87429 DMO o-Anisic Acid Substrate Specificity

Dicamba mono-oxygenase (DMO) is an enzyme that catalyzes the demethylation of the herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) to the non-herbicidal 3,6-dichlorosalycilic acid (DCSA) (Chakraborty et al., 2005). In order to evaluate the selectivity of DMO for dicamba herbicide as compared to potential endogenous substrates, a series of *in vitro* studies were previously performed and reported in MON 87708 USDA-AHPIS Petition #10-188-01p. Endogenous compounds evaluated in those studies included ferulic acid, *o*-anisic acid, sinapic acid, syringic acid, and vanillic acid. These substrates were chosen because they are structurally similar to dicamba and are found in plants. The results demonstrated that DMO did not catabolize the potential endogenous substrates tested and supported the conclusion that DMO has a high specificity for dicamba. The *Escherichia coli* (*E. coli*)-produced DMO used in MON 87708 USDA-AHPIS Petition #10-188-01p had an identical amino acid sequence to the wild-type DMO found in *Stenotrophomonas maltophilia*, with the exception of a histidine-tag at the N-terminus.

MON 87429 maize contains a *dmo* expression cassette that results in two forms of the DMO protein, DMO+0 and DMO+1, collectively referred to herein as MON 87429 DMO. The DMO+0 differs from the wild-type DMO by only a leucine added at position 2 (Wang et al., 2016). The DMO+1 has one additional cysteine on the N-terminus of the DMO+0, which is derived from a chloroplast transit peptide. Due to the close similarity of the DMO protein used in MON 87708 USDA-AHPIS Petition #10-188-01p and MON 87429 DMO, the two enzymes were expected to exhibit the same level of substrate specificity. This was evaluated by testing for potential catabolism of *o*-anisic acid by *E. coli*-produced MON 87429 DMO, using the same qualitative assay reported in MON 87708 USDA-AHPIS Petition #10-188-01p. Although *o*-anisic acid is not known to be present in corn, this substance was chosen for this confirmatory experiment since, among the five substrates used in the MON 87708 USDA-AHPIS Petition #10-188-01p, this is the substrate that is most structurally similar to dicamba. The results were evaluated qualitatively. For *o*-anisic acid, similarly to the previously reported results in MON 87708 USDA-AHPIS Petition #10-188-01p, no new peaks that would be indicative of the predicted demethylated product were observed, confirming that the MON 87429 DMO did not catabolize *o*-anisic acid. These results taken together with the previously reported results demonstrate that DMO has a high specificity for dicamba as a substrate.

E.2. Materials and Methods for MON 87429 DMO o-Anisic Acid Substrate Specificity

MON 87429 produces two forms of the DMO protein, DMO+0 and DMO+1, collectively referred to herein as MON 87429 DMO. The MON 87429 DMO used in the in vitro enzyme assays was co-expressed in *E. coli* using a single expression vector designed to

match the DMO+0 and DMO+1 produced in MON 87429. The DMO+0 has an identical amino acid sequence to the wild-type DMO protein isolated from *S. maltophilia*, with the exception of a leucine added at position 2 (Wang et al., 2016). The DMO+1 has an identical amino acid sequence to the DMO+0, with the exception of one additional cysteine on its N-terminus derived from a chloroplast transit peptide.

The compounds tested and standards used in the in vitro enzyme assays are shown in Table E-1.

Table E-1. Compounds Used in Specificity In Vitro Enzyme Assays

Manufacturer/ Retailer	Compound	Common Name
Compounds Tested:		
Millipore	2-methoxybenzoic acid	*o*-anisic acid
Sigma	3,6-dichloro-2-methoxybenzoic acid	dicamba

E.3. In Vitro Enzymatic Reaction Method

The reaction of the *E. coli*-produced MON 87429 DMO with dicamba or *o*-anisic acid was carried out using the three enzymes necessary for demethylation: 0.077 µg/µL reductase (lot 11406970), 0.02 µg/µL ferredoxin (lot 11423290), and 0.005 µg/µL *E. coli*-produced MON 87429 DMO (lot 11472501), 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 FeSO4, and the tested compound at 0.3 mM. Control assays were run by substituting dilution buffer (25 mM KPi, pH 7.2, 10 mM MgCl2) for DMO in equal volume for the above reactions. The final volume for each assay sample was 200 µl. Each assay sample was incubated for 15 min at ~30ºC before quenching the reaction by the addition of 5% H_2SO_4. The activity of DMO with dicamba provided a positive control for DMO functional activity.

E.4. Liquid Chromatography Separation Method

Each reaction mixture was separated by UPLC using an ACQUITY UPLC BEH C18 Column containing 1.7 µm Bridged Ethyl Hybrid (BEH) particles. The column was heated to and maintained at 40°C. The substrates and products formed were detected by ACQUITY UPLC UV detector with optimized wavelengths (dicamba at 226 and 280nm, anisic acid at 210 and 280 nm) (LC-UV). The chromatography was performed at 0.05 ml/min and following the separation the column effluent was then directed to the mass spectrometer. Both mobile phase A (H_2O) and solvent B (acetonitrile) contained 0.1% v/v formic acid. Gradients used were as following:

The gradient was run from 40 to 60% solvent B in 6 min, 60 to 100% solvent B in 4 min and then kept at 100% solvent B for 2 min before returning to 40% solvent B in 0.1 min. DCSA was used as a standard to determine product retention time.

A 5 µl injection of reaction mixture was used to monitor the presence of the tested compound.

E.5. Mass Spectrometry 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 2500 V, extraction cone of 1.5 V, source temperature of 80°C, and the desolvation temperature was 120°C. The sample cone voltage was 25 V for dicamba, DCSA, and *o*-anisic acid. 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 scanned for dicamba and DCSA was from 160 to 224 from 0 to 15 min. The m/z at 175, which is a fragment ion of dicamba, was used as the detection ion 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 scanned for *o*-anisic acid was from 120 to 230 within 15 min. The m/z at 151 was used to detect *o*-anisic acid, and the m/z at 137 was used to look for a new peak that would be indicative of a potential demethylation product of *o*-anisic acid.

E.6. Results and Discussion

The potential catabolism of *o*-anisic acid by *E. coli*-produced MON 87429 DMO was evaluated using the same qualitative enzymatic assay reported in MON 87708 USDA-AHPIS Petition #10-188-01p. The conversion of dicamba to DCSA was also assessed. Mass spectrometry scans from 120 m/z to 230 m/z were analyzed to cover the range of tested compounds and all potential demethylated products formed by the reaction of *o*-anisic acid and *E. coli*-produced MON 87429 DMO. For *o*-anisic acid, no additional peaks that might be associated to the predicted demethylated product were observed in the mass spectrometry scans from 120 m/z to 230 m/z. Figure E-1 shows a representative result from the reaction mixtures containing *o*-anisic acid, with and without *E. coli*-produced MON 87429 DMO. *o*-Anisic acid is shown at its respective detection mass (m/z 151; Figure E-1B). A single demethylation of *o*-anisic acid to produce a compound with m/z 137 was predicted based on the known mode of action of DMO. No new peaks were observed at m/z 137 in the chromatograms (Figure E-1C) for the samples containing DMO as compared to the negative controls, which did not contain DMO. As expected, a novel peak was present at the respective m/z (205) for DCSA in reactions containing both dicamba and DMO (Figure E-2C), confirming that the *E. coli*-produced MON 87429 DMO used for this analysis was functionally active. The data demonstrate that *o*-anisic acid was not catabolized by *E. coli*-produced MON 87429 DMO.

E.7. Conclusions

The data reported herein, together with the previously reported data (MON 87708 USDA-AHPIS Petition #10-188-01p) demonstrate that DMO, including *E. coli*-produced MON 87429 DMO, is selective for its substrate, dicamba.

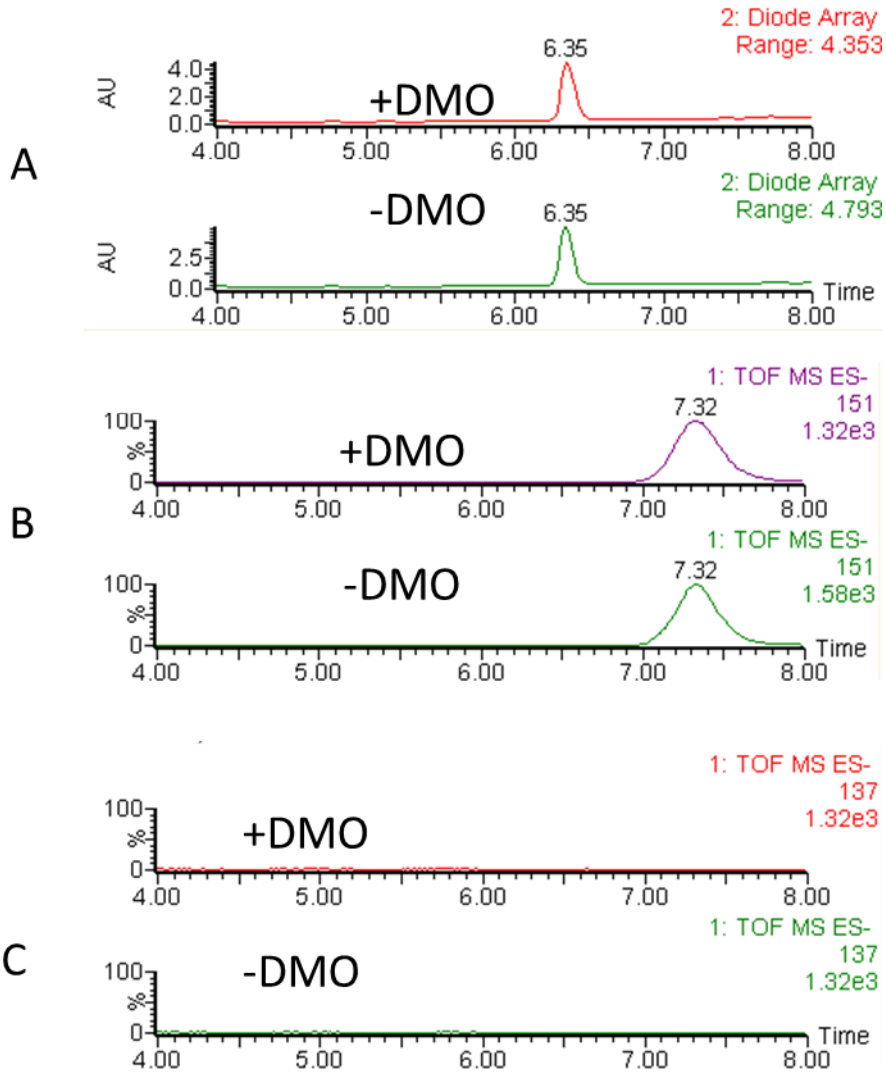


Figure E-1. Representative Assay Results for *o*-Anisic Acid

0.3 mM of *o*-anisic acid was included in a reaction mixture made with (+DMO, upper) or without (-DMO, lower) MON 87429 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 5 µl injection show the monitored m/z (137) for the predicted product. Only peaks of interest that eluted between 4-8 min were shown in the figure.

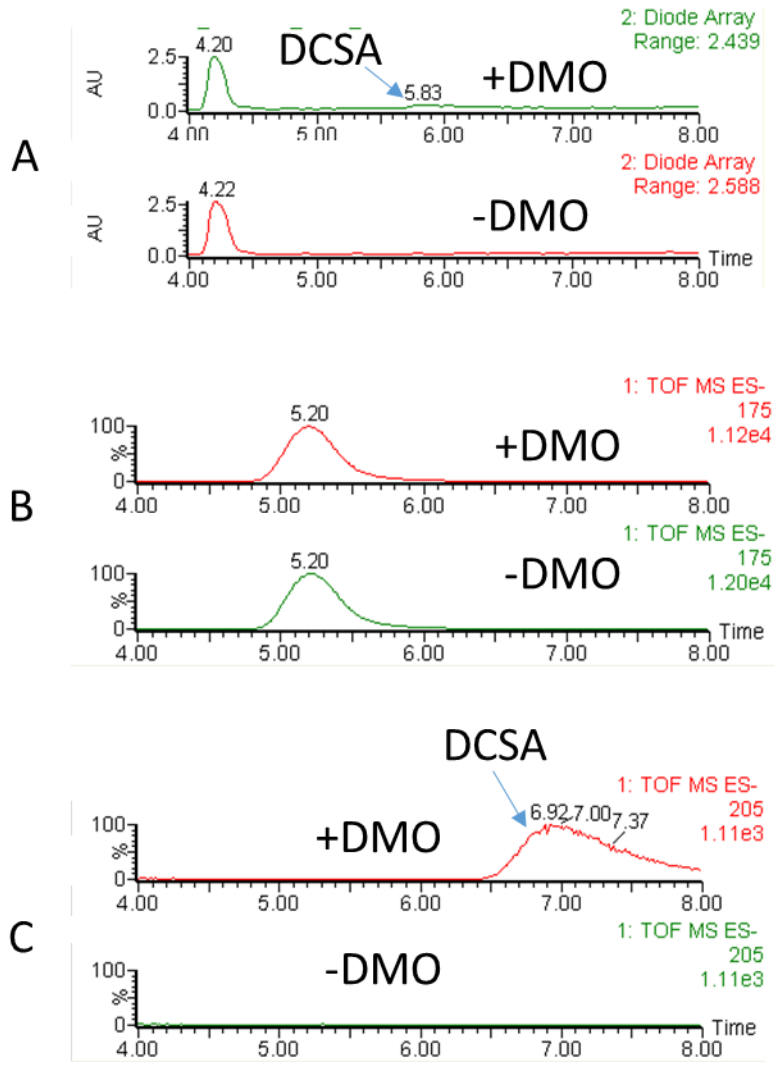


Figure E-2. Representative Assay Results for Dicamba

0.3 mM of dicamba was included in a reaction mixture made with (+DMO, upper) or without (-DMO, lower) MON 87429 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 5 µl injection show the monitored m/z (205) for DCSA. The peak corresponding to DCSA is indicated by arrow in panels A and C. Only peaks of interest that eluted between 4-8 min were shown in the figure.

References for Appendix E

Chakraborty, S., M. Behrens, P.L. Herman, A.F. Arendsen, W.R. Hagen, D.L. Carlson, X.Z. Wang and D.P. Weeks. 2005. A three-component dicamba O-demethylase from Pseudomonas maltophilia, strain DI-6: purification and characterization. Arch Biochem Biophys 437:20-28.

Burzio, L. and McCann, M. 2010. Specificity of Dicamba Mono-Oxygenase for Potential Endogenous Substrates. RPN-10-365.

Wang, C., K.C. Glenn, C. Kessenich, E. Bell, L.A. Burzio, M.S. Koch, B. Li and A. Silvanovich. 2016. Safety assessment of dicamba mono-oxygenases that confer dicamba tolerance to various crops. Regul Toxicol Pharmacol 81:171-182.

Appendix F: Materials and Methods Used for the Analysis of the Levels of DMO, PAT, CP4 EPSPS and FT_T Proteins Expressed in MON 87429

F.1. Materials

MON 87429 forage, leaf, root, grain and pollen tissue samples were harvested from five sites grown in United States during the 2017 growing season from starting seed lot 11464934. CP4 EPSPS (10000739), PAT (11383593), FT_T (11462796) and DMO (11402310) were used as the analytical reference standards.

F.2. Characterization of the Materials

The identity of the test substance was confirmed by analysis of the starting seed DNA by an event-specific polymerase chain reaction method.

F.3. Field Design and Tissue Collection

The test substance was planted in four replicated plots at five sites using randomized complete block design.

F.4. Tissue Processing and Protein Extraction

The CP4 EPSPS, PAT, FT_T and DMO proteins were extracted from tissue samples as described in Table F-1.

Table F-1. DMO, PAT, FT_T and CP4 EPSPS Extraction Parameter

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

[1] All proteins analyzed by Multiplexed Immunoassay were extracted by adding the appropriate volume of extraction buffer, stainless steel beads, and shaking in a Geno Grinder (SPEX Inc., Metuchen, NJ). The extracted samples were clarified by centrifugation.

[2] Tris borate buffer (pH 7.8) (0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.005 M $MgCl_2$, 0.05% (v/v) Tween 20)

F.5. Immunoassay Reagent and Methods

F.5.1. CP4 EPSPS Antibodies

Mouse monoclonal antibody clone 39B6.1 specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein A affinity chromatography. The concentration of the purified antibody was determined to be 5.9 mg/ml by spectrophotometric methods. The purified antibody (lot G-893017) was stored in a

phosphate buffered saline (1 × PBS, 15 mM NaN_3) and was used as the capture antibody in the multiplexed immunoassay.

Mouse monoclonal antibody clone 39B10.1.28 specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein A affinity chromatography. The purified antibodies were coupled with biotin (Thermo Fisher Scientific) per the manufacturer's instructions and assigned lot 209368-A. The detection reagent was streptavidin conjugated to R-Phycoerythrin.

F.5.2. PAT Antibodies

Purified mouse monoclonal antibody clone PAT (*pat*)-4 specific for PAT (*pat*) protein was purified by Protein G affinity chromatography. The concentration of the purified IgG was determined to be 0.9 mg/ml by spectrophotometric methods. The purified antibody (lot 276600E) was stored in 1 × PBS and was used as the capture antibody in the multiplexed immunoassay.

Purified rabbit polyclonal anti-PAT (*pat*) antibodies specific for the PAT (*pat*) protein were purified from serum using Protein G affinity chromatography and coupled with biotin per the manufacturer's instructions and assigned lot 351209. The detection reagent was streptavidin conjugated to R-Phycoerythrin.

F.5.3. FT_T Antibodies

Mouse monoclonal antibody clone 6-34.2.5 specific for the FT_T protein was purified by Protein G affinity chromatography. The concentration of the purified IgG was determined to be 2.95 mg/ml by spectrophotometric methods. The purified antibody (lot 546797) was stored in a phosphate buffered saline (1 × PBS, 15 mM NaN_3).

Mouse monoclonal antibody clone 6-35.2.11 specific for the FT_T protein was purified using Protein G affinity chromatography. The detection antibody was coupled with biotin (Thermo Fisher Scientific), per the manufacturer's instructions and assigned lot 546735.

F.5.4. DMO Antibodies

Mouse monoclonal antibody clone D712 specific for the DMO protein was purified from mouse ascites fluid using Protein A affinity chromatography. The concentration of the purified antibody was determined to be 3.3 mg/ml by spectrophotometric methods. The purified antibody (lot 151586) was stored in a phosphate buffered saline (1 × PBS, 15 mM NaN_3) and was used as the capture antibody in the multiplexed immunoassay.

Mouse monoclonal antibody clone 1-10C3.1.16.2 specific for the DMO protein was purified from mouse ascites fluid using Protein G affinity chromatography. The purified antibodies were coupled with biotin per the manufacturer's instructions and assigned lot 125874. The detection reagent was streptavidin conjugated to R-Phycoerythrin (Thermo Fisher Scientific).

F.6. Multiplexed Immunoassay Method

All CP4 EPSPS, PAT, FT_T and DMO proteins were analyzed using a validated multiplexed immunoassay method. Capture antibodies were covalently coupled to xMAP beads (Luminex Corp., Austin, TX) using the Antibody Coupling Kit (Luminex Corp., Austin, TX) as per the manufacturer's instructions. Antibody-coupled xMAP beads specific to each protein were diluted in sample buffer (1 × PBS containing 1% BSA (w/v)) to a final concentration of 50 beads/µl. Standards, QCs, and tissue samples were added to wells as appropriate followed by diluted beads and incubated for 30-60 minutes (min) at room temperature (RT) while shaking on a plate shaker at ~800. Plates were washed with 1 × PBS containing 0.05 % (v/v) Tween 20 (1 × PBST). A cocktail of biotinylated secondary antibodies was prepared in sample buffer containing 0.5% NFDM (w/v) and 0.5 mg/ml of mouse, rabbit, and goat IgG. The biotinylated antibody cocktail was added and incubated for 30-60 min at RT while shaking on a plate shaker at ~800. Plates were washed with 1 × PBST. Streptavidin RPE conjugate was added at a final concentration of 4 µg/ml and incubated for 30-60 min at RT by shaking on a plate shaker at ~800. Plates were washed with 1 × PBST. The beads were re-suspended by adding sample buffer and shaking on a plate shaker at ~800 for at least 10 min at RT. Quantification of each of the proteins was accomplished by interpolation from each of the protein standard curves.

F.7. Data Analyses

Multiplexed immunoassay plates for DMO, PAT, FT_T and CP4 EPSPS proteins were analyzed on the FLEXMAP 3D (Luminex Corp.). Plates were run as a batch on FLEXMAP 3D using appropriate methods, standards and control definitions. Data reduction analyses were performed using Milliplex Analyst software. Protein standard concentrations for each of the reference standards were fitted to a curve by the software using a best fit analysis (i.e. multiple models are fitted and the statistical best fit is used).

Following the interpolation from the standard curve, for data that determined to be greater than or equal to the LOQ, the protein levels (ng/ml) in the tissues were converted to a µg/g dw value. For each protein, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio in Core Informatics Laboratory Information Management System (LIMS, version 5.1.28).

The across-site sample means, standard errors (SEs), and ranges were calculated by Microsoft Excel 2016.

One grain sample from IAAU site of MON 87429 resulted in an unexpectedly negative result for CP4 EPSPS protein. The sample was re-extracted twice and re-analyzed for CP4 EPSPS protein to confirm the results. The sample was found to be expressing slightly above LOQ and was included in calculations.

Appendix G: Mode of Action and Specificity of MON 87429 FT_T Protein

G.1. Materials and Methods Used for FT_T Enzyme Kinetics Characterization and Protein Melting Temperature Determination

Enzyme kinetics characterization was conducted using a modified phenolic-based colorimetric detection assay (Fukomori, F. et al. 1993). Purified proteins were tested with selected substrates. All substrates were obtained from Sigma Aldrich in the racemic form, except where indicated; all other reaction components were obtained from Sigma Aldrich. The reaction buffer contains 20 mM MOPS pH 6.75, 50 µM (NH4)2 $Fe(SO_4)_2$, 50 µM Ascorbic Acid (Na salt), and 1 mM alpha-ketoglutarate. Reactions were performed in 150 µl final reaction volumes in 96-well assay plates. All reactions for kinetic characterization were completed with four replications. Reactions were stopped with 15µl of stop solution (50 mM Boric Acid, 50 mM KCl, pH 10.0 (KOH) with 2% 4-aminoantipyrine w/v (in water) freshly diluted 1:10 into stop buffer). Color development agent was added (15 ul of an 8% potassium ferricyanide w/v (in water), freshly diluted 1:10). Following color development, assays were recorded with a SpectraMax spectrometer at an absorbance of 510 nm. Back calculations were completed with product standards in mock reactions (standards from Sigma Aldrich or custom synthesis). Control reactions without alpha-ketoglutarate were inactive, and reactions with purified enzyme without $(NH_4)_2$ $Fe(SO_4)_2$ and ascorbic Acid were significantly inhibited. Unless indicated, all reactions were completed at 23 °C.

Protein melting temperatures were determined by fluorescence tracking of the hydrophobic protein binding dye, SYPRO Orange (Invitrogen) with progressive increases in temperature. The assays were run in a 20 mM Tris, pH 7.75 and 150 mM NaCl buffer (with or without the addition of $(NH_4)_2$ $Fe(SO_4)_2$ and Ascorbic Acid, as in the enzyme reaction) containing the FT_T protein and a 1:100 dilution of SYPRO Orange dye in a Bio-Rad CFX96 PCR thermocycler with a fluorescence detector. The temperature was progressively increased from room temperature to full protein denaturation. The temperature at which the protein was 50% denatured was calculated.

Table G-1. Characterization of FT_T Enzyme

			RdpA	FT_T
	Protein melting	Buffer	43	58
		Buffer (Fe and αKG)	53	62
FOPs	Quizalofop	V_{max}	2.76 (0.11)	1.62 (0.05)
		K_m	0.09 (0.01)	0.12 (0.01)
	Haloxyfop	V_{max}	2.37 (0.05)	1.05 (0.02)
		K_m	0.06 (0.01)	0.04 (0.004)
Synthetic auxins	Dichlorprop	V_{max}	5.32 (0.09)	2.09 (0.03)
		K_m	0.06 (0.004)	0.03 (0.002)
	2,4-D	V_{max}	0.25 (0.01)	1.17 (0.01)
		K_m	0.13 (0.02)	0.03 (0.002)
	MCPA	V_{max}	0.20 (0.01)	1.23 (0.02)
	Mecoprop	V_{max}	4.11 (0.15)	3.04 (0.07)

FT_T and RdpA as a control are characterized for enzyme temperature stability and enzyme kinetics with FOP and synthetic auxin herbicides. Protein melting is recorded as the temperature (C) in which the protein is 50% denatured in buffer alone or with supplemental Fe_2 and α-ketoglutarate. Enzyme kinetic parameters are recorded for representative FOP and synthetic auxin herbicides with V_{max} (µmol mg^{-1} min^{-1}) and K_m (mM) shown. Standard error shown in parenthesis. ND, not determined.

G.2. Materials and Method for Endogenous Substrate Specificity Testing

The endogenous substrate screen utilized a computational screening method with chemical similarity and chemical docking screens followed by *in vitro* screening of the compounds that passed both computational screens and were thus the most likely candidates for substrates to the FT_T enzyme. The computational nature of the first two steps in this three-step screening process enabled screening of large datasets of small plant-based molecules in an efficient and robust manner. For these screens, a plant specific small molecule dataset from NAPRALERT (https://www.napralert.org/) (Bisson et al., 2016) was used (as of March 1, 2016). NAPRALERT is a relational database of natural products from different sources. Over 900 small molecules from maize, canola, cotton, wheat and soybean were identified from the literature and cataloged in this NAPRALERT dataset. Small molecules were curated digitally using publicly available tools (https://pubchem.ncbi.nlm.nih.gov/ and downloading the 2D sdf file).

A control database was also curated. The herbicide control database included 11 known substrates for the FT enzymes, including chemical ID: 15118048 (Cyhalofop), 7153 (Mecoprop), 7204 (MCPA), 86134 (Fenoxaprop), 91701 (Fluazifop), 1486 (2,4-dichlorophenoxyacetic acid), 41428 (Triclopyr), 50465 (Fluroxypyr), 50895 (Haloxyfop), 178795 (Quizalofop), 8427 (Dichlorprop). These compounds are not plant metabolites but are synthetic herbicide compounds.

For Step 1 of the screen (the compound similarity search) we internally solved the structure of FT_T bound to its substrate, dichlorprop. The details of the software parameters and coding are provided for computation methods below. The computational methods summarized here provide an overview of the process. The dichlorprop structure was used as the query with Blaze search engine from Cresset. The Blaze software searched our curated plant small molecule database for similar compounds based on electrostatic characteristics and shape (field print). The top 50% of the field print search from databases were refined using Clique and followed by simplex refinement using the top 50% from Clique refinement. The field similarity and 2D Tanimoto similarity were determined. From the output, only entries with field similarity greater than 0.6 and a Tanimoto coefficient greater than 0.1 were selected using Forge (no refinement was performed). These thresholds, which were chosen such that all herbicide control compounds were recovered, returned a total of 71 novel compounds from the original database.

Step 2 of the screens included docking and analysis of the selected compounds. To leverage the structural information of FT_T, we used protein-small molecule docking in Rosetta to evaluate the fitness of 71 candidate molecules within the active site of FT_T. The candidate molecules were superimposed onto the query (Dichlorprop) from Blaze. Conformations of the candidate molecule were generated from OpenBabel and Frog2. We used default methods from Rosetta Macromolecule Modeling Suite to generate molecular mechanics parameters and performed local docking and refinement of candidate molecules within the active site. A total of 1000 refinement decoys were generated using Rosetta ligand docking. Since the goal was to evaluate the fitness of

aligned candidate molecules within the protein active site after refinement, we selected the top 1% (10 decoys) with least centroid movement while maintaining favorable (negative) interaction energy between protein and ligand. We then analyzed several interface features between FT_T and the selected small molecules, including repulsion energy between protein and small molecule, coulombic interaction energy, and buried SASA (solvent accessible surface area). Using the averages of these three features, we selected cutoff values to recover all herbicide compounds. This process resulted 38 novel compounds that were as compatible as the putative substrate, and were selected as candidates for *in vitro* enzymatic screening.

All 38 compounds from the computational screening plus 11 control herbicide compounds and cinnamate (a compound identified as a marginal substrate in a similar enzyme family (Griffin, S. L. et al, 2013) were ordered through www.chemnavigator.com or Sigma Aldrich. Six of the compounds selected from Step 2 of the computational screens could not be screened *in vitro* because they were not commercially available or available from custom synthesis groups. Therefore, the in vitro enzymatic screening was conducted with a total of 44 compounds (11 herbicides and 32 compounds that passed the in-silico screen and cinnamate) (Table V-1).

For the final *in vitro* enzymatic screening of oxidation by FT_T of the selected compounds, we used a coupled enzyme screen because it uses a common detection system that does not require standards of the final reaction products. This assay was adapted from a coupled assay previously described (Luo et al. 2006). The reaction mixture contained 100 mM MOPS (pH 7.0), 1 mM PEP, 0.4 mM NADH, 0.4mM coenzyme A, 0.4 mM ATP, 0.1 mM $MgCl_2$, 0.1 mM $(NH_4)_2Fe\ (SO_4)_2$, 0.2 mM Na ascorbate, 1 µM succinate CoA synthetase, 6-10 units pyruvate kinase, 6-10 units lactate dehydrogenase, 2 µg recombinant purified FT_T and 200 µM of each test compound diluted from a 5 mM DMSO. All reagents were purchased from Sigma Aldrich except succinyl CoA Synthetase, which was purchased from Megazyme. Reactions were arrayed in a 96-well plate and pre-incubated at room temperature for 10 min. The reaction was started by the addition of 1 mM α-ketoglutarate. For measurement of both herbicides and putative endogenous substrates, the assay was performed in triplicate (each with 3 technical replicates for a total n=9, with fenoxaprop assayed twice for a total n=18) by measuring absorbance at 340 nm continuously for up to one hour using a TECAN spectrometer. A positive (2,4-D) and negative (blank; DMSO without a test compound) control were included on each plate.

To screen for false positive, all positive reactions were confirmed with control reactions using either no α-ketoglutarate or no FT_T enzyme reactions. In addition, all the compounds were monitored for solubility and absorbance spectra before testing to ensure that did not interfere with the assay. To ensure that any change in A_{340} was due to the oxidation of NADH rather than the direct oxidation of the compounds, all the compounds were tested in the assay without the coupling system. To ensure that the compound did not inhibit the coupling system producing a false negative effect, 1 mM succinate was added to each well that had a negative result and the A_{340} was compared to a control without additional succinate.

References for Appendix G

Bisson, J., J. McAlpine, J. Graham, and G.F. Pauli. 2016. NAPRALERT, from an historical information silo to a linked resource able to address the new challenges in natural products chemistry and pharmacognosy. CEUR Workshop Proceedings 1747

Fukomori, F. and R. P. Hausinger. 1993. Purification and characterization of 2,4-dichlorophenoxyacetate/α-ketoglutarate dioxygenase. The Journal of Biological Chemistry 268:24311-24317

Griffin, S. L., J. A. Godbey, T. J. Oman, S. K. Embrey, A. Karnoup, K. Kuppannan, B. W. Barnett, G. Lin, N. V. J. Harpham, A. N. Juba, B. W. Schafer, and R. M. Cicchillo. 2013. Characterization of Aryloxyalkanoate Dioxygenase-12, a Nonheme Fe(II)/α-Ketoglutarate-Dependent Dioxygenase, Expressed in Transgenic Soybean and Pseudomonas fluorescense. Journal of Agricultural and Food Chemistry 61:6589-6596

Luo, C., X. Wang, J. Long and J. Liu . 2006. An NADH-tetrazolium-coupled sensitive assay for malate dehydrogenase in mitochondria and crude tissue homogenates. J Biochem Biophys Methods 68(2):101-111

Appendix H: Materials and Methods for Seed Germination and Dormancy Assessment of MON 87429

H.1. Materials

Seed germination and dormancy characteristics were assessed on seed from MON 87429 and the conventional control harvested from one 2017 field production site in Kihei, Hawaii, and on four reference maize hybrids obtained from commercial sources (Table H-1)

H.2. Characterization of the Materials

The presence of the MON 87429 event in the starting seed of the test material and the absence of the MON 87429 event in the starting seed of the conventional control were verified by event-specific polymerase chain reaction (PCR) analyses.

H.3. Germination Testing Facility and Experimental Methods

Germination and dormancy evaluations were conducted at SGS North America, Inc, Brookings, SD. 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, 2017a; b; AOSA, 2009; AOSA/SCST, 2010).

The seed lots of MON 87429, the conventional control, and four reference hybrids were tested under two temperature regimes. The optimum temperature regime consisted of alternating temperatures of approximately 20°C for 16 hours followed by 30°C for 8 hours and was maintained for seven days (AOSA, 2017a; b). The suboptimum temperature regime consisted of constant temperatures of approximately 10°C for seven days followed by 25°C for four days (AOSA, 2009). One germination chamber was used for each temperature regime. Each chamber was maintained dark. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Rolled germination towels were assembled for each material by placing approximately 50 seeds on a pre-moistened and labeled germination towel. An additional pre-moistened germination towel was placed on top of the seed, and the towels and seed were rolled up. Approximately 100 seeds (a set of two germination towel rolls secured together with a rubber band) of each material were placed into a bucket to form a replication. Each temperature regime constituted a separate experiment that was conducted using a randomized complete block design with four replications (*i.e.*, buckets).

Each set of rolled germination towels in each temperature regime was assessed at prescribed timings for germinated, dead, firm swollen (viable and nonviable), and hard (viable and nonviable) seed. Additional details for each germination characteristic evaluated and the timing of evaluations are presented in Table VII-1. Seeds placed under the optimum temperature regime were evaluated according to AOSA standards for testing

of maize (AOSA, 2017a; b). Seeds placed under the suboptimum temperature regime were evaluated according to AOSA standards for cold testing of maize (AOSA, 2009).

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

The percentage of seed in each assessment category was based on the number of seeds evaluated. Across temperature regimes, the total number of seeds evaluated from each set of rolled germination towels was approximately 100.

H.4. Statistical Analysis

For each temperature regime, ANOVA was conducted according to a randomized complete block design using SAS® (SAS, 2012) to compare MON 87429 to the conventional control for the germination and dormancy characteristics. If analysis of variance assumptions were not satisfied, Fisher's Exact test was conducted for that characteristic using SAS (SAS, 2012). The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). The reference range for each characteristic was determined from the minimum and maximum mean values among the four references.

Table H-1. Starting Seed for Germination and Dormancy Assessment of MON 87429

Material Name	Regulatory Lot Number	Phenotype	Material Type
MON 87429	11478364	Herbicide Tolerant	Test
LH244+HCL617	11478363	Conventional	Control
Dekalb DKC64-85	11464651	Conventional	Reference
Dekalb DKC 61-52	11427255	Conventional	Reference
Kruger K-0708	11427267	Conventional	Reference
Agrigold A 6300	11446938	Conventional	Reference

References for Appendix H

AOSA. 2009. Seedling evaluation. Rules for Testing Seeds. Volume 4. Association of Official Seed Analysts, Ithaca, New York.

AOSA. 2017a. AOSA Rules for testing seeds. Volume 1: Principles and procedures. Association of Official Seed Analysts, Washington, D.C.

AOSA. 2017b. AOSA Rules for testing seeds. Volume 4: Seedling evaluation. Association of Official Seed Analysts, Washington, D.C.

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

SAS. 2012. Software Release 9.4 (TS1M4). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Appendix I: Material and Methods for Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87429 under Field Conditions

I.1. Materials

Agronomic, phenotypic, and environmental interaction characteristics were assessed for MON 87429, the conventional control, and reference hybrids grown under similar agronomic conditions. Four reference hybrids were planted per site (Table I-1). A total of 16 unique reference hybrids were evaluated among the eight sites.

I.2. Characterization of the Materials

The presence of the MON 87429 event in the starting seed of the test material and the absence of the MON 87429 event in the starting seed of the conventional control were verified by event-specific polymerase chain reaction (PCR) analyses.

I.3. Field Sites and Plot Design

Field trials in 2017 at eight sites provided a range of environmental and agronomic conditions representative of U.S. maize growing regions. The Principal Investigator at each site was familiar with crop growth and production and evaluation of crop characteristics. The study was established at each site in a randomized complete block design with four replications. Plot and row dimensions are listed in Table I-2.

I.4. Planting and Field Operations

Planting information, soil description, and cropping history of the sites are listed in Table I-2. The Principal Investigator at each site followed local agronomic practices including those related to seed bed preparation and trial maintenance such as application of agricultural chemicals, fertilizer, and irrigation. All maintenance operations were performed uniformly across all plots within each site.

Table I-1. Starting Seed for Phenotypic, Agronomic, and Environmental Interactions Assessment of MON 87429

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype	Material Type
All	MON 87429	11464934	Herbicide Tolerant	Test
All	LH244+HCL617	11464933	Conventional	Control
IAAU	Dekalb DKC61-52	11427255	Conventional	Reference
IAAU	Lewis 1407	11427264	Conventional	Reference
IAAU	Golden Harvest G12J11-A	11446928	Conventional	Reference
IAAU	LG Seeds LG2549	11446934	Conventional	Reference
IARL	Lewis 1613	11427265	Conventional	Reference
IARL	Stone 5820	11427266	Conventional	Reference
IARL	Agrigold A6472	11446939	Conventional	Reference
IARL	Agrigold A6574	11446940	Conventional	Reference
ILCX	Dekalb DKC64-85	11427257	Conventional	Reference
ILCX	Lewis 1613	11427265	Conventional	Reference
ILCX	Kruger K-0708	11427267	Conventional	Reference
ILCX	Mycogen Seeds MY09V40	11446930	Conventional	Reference
ILMN	Dekalb DKC61-52	11427255	Conventional	Reference
ILMN	Dekalb DKC65-18	11427258	Conventional	Reference
ILMN	Golden Harvest G09C43	11446927	Conventional	Reference
ILMN	Agrigold A6574	11446940	Conventional	Reference
INSH	Kruger K-0708	11427267	Conventional	Reference
INSH	Mycogen Seeds 2H721	11446932	Conventional	Reference
INSH	LG Seeds LG2549	11446934	Conventional	Reference
INSH	LG Seeds LG2636	11446935	Conventional	Reference
NESW	Lewis 1407	11427264	Conventional	Reference
NESW	Lewis 1613	11427265	Conventional	Reference
NESW	Golden Harvest G12J11-A	11446928	Conventional	Reference
NESW	Mycogen Seeds MY09V40	11446930	Conventional	Reference
NEYO	Dekalb DKC64-85	11427257	Conventional	Reference
NEYO	Golden Harvest G12J11-A	11446928	Conventional	Reference
NEYO	LG Seeds LG2549	11446934	Conventional	Reference
NEYO	Agrigold A6574	11446940	Conventional	Reference
OHTR	Dekalb DKC62-06	11427256	Conventional	Reference
OHTR	Dekalb DKC64-85	11427257	Conventional	Reference
OHTR	Kruger K-0708	11427267	Conventional	Reference
OHTR	Golden Harvest G09C43	11446927	Conventional	Reference

Note: Starting seed of test and control materials were produced in Kihei, HI in 2017.

[1] Site codes: IAAU = Audubon County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; NESW = Seward County, NE; NEYO = York County, NE; OHTR = Miami County, OH.

Table I-2. Field Information for Phenotypic, Agronomic, and Environmental Interactions Assessment of MON 87429

Site Code[1]	Planting Date[2]	Harvest Date[2]	Planting Rate (seeds/m^2)	Rows/ Plot	Inter-row Distance (cm)	Row Length (m)	Plot Size (m^2)	Soil Texture	Organic Matter (%)	Previous Crop 2016
IAAU	05/13/2017	11/01/2017	9.5	8	76	6.1	37.2	Silty Clay Loam	2.8	Soybean
IARL	05/05/2017	10/05/2017	9.5	8	76	6.1	37.2	Silty Clay Loam	3.4	Soybean
ILCX	06/01/2017	10/10/2017	8.6	8	76	6.2	38.1	Clay Loam	4.0	Maize
ILMN	05/09/2017	10/09/2017	9.5	8	76	6.1	37.2	Clay Loam	5.1	Soybean
INSH	05/18/2017	10/31/2017	9.5	8	76	6.1	37.2	Loam	2.2	Maize
NESW	05/08/2017	10/12/2017	9.5	8	76	6.1	37.2	Loam	3.1	Maize
NEYO	05/08/2017	10/19/2017	9.5	8	76	6.1	37.2	Loam	3.1	Soybean
OHTR	06/07/2017	11/17/2017	9.5	8	76	6.4	39.0	Loam	1.8	Maize

Note: Planting rate and plot/row dimensions are approximate.

[1] Site codes: IAAU = Audubon County, IA; IARL = Jefferson County, IA; ILCX = Vermilion County, IL; ILMN = Warren County, IL; INSH = Boone County, IN; NESW = Seward County, NE; NEYO = York County, NE; OHTR = Miami County, OH.

[2] Date format = mm/dd/yyyy.

I.5. Phenotypic Characteristic Assessments

Phenotypic characteristics assessed and the timing of each assessment are listed in Table VII-1.

I.6. Environmental Interaction Assessments

The test, conventional control, and commercial references were evaluated at each site for differences in plant responses to abiotic stressors, diseases, and arthropod pests. Evaluations were performed four times during the growing season at the following growth stages: V5 – V8, V12 – R1, R1 – R3, and R4 – R5.

The Field Co-operator at each site identified abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the plots or likely to occur in maize during a given observation period. Stressors assessed often varied among observations and sites.

Ratings were made 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

I.7. Statistical Analysis/Data Summarization

A combined-site analysis was conducted according to a randomized complete block design using SAS® (SAS, 2012) to compare the test to the conventional control for the phenotypic characteristics listed in Table VII-1. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). Descriptive statistics are provided for one characteristic, seed loss, that had insufficient variability for formal statistical analysis. The reference range for each phenotypic characteristic was determined from the minimum and maximum mean values among the 16 references, where each mean was combined over all the sites at which the reference was planted. There were no plots for which data were excluded from the study.

The environmental interactions data consisting of plant responses to abiotic stressors, diseases, and arthropod pests are categorical and 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 four replications within an observation.

I.8. Detailed Results for Environmental Interactions Assessments for MON 87429

No differences were observed between the test and the conventional control for any of the 96 comparisons of plant responses to the assessed abiotic stressors: cold temperatures, drought, excessive rain (i.e. waterlogging), hail, high temperatures, high winds (lodged plants), nutrient deficiency, soil compaction, and sun scald (Table I-3).

No differences were observed between the test and the conventional control for any of the 96 comparisons of damage from the assessed diseases: anthracnose, corn stunt, eyespot, Goss's bacterial wilt, gray leaf spot, leaf blight, northern leaf spot, rust, smut (head and ear), and Stewart's wilt (Table I-4).

No differences were observed between the test and the conventional control for any of the 96 comparisons of damage from the assessed arthropods: aphids, armyworms, billbugs, corn earworms, corn flea beetles, corn rootworm beetles, cutworms, European corn borers, grasshoppers, Japanese beetles, sap beetles, slugs, spider mites, and stink bugs (Table I-5).

The results of the assessed environmental interactions support the conclusion that MON 87429 is not expected to pose increased plant pest risk compared to conventional maize.

Table I-3. Summary of Abiotic Stressor Response Evaluations for MON 87429 and the Conventional Control in 2017 U.S. Field Trials

Abiotic Stressor	Total Observations across Sites	Number of Observations without Differences between the Test and the Conventional Control
Total	96	96
Cold Temperatures	1	1
Drought	13	13
Excessive Rain (i.e. waterlogging)	14	14
Hail	10	10
High Temperatures	10	10
High Winds (lodged plants)	26	26
Nutrient deficiency	12	12
Soil compaction	3	3
Sun scald	7	7

Notes: No differences were observed between the test and the conventional control during any observation for responses to any of the assessed abiotic stressors. Categorical data were summarized across sites and observation times.

Table I-4. Summary of Disease Damage Evaluations for MON 87429 and the Conventional Control in 2017 U.S. Field Trials

Disease	Total Observations across Sites	Number of Observations without Differences between the Test and the Conventional Control
Total	96	96
Anthracnose	8	8
Corn stunt	4	4
Eyespot	8	8
Goss's bacterial wilt	12	12
Gray leaf spot	20	20
Leaf blight[1]	13	13
Northern leaf spot	3	3
Rust[2]	18	18
Smut (head and ear)	8	8
Stewart's wilt	2	2

Notes: No differences were observed between the test and the conventional control during any observation for damage caused by any of the assessed diseases. Categorical data were summarized across sites and observation times.
[1] Includes Northern.
[2] Includes common.

Table I-5. Summary of Arthropod Damage Evaluations for MON 87429 and the Conventional Control in 2017 U.S. Field Trials

Arthropod	Total Observations Across Sites	Number of Observations without Differences between the Test and the Conventional Control
Total	96	96
Aphids (Aphididae)	12	12
Armyworms (Noctuidae)	13	13
Billbugs (weevil)	1	1
Corn earworms (*Helicoverpa zea*)	7	7
Corn flea beetles (*Chaetocnema pulicaria*)	7	7
Corn rootworm beetles (*Diabrotica* spp.)	12	12
Cutworms (Noctuidae)[1]	7	7
European corn borers (*Ostrinia nubilalis*)	2	2
Grasshoppers (*Melanoplus* spp.)	15	15
Japanese beetles (*Popillia japonica*)	10	10
Sap beetles (Nitidulidae)	1	1
Slugs[2]	1	1
Spider mites (*Tetranychus* spp.)	2	2
Stink bugs (Pentatomidae)	6	6

Notes: No differences were observed between the test and the conventional control during any observation for damage caused by any of the assessed arthropods. Categorical data were summarized across sites and observation times.

[1] Includes Western bean cutworm.

[2] Slugs are not arthropods but are occasional pests in maize.

References for Appendix I

SAS. 2012. Software Release 9.4 (TS1M4). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Appendix J: Materials and Methods for Pollen Morphology and Viability Assessment

J.1. Plant Production

Pollen Morphology and Viability were assessed for MON 87429, the conventional control, and four conventional reference hybrids grown under similar agronomic conditions in a field trial in Warren County, Illinois in 2017 (Table J-1). The trial was arranged in a randomized complete block design with four replications. Each plot consisted of 8 rows approximately 6 m in length.

J.2. Pollen Collection and Pollen Sample Preparation

Pollen was collected from the three plants (tassels) per plot. The pollen collected from an individual tassel represented a subsample. Each subsample was transferred to a uniquely labeled tube containing approximately 400 µl of Alexander's stain solution (Alexander, 1980) diluted 1:5 with distilled water. The tubes were closed and shaken until thoroughly mixed. Pollen was allowed to stain and fix for at least 20 hours at ambient temperatures before assessments.

J.3. Data Collection

Slides were prepared by aliquoting suspended pollen/stain solution onto a slide. Pollen characteristics were assessed under an Olympus[Ó] BX53 light microscope equipped with an Olympus[©] DP72 digital color camera at a target magnification of 200x. The microscope and camera were connected to a computer running Microsoft Windows XP[Ò] and installed with Olympus© cellSens version 1.4.1 software.

J.3.1. Pollen Viability

When pollen grains are exposed to the staining solution, viable pollen grains stain red to purple due to the presence of living cytoplasmic content. Non-viable pollen grains stain light blue to green or colorless, and the shape can appear 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 125 pollen grains were counted for each of the three subsamples per plot. Mean pollen viability for each plot was calculated from the subsamples and expressed as a percentage of total number of evaluated pollen grains.

[©] Olympus Corporation.

[®] Windows XP is a registered trademark of Microsoft Corporation.

J.3.2. Pollen Diameter

For a single predetermined subsample from each plot, pollen grain diameter was measured along two perpendicular axes for 10 representative pollen grains. Mean pollen diameter for each plot was calculated from the total of 20 diameter measurements.

J.3.3. General Pollen Morphology

General pollen morphology was observed from micrographs of the test, control, and the reference materials that were also used for the pollen diameter measurements.

J.3.4. Statistical Analysis

An analysis was conducted according to a randomized complete block design using SAS® (SAS, 2012) to compare the test to the conventional control for percent viable pollen and pollen grain diameter. The level of statistical significance was predetermined to be 5% (α=0.05). The reference range for each phenotypic characteristic was determined from the minimum and maximum mean values among the four references. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations. There were no plots for which data were excluded from the study.

J.3.5. General Pollen Morphology Results

No visual differences in general pollen morphology were observed between MON 87429 and the conventional control (Figure J-1).

®SAS is a registered trademark of SAS Institute, Inc.

Table J-1. Starting Seed for Pollen Morphology and Viability Assessment

Material Name	Material Type	Phenotype	Regulatory Lot Number
MON 87429	Test	Herbicide Tolerant	11464934
LH244+HCL617	Control	Conventional	11464933
Agrigold A6574	Reference	Conventional	11446940
Dekalb DKC61-52	Reference	Conventional	11427255
Dekalb DKC65-18	Reference	Conventional	11427258
Golden Harvest G09C43	Reference	Conventional	11446927

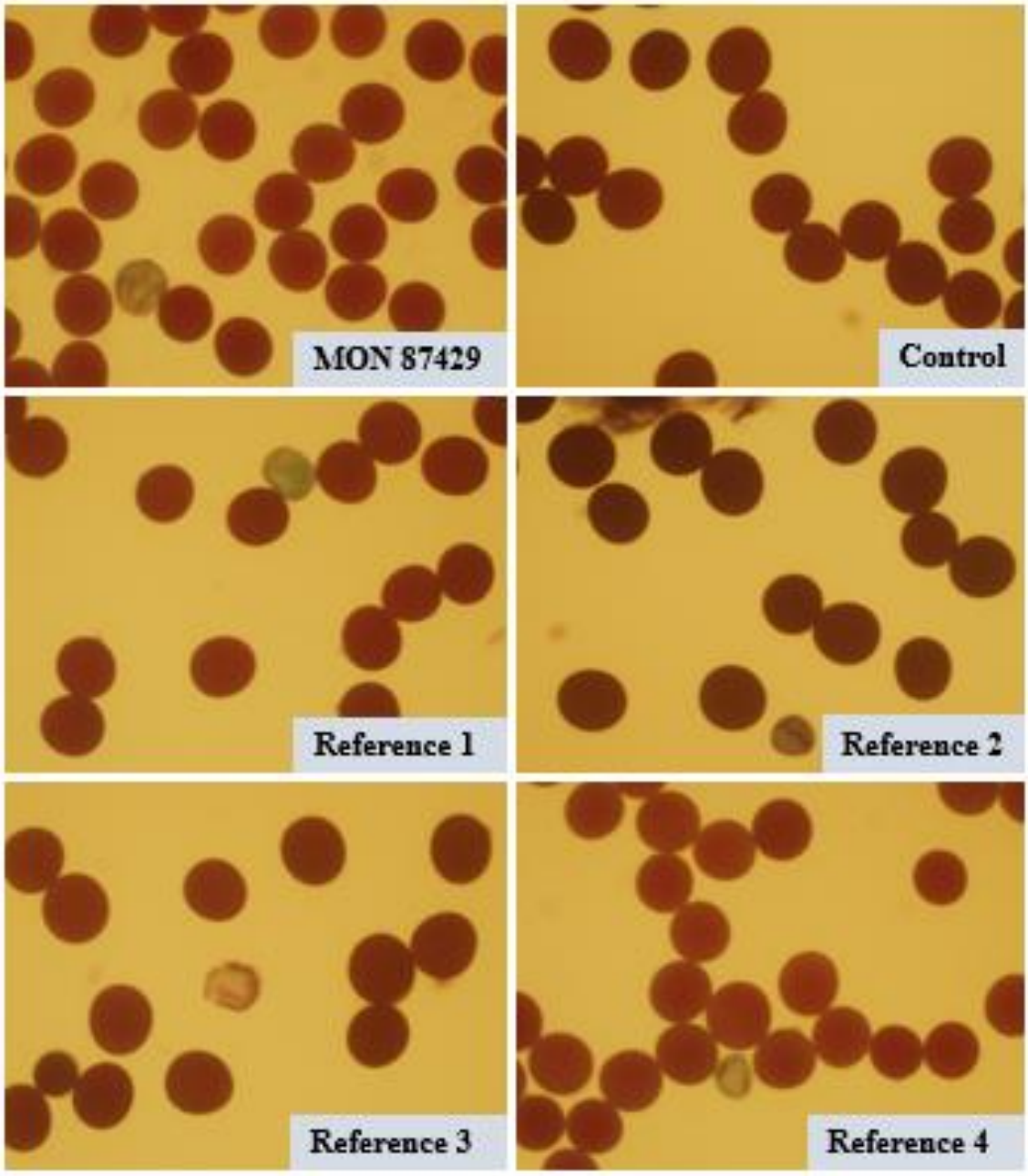


Figure J-1. General Morphology of Pollen from MON 87429, the Conventional Control, and Commercially Available Conventional Reference Materials under 200X Magnification

Viable pollen grains stained red to purple, while non-viable pollen grains stained lighter or colorless and the shape appeared round to collapsed depending on the degree of hydration.

References for Appendix J

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55:13-18.

SAS. 2012. SAS/STAT software version 9.3. SAS Institute, Inc., Cary, North Carolina.

Appendix K: Herbicide Resistance

K.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. 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., 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)[11], 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, 2015; Norsworthy et al., 2012; WSSA, 2019)[12] and are being implemented by growers.

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of dicamba, glufosinate, quizalofop, 2,4-D and glyphosate 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 implementation of good management practices by growers and associated research and education. These activities are key elements of Monsanto's approach to providing stewardship of dicamba, glufosinate, quizalofop,

[11] An allele is any of several forms of a gene, usually arising through mutations, that are responsible for hereditary variation.

[12] The Herbicide Resistance Action Committee (HRAC; www.hracglobal.com) 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.

2,4-D and glyphosate used on MON 87429 maize that will be combined with deregulated full plant glyphosate tolerance traits, such as NK603. Monsanto will continue to 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, glufosinate, quizalofop, 2,4-D and glyphosate in maize production. This appendix provides an overview of Monsanto's approach to the development of best management practices to delay selection for weed resistance to dicamba, glufosinate, quizalofop, 2,4-D and/or glyphosate herbicides. 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. An example of this collaboration is the development and posting of herbicide-resistance 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 recommends pesticide registrants to provide growers with information regarding an herbicide's sites-of-action and resistance management practices via labeling 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) Notices 2017-1 and 2017-2 (U.S. EPA, 2017b; U.S. EPA, 2017c). Monsanto incorporates EPA's guidelines for pesticide site-of-action and resistance management labeling on its agricultural herbicide labels and will continue to do so in the future.

K.2. The Herbicides Dicamba and 2,4-D

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is classified as a benzoic acid herbicide belonging to the synthetic auxin group of herbicides (WSSA, 2018). 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. 2,4-D (2,4-Diclorophenoxy acetic acid) is classified as a phenoxy carboxylic acid herbicide (WSSA, 2018). As they regulate plant growth through an auxin receptor site-of-action, dicamba, 2,4-D and other synthetic auxin herbicides are classified in Herbicide Group 4 by the Weed Science Society of America (WSSA, 2018). Most herbicides in Group 4 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, approximately 50 perennial broadleaf species and control or suppression of over 50 woody species (Bayer CropScience, 2018). 2,4-D provides control of approximately 70 annual broadleaf and approximately 30 perennial broadleaf weed species (Dow, 2017). Dicamba and 2,4-D are not active on grass weeds and are often used in combination with other herbicides to provide broad spectrum weed control.

Dicamba was commercialized in the U.S. for agricultural use in 1967 and 2,4-D has been used as an herbicide since mid-1940s. They are currently labeled for use preplant and/or preemergence and/or postemergence to control emerged weeds in maize, sorghum, rice, small grains (wheat, barley, rye and oats), millet, pasture, rangeland, asparagus, sugarcane, turf, grass grown for seed, various other crops, conservation reserve program land, fallow cropland, and/or for non-crop uses (U.S. EPA, 2005; U.S.EPA, 2009). In addition, dicamba and 2,4-D are approved for preplant application in conventional soybean and cotton. Dicamba is approved for postemergence in-crop use in dicamba-tolerant soybean and cotton (Bayer CropScience, 2018), and 2,4-D is approved for postemergence in-crop use in 2,4-D-tolerant soybean, cotton and maize (Dow, 2017). Dicamba and 2,4-D are sold as standalone formulations which can be tank-mixed, based on the approved label, with one or more active ingredients depending upon the specific herbicide, the crop and the weed spectrum. Dicamba and 2,4-D are each sold as premix formulations with certain other herbicides.

K.3. The Herbicide Glufosinate

Glufosinate [2-amino-4-(hydroxymethylphosphinyl) butanoic acid] is classified by the Weed Science Society of America as a phosphinic acid herbicide belonging to the Group 10 glutamine synthetase inhibitor group of herbicides (WSSA, 2018). Bialaphos is the only other herbicide belonging to the phosphinic acid chemical family. Glufosinate provides control of approximately 70 annual broadleaf, 30 annual grass weed species and control or suppression of over 30 biennial and perennial grass and broadleaf weed species (Bayer CropScience, 2016).

Glufosinate was first registered for use in the U.S. in 1989 (U.S. EPA. 1989) and is currently labeled for non-crop uses, preplant burndown for glufosinate-tolerant and non-glufosinate-tolerant crops and/or in-crop postemergence weed control in glufosinate-tolerant canola, maize, cotton, and soybean (Bayer CropScience, 2016). 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.

K.4. The Herbicide Quizalofop

Chloroplastic acetyl-CoA carboxylase (ACCase) inhibitors Aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides have been widely used to control grass weed species since their introduction in the 1970s and 1980s, respectively (Kraehmer et al., 2014). These AOPP and CHD post-emergence grass herbicides are frequently referred to as “fops” and “dims”, respectively. While “fops” and “dims” herbicides are two chemically dissimilar classes of herbicides, they both inhibit the ACCase enzyme, which catalyzes the first committed step in fatty acid biosynthesis, causing plant death (Kraehmer et al 2014). Quizalofop-p-ethyl (Ethyl(*R*)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionic acid), is a systemic herbicide absorbed by the leaves with translocation throughout the plant (Mantzos et al., 2016). Quizalofop is classified as ACCase-inhibiting “fops” herbicide

belonging to the Herbicide Group 1 by the Weed Science Society of America (WSSA, 2018).

Quizalofop-p-ethyl was first approved for use as a registered product in 1990 (Assure® II: EPA Reg No. 352-541). It is used to selectively control post-emergent annual and perennial grass weeds in dicot crops such as potatoes, soybeans, peanuts, sugar beet, oilseed rape, sunflower, vegetables, cotton and flax and in quizalofop-tolerant maize (Wright et al., 2010; U.S. EPA, 2018).

K.5. The Herbicide Glyphosate

Glyphosate [N-(phosphonomethyl) glycine] is classified by the Weed Science Society of America as an EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) inhibitor herbicide belonging to the Group 9 (WSSA, 2018). Glyphosate is the only herbicide within Group 9 and is a non-selective, systemic, postemergence herbicide that translocates primarily to metabolic sinks, killing meristematic tissues away from the application site. Its phloem-mobile properties and slow action in killing weeds allow the herbicide to move throughout the plant, making it effective for weed control including perennial weeds (Duke 2018). Glyphosate provides control of approximately 100 annual weed species (grass and broadleaf), over 60 perennial weed species (grass and broadleaf) and control or suppression of approximately 65 woody brush, trees and vines (e.g., Roundup PowerMax® II; EPA Reg. No. 524-537).

Glyphosate was first registered for use in the U.S. in 1974 and has been extensively used globally (Duke, 2018). It is currently labeled for non-crop uses, preplant burndown to glyphosate- and non-glyphosate-tolerant crops and/or in-crop postemergence weed control in glyphosate-tolerant canola, maize, cotton, soybean, sugarbeet and alfalfa (Monsanto, 2017). Glyphosate 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. Glyphosate is also sold as premix formulations with certain other herbicides.

K.6. 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 (*Commelina diffusa*) biotype resistant to 2,4-D, was identified in Hawaii (Heap, 2019). Through October 2018, there are approximately 160 unique cases of herbicide resistance by weed species with known herbicide-resistant biotypes to one or more herbicides in the U.S (Heap, 2019). 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, 2019). An herbicide-tolerant weed species is one that is naturally tolerant to an herbicide, for example a grass species is not killed by the application of a broadleaf herbicide (WSSA, 2019). 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 sites-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 build-up 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; HRAC, 2015).

Recent research indicates that herbicide mixtures offer a better management option than rotating herbicides. However, the effectiveness of herbicide mixtures depends on their similar efficacies, soil residual activity and/or different propensities for resistance selection in the target weed species (Beckie and Reboud, 2009; Lagator et al., 2013; Evans et al., 2016; Beckie and Harker, 2017). Simultaneously using two herbicides with different sites-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). Use of multiple methods of weed control including multiple herbicides with different sites-of-action in a single field 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; Andrew et al., 2015). While herbicide mixtures have been shown to be an effective strategy to delay the development of herbicide resistance, given that some weeds already evolved resistance to multiple previously effective herbicides, it could become challenging to find effective herbicide mixtures that individually suppress a specific weed species in the future (Evans et al., 2016). Broad use of herbicide mixtures may increase the chance of development of cross-resistance, especially if use rates are below the effective rates approved on the herbicide label (Evans et al., 2016). These challenges highlight the need to follow each best management practice described in the paragraph

above (a-l), including applying label rates of herbicides and using other cultural practices to assist in delaying the development of herbicide resistance.

K.7. 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, 2019; Figure K-1). The graph in Figure K-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.

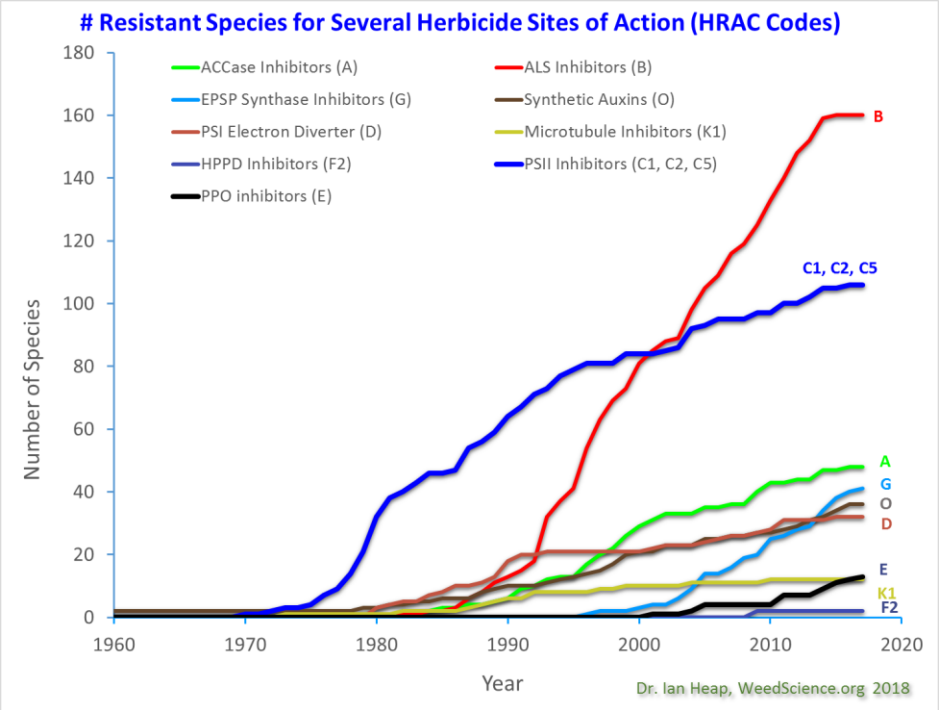


Figure K-1. Global Weed Resistance to Various Herbicide Families

Weed resistance to the synthetic auxins (O) 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 in this Section K-8.

K.8. Weeds Resistant to Dicamba, Glufosinate, Quizalofop, 2,4-D or Glyphosate Herbicides

As noted earlier in Section K.7, 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 North America after over 40 years of use: common hempnettle, kochia, prickly lettuce and wild mustard (Heap, 2019). Additionally, a population of common lambsquarters has been confirmed to be resistant in New Zealand, wild mustard in Turkey, cornflower in Poland and smooth pigweed in Argentina have been confirmed to be resistant for a total of seven species worldwide with confirmed resistant biotypes to dicamba. For the synthetic auxin group of herbicides, there exist a total of appropriately 30 broadleaf species globally with biotypes having confirmed resistance to at least one member of this group, but only seven broadleaf species in the U.S. and six broadleaf species in Canada (Heap, 2019). Broadleaf weed species that are resistant to synthetic auxins in the U.S. include Palmer amaranth, tall waterhemp, yellow starthistle (*Centaurea solstitialis*), wild carrot, kochia, prickly lettuce and buckhorn plantain. In Canada, kochia, wild mustard, wild carrot, common hempnettle, spotted knapweed (*Centaurea maculosa*) and false cleavers (*Galium spurium*) were reported to be resistant to synthetic auxins. For 2,4-D herbicide, a total of 5 broadleaf weeds (Palmer amaranth, waterhemp, prickly lettuce, wild carrot, and buckhorn plantain) have been confirmed to be resistant in the U.S. All the broadleaf populations are found in the Midwest (Palmer amaranth in KS, waterhemp in NE and IL and wild carrot in OH and MI, and buckhorn plantain in IN), western U.S. (prickly lettuce in WA) 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 because of exposure to a similarly acting herbicide). Therefore, consideration must be given to the possibility that dicamba and/or 2,4-D 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; Tehranchian et al., 2017). However, because of differences in sites-of-action among the chemistry families within the synthetic auxin herbicide group (i.e., benzoic acids compared to pyridine-carboxylic acids), cross-resistance between them is uncommon (Beckie and Tardif, 2012).

MON 87429 maize will be combined with deregulated glyphosate tolerance traits, such as NK603, where dicamba or 2,4-D can be applied in combination with glyphosate and glufosinate. It is important to note that in the U.S., kochia is the only broadleaf species with biotypes with herbicides resistance to dicamba, 2,4-D and glyphosate (LeClere et al., 2018; Heap, 2019). In addition, dicamba (only) resistant kochia biotypes were reported in 4 other states (Nebraska, Colorado, Idaho and North Dakota). In Kansas, 2 separate kochia biotypes, one with multiple resistance to dicamba, glyphosate, atrazine and chlorsulfuron and another with multiple resistance to dicamba, fluroxypyr and glyphosate were reported. A kochia biotype in Montana was reported to be resistant to dicamba and fluroxypyr (Heap, 2019). A biotype of waterhemp in Illinois was reported to be resistant to 2,4-D, HPPD, PS II, ALS and PPO inhibitors (Heap, 2019). In Nebraska, a waterhemp biotype was reported to be resistant to 2,4-D, aminopyralid, atrazine, chlorimuron,

imazethapyr and picloram (Heap, 2019). Biotypes of waterhemp with resistance to 2,4-D or glyphosate exist both in Illinois and Kansas (Heap 2019), and there is a high potential for biotypes with resistance to both herbicides to occur via crossing in this cross-pollinated dioecious species. A Palmer amaranth biotype in Kansas was reported to be resistant to both 2,4-D and glyphosate (also resistant to HPPD, PS II and ALS inhibitors) (Heap, 2019). In addition, a prickly lettuce biotype was reported to be resistant to 2,4-D and dicamba and MCPA in Washington state, outside of the main corn-growing regions of the U.S. (Heap, 2019). MON 87429 maize hybrids combined with deregulated glyphosate tolerance traits, such as NK603, provide tools to reduce the likelihood of resistance development in many broadleaf weed species and/or delay the likelihood of further selection or spread of biotypes that are already resistant to one or more herbicides with different sites-of-action. For example, growers would have options to rotate herbicides or use herbicide mixtures with dicamba or 2,4-D, glyphosate, and/or glufosinate, each with a distinct site-of-action, based on the approved label and the weed spectrum (Table VIII-7) to reduce the likelihood of resistance development. If additional weed populations with resistance to both glyphosate and dicamba or 2,4-D herbicides were to occur there are other preplant and/or postemergence herbicide options for managing the weed in maize and in its rotational crops (Table K-1).

There is no known broadleaf weed species reported to be resistant to glufosinate in the U.S (Heap, 2019). To date there are three grass weed species with confirmed resistance to glufosinate globally: goosegrass in Malaysia, rigid ryegrass in Greece, perennial ryegrass in New Zealand and Italian ryegrass in New Zealand and the U.S. (in Oregon and California states) (Heap, 2019). In the case of goosegrass (*Eleusine indica*), a population was found in Malaysia that is resistant to glufosinate and paraquat herbicides. Resistance to glufosinate evolved likely due to the repeated use of glufosinate for at least six times a year and for more than four consecutive years as standalone or in combination with paraquat in a bitter gourd field. It is speculated that paraquat resistance in this goosegrass population might have evolved earlier than glufosinate resistance since this vegetable field which was originally a rubber plantation had exposure to paraquat treatment since the 1970s (Seng et al., 2010). In rigid ryegrass (*Lolilum rigidum* Gaud.), glufosinate resistance was confirmed in Greece with the level of resistance ranging from three-to seven-fold. It was also demonstrated that the level of glufosinate-resistance of rigid ryegrass was dependent on the growth stage at which it was applied (Travlos et al., 2018). In the case of an observed Italian ryegrass biotype (*Lolium perenne ssp. multiflorum*), glufosinate resistance was discovered in populations not previously exposed to the herbicide. Avila-Garcia and Mallory-Smith (2011) 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. No resistance to glufosinate in a broadleaf species has been reported to date (Heap, 2019).

In the case of AOPP ACCase inhibitor “fops” (i.e. quizalofop) herbicides, johnsongrass, Italian ryegrass, giant foxtail, cheatgrass and common wild oat have been reported to be resistant in the U.S. (Heap, 2019). Certain biotypes of johnsongrass, Italian ryegrass, giant foxtail and cheatgrass were confirmed to be resistant to “fops” and/or “dims”

ACCase inhibitor herbicides. ACCase-resistant johnsongrass biotypes in the U.S. were reported in 5 states (Mississippi, Kentucky, Tennessee, Virginia and Louisiana), with 2 of these states (Mississippi and Louisiana) having different biotypes that are resistant to glyphosate (Heap, 2019). In addition, ACCase-resistant Italian ryegrass biotypes in the U.S. were reported in 13 states and biotypes in California were also resistant to glyphosate (Heap, 2019). However, these weeds are controlled by using other effective herbicides such as photosystem I and II inhibitors, HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitors, long-chain fatty acid inhibitors or glufosinate and/or glyphosate on non-glyphosate-tolerant grasses depending upon the crop and the weed spectrum. See Table K-1 for other herbicide options in maize and its rotational crops to control "fops"-resistant grass weeds.

For glyphosate, there are 44 weed species reported to be resistant globally (Heap, 2019). In the U.S. there were 17 weed species reported to be glyphosate resistant, six of those being grass species (junglerice, goosegrass, Italian ryegrass, rigid ryegrass, annual bluegrass and johnsongrass) and 11 broadleaf weed species (Palmer amaranth, waterhemp, common ragweed, giant ragweed, kochia, spiny amaranth, horseweed, hairy fleabane, common sunflower, ragweed parthenium and Russian thistle) (Heap, 2019). Many of the glyphosate-resistant broadleaf weeds, particularly, Palmer amaranth, horseweed, waterhemp, common ragweed, giant ragweed and kochia were found in at least 10 states in the U.S (Heap and Duke, 2018). Palmer amaranth was predominantly found in the southern U.S. states and glyphosate-resistant Palmer amaranth biotypes have been found in 24 states in the U.S. (Heap and Duke, 2018; Heap, 2019). Furthermore, waterhemp was more commonly seen in northern U.S. states (Heap and Duke, 2018) with biotypes resistant to glyphosate reported in 18 states (Heap, 2019).

K.9. Mechanisms of Resistance and Inheritance of Resistance

To date, three basic mechanisms by which weed species develop resistance to an herbicide have been identified: 1) target site alteration (point mutations and/or increased expression), 2) enhanced metabolism of the herbicides (via chemical modification of the herbicide either by conjugation or degradation), and 3) reduced herbicide access to the site-of-action within the plant cell (exclusion) (Sammons et al., 2007; Sammons and Gaines 2014). Avoidance was proposed as an additional mechanism of resistance whereby weeds avoid toxic effect by its biochemical ability to handle the toxic agent produced by the herbicide (Sammons and Gaines 2014).

Over 200 distinct weed biotypes worldwide have developed herbicide resistance with the most common resistance mechanism occuring via target site alteration (Devine and Shukla, 2000). It has been found that target site resistance is the most common mechanism for ALS inhibitors, ACCase inhibitors, and triazines, but is less common for other herbicide groups, such as glyphosate and synthetic auxins (Devine and Shukla, 2000; Sammons et al., 2007; Sammons and Gaines, 2014; Powles and Yu, 2010; Busi et al 2017). 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 (Devine and Shukla, 2000). Recently five species (*Bassia scoparia*, *Amaranthus spinosus*, *Amaranthus palmeri*, *Amaranthus tuberculatus*, *Lolium perenne* ssp *multiflorum*) have managed to duplicate the EPSPS gene creating much higher expression levels of EPSPS protein (Sammons and Gaines, 2014; Koo et al., 2018a; Koo et al., 2018b).

In the case of synthetic auxin herbicides, there is limited evidence for any single mechanism conferring weed resistance and known evidence indicate different resistance mechanisms between weed species and/or biotypes within a weed species. Resistance to synthetic auxins has been speculated to be due to mutation(s) in genes encoding an auxin-binding protein, *ABP1* causing reduced herbicide binding or due to impaired auxin signal transduction pathway, including transport inhibitor response gene, *TIR1* (Zheng and Hall, 2001; Goss and Dyer, 2003; Grossmann, 2010). In several studies, differential herbicide absorption, translocation, and metabolism were ruled out as possible mechanisms of resistance to certain auxinic herbicides in kochia (Cranston et al., 2001) and in wild mustard (Zheng and Hall, 2001). In a recent study, it was demonstrated that a single amino acid change from glycine to asparagine in a conserved region within *KsIAA16* gene (a homologue of *IAA16* in arabidopsis) in a kochia biotype found in Nebraska, could result in cross-resistance between dicamba, 2,4-D and fluroxypyr herbicides (LeClere et al., 2018). This conserved protein domain in IAA16 was previously shown to be important for auxin binding and interaction between TIR1/F-box proteins and AUX/IAA in Arabidopsis (Tan et al., 2007). Pettinga et al., (2017) have shown that increased expression of *CHS* (*chalcone synthase*) gene in a resistant kochia biotype found in Nebraska (the same biotype studied by LeClere et al., 2018), co-segregated with dicamba resistance primarily by reducing dicamba translocation while no change was observed in herbicide uptake. The 2,4-D-resistant prickly lettuce biotype appears to have evolved resistance to other auxins in the phenoxy-carboxylate and benzoate subgroups, including MCPA and dicamba (Lyon and Burke 2016). Inheritance of 2,4-D resistance in prickly lettuce was governed by a single codominant gene and it was observed that the resistant biotype showed reduced translocation of 2,4-D in the treated leaf or crown but showed no difference in rate of 2,4-D metabolism compared to the susceptible biotypes (Riar et al 2011). 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. Jasieniuk et al. (1995) reported results indicating that inheritance of dicamba resistance in wild mustard is determined by a single, completely dominant nuclear allele. While wild mustard biotype was highly resistant to dicamba, it was moderately resistant to 2,4-D indicating differences in resistance phenotypes for herbicides within the synthetic auxin site-of-action (Zheng and Hall, 2001). However, Cranston et al. (2001) reported results indicating that dicamba resistance in kochia biotype (found in Montana) is 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 a quantitative trait (Cranston et al., 2001). It is also demonstrated that reduced

fitness due to the auxin resistance in the kochia biotype found in Nebraska may be partially responsible for the rarity of auxin resistant weeds (LeClere et al., 2018).

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 collected from Oregon 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. In another study conducted with an Italian ryegrass biotype resistant to glufosinate, it was shown that resistance to glufosinate was due to the target site mutation in plastidic *GS2* (*Glutamine Synthetase2*) gene causing a single amino acid change from asparagine to aspartic acid (Avila-Garcia et al., 2012). In a more recent study, Jalaludin et al. (2017) demonstrated that glufosinate resistance in resistant goosegrass population was not due to an insensitive glutamine synthase (GS), or increased GS activity, or altered glufosinate uptake and translocation, or enhanced glufosinate metabolism. They excluded the possibility of target-site resistance to glufosinate in goosegrass, however, the exact resistance mechanism(s) remain to be determined.

For ACCase inhibitors including “fops” and “dims” different patterns of resistance may be conferred by separate mutations in the gene for plastidic ACCase (Devine and Shulka 2000). A single modification (a single isoleucine-to-leucine substitution) confers cross-resistance to the “fops” and “dims” herbicides in wild oat (Christoffers et al., 2002), rigid ryegrass (Delye et al., 2002b; Tal and Rubin 2004; Zagnitko et al., 2001), green foxtail (Delye et al., 2002c), and blackgrass (*Alopecurus myosuroides* Huds.) (Delye et al., 2002a; Delye et al., 2002b). This isoleucine-to-leucine substitution at the 1781 position in *ACCase* gene in blackgrass confers resistance to certain “fops” (fenoxaprop and diclofop) and to “dims” (cycloxydim) and does not confer resistance to haloxyfop and clodinafop, nor to clethodim (Delye et al., 2002a; Delye et al., 2003b). A second substitution (an isoleucine-to-asparagine substitution) at position 2041 was associated with resistance to “fops” (fenoxaprop, diclofop, clodinafop and haloxyfop) but not “dims” (cycloxydim and clethodim) in blackgrass (Delye et al., 2003a). Both isoleucine-to-leucine substitution at the 1781 position and isoleucine-to-asparagine substitution at position 2041 in *ACCase* gene were dominant alleles (Delye et al., 2002a; Delye et al., 2003a) and arose independently in geographically distant blackgrass populations (Delye et al., 2003b). A recent study on large crabgrass (*Digitaria sanguinalis*) biotype collected from Ontario, Canada revealed that over expression of the ACCase enzyme conferred resistance to the ACCase inhibitor herbicides (both “fops” and “dims”) and that over expression of the ACCase enzyme in the resistant biotype was likely due to *ACCase* gene duplication (Laforest et al., 2017). Another large crabgrass biotype collected from South Australia that is resistant to “fops” (fluazifop, haloxyfop and quizalofop) and has reduced sensitivity to sethoxydim herbicides, was studied for mechanism of resistance to fluazifop. This study revealed that an enhanced metabolism of fluazifop acid conferred resistance to fluazifop herbicide rather than reduction in ACCase enzyme sensitivity, reduced absorption or translocation (Hidayat and Preston, 1997).

Studies of resistance to glyphosate have revealed a wider range of molecular mechanisms of resistance than are known for any other herbicide site-of-action (Sammons and Gaines, 2014), including herbicide sequestration in the vacuole, reduced translocation presumably due to a rapid cell death response, genetic polymorphisms in the herbicide target (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS) and increased copy number for the gene encoding EPSPS. It was further observed that resistance mechanism due to copy number of *epsps*, had two distinct molecular mechanisms, a tandem duplication in kochia (Jugulam et al., 2014) and a large extrachromosomal circular DNA (eccDNA) that is tethered to the chromosomes and passed to gametes at meiosis in Palmer amaranth (Koo et al., 2018a). Target-site mutations in *epsps* have been documented at amino acid position Pro106 (to primarily Ser, Thr or Ala) in few weed species including *Poa annua* (Cross et al., 2015), *Eleusine indica, Lolium rigidum, Lolium multiflorum, Echinochloa colona, Digitaria insularis, Amaranthus tuberculatus* (Sammons & Gaines, 2014), at Thr102Ser in *Tridax procumbens* (Li et al., 2018), at Thr102Ile and Pro106Ser in combination in *Eleusine indica* (Yu et al., 2015), and recently a triple mutation involving Thr102Ile, Ala103Val, and Pro106Ser in *Amaranthus hybridus* (Perotti et al., 2019). The simultaneous occurrence of multiple mutations within the same allele appears to be unique to glyphosate resistance and *epsps* (Gaines et al., 2019).

K.10. Sustainable Use of Dicamba, Glufosinate, Quizalofop, 2,4-D and Glyphosate as a Weed Management Option in Maize

Diversified use of herbicides with different and effective sites-of-action is one of the key weed management strategies to minimize selection for herbicide resistance. MON 87429 maize will likely be sold in maize hybrids that also contain other herbicide-tolerant traits, including glyphosate-tolerance. Maize hybrids containing both MON 87429 and glyphosate tolerance traits, such as NK603, will enable dicamba, glufosinate, quizalofop or 2,4-D herbicides to be applied with glyphosate and/or other maize herbicides depending upon the specific herbicide use labels and the weed spectrum in an integrated weed management program. Either dicamba or 2,4-D will likely be used in mixtures with glyphosate 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 translocated to growing parts of the plant) and reduced efficacy of glyphosate on susceptible weed species. Either dicamba or 2,4-D and glufosinate applications on MON 87429 maize 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. Either dicamba or 2,4-D and glufosinate (Ganie and Jhala, 2017; Joseph et al., 2018) will also help delay development and/or combat existing weed resistance issues that can limit the use of other herbicides such as glyphosate. Likewise, dicamba or 2,4-D with or without glyphosate (depending on the presence of glyphosate- resistant weeds) may help delay or minimize potential evolution of resistance to glufosinate in broadleaf species and similarly, glufosinate may delay or minimize the potential evolution of broadleaf species biotypes resistant to dicamba, 2,4-D or glyphosate (Ganie and Jhala, 2017; Joseph et al., 2018). In addition, residual herbicides also will be recommended for use, to provide early season weed

control and to supplement dicamba or 2,4-D and glufosinate activity on certain hard-to-control and glyphosate-resistant weed biotypes, such as glyphosate-resistant Palmer amaranth where weed population density can be very substantial. However, over-reliance on any herbicide(s) for weed control including dicamba, 2,4-D, glufosinate, "fops" or glyphosate should be avoided and a diversity of herbicide sites-of-action must be maintained by using herbicide tank-mixtures or premixes with multiple effective sites-of-action, along with non-chemical weed control methods including crop rotation, tillage, competitive cultivars, weed seed destruction, and cover crops, among others (Norsworthy et al., 2012; Ganie and Jhala, 2017).

MON 87429 maize in combination with a deregulated glyphosate tolerance trait, such as NK603, will also enable use of quizalofop to control a broad spectrum of grass weed species in addition to non-selective herbicides, glufosinate and glyphosate. Quizalofop can be used either in mixture with glyphosate or in sequence with glyphosate or glufosinate to provide options for postemergence control of grass weed species. Many mixtures of graminicides including ACCase herbicides with glufosinate have been demonstrated to exhibit antagonism (Gardener et al., 2006; Burke et al., 2005). Either quizalofop or glufosinate applications on MON 87429 maize can provide control of glyphosate-resistant grass weeds and improve the control of annual and perennial grass weed species, some of which are difficult to control with glyphosate. Combinations of these application options may help delay potential evolution of resistance to quizalofop, glufosinate or glyphosate in grass species.

Dicamba, glufosinate, quizalofop and 2,4-D, as complementary herbicides to glyphosate, will provide additional 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 would be complementary to and strengthen the utility and sustainability of dicamba, glufosinate, quizalofop or 2,4-D as weed control tools for the combined commercial hybrid product containing MON 87429 and a deregulated glyphosate tolerance trait, such as NK603.

In the event there is known or suspected resistance to dicamba, glufosinate, quizalofop, 2,4-D, or glyphosate, or other herbicides, cultural and mechanical weed control options are available to the grower in the U.S. for managing resistant biotypes. There are multiple preplant and postemergence herbicide options (including soil residuals) for managing weed populations that are resistant or may potentially develop resistance to dicamba, glufosinate, quizalofop, 2,4-D or glyphosate in maize, as well for crops grown in rotation with maize. A representative short list of the effective herbicide options are noted in Table K-1 for a sub-set of common weed species in maize in the U.S., chosen based on if a weed species has biotype(s) that are resistant to herbicide(s) tolerated by MON 87429 maize (dicamba, glufosinate, 2,4-D and/or "fops") and biotypes of these species reported to have resistance to glyphosate either as multiple resistance (kochia, Palmer amaranth, Italian ryegrass) or resistance to glyphosate exist separately in biotypes with high potential for biotypes with combined resistance development (waterhemp, johnsongrass) (Heap, 2019; see Table VIII-6 and Appendix K.8). Table K-1, however, is

not intended to provide extensive set of weed control recommendations for any specific biotype(s) with unique cases of resistance present in a geography (county or state) that may include resistance to herbicide site(s)-of-action not tolerated by MON 87429 maize. Geography- and biotype-specific weed control recommendations can be obtained through state Cooperative Extension Service (CES) and/or through state-specific weed control guides published by the universities, where available (e.g., Kansas State University, 2019, Mississippi State University, 2019; University of Nebraska, 2019).

Table K-1. Representative Management Recommendations for Control of Common Weeds in Maize with Biotypes Resistant to Herbicide(s) Tolerated by MON 87429 and to Glyphosate

Weed Species[1,2]	Primary Crop Maize	Rotational Crops			
		Soybeans	Cotton	Sorghum	Wheat
Kochia	Paraquat+ Atrazine[a] Isoxaflutole+ Atrazine[a] Thiencarbazone+ Tembotrione[a] Mesotrione[a]	Sulfentrazone+ Metribuzin[a] Flumioxazin+ Pyroxasulfone+ Chlorimuron[a] Saflufenacil+ Imazethapyr[a] Paraquat[a] Glufosinate[a,3]	Glufosinate[b,4]	Mesotrione+ s-Metolachlor + Atrazine[a] Atrazine[a] Acetochlor[a] Saflufenacil[a]	Carfentrazone+ 2,4-D[a] Triasulfuron+ Fluroxypyr[a] Pyrasulfotole+ Bromoxynil+ MCPA[a]
Tall Waterhemp	Topramezone[a] Saflufenacil[a] Isoxaflutole+ Atrazine[a] Mesotrione[a] Tembotrione[a] Glufosinate[a,4]	Sulfentrazone+ Metribuzin[a] Glyphosate+ Lactofen[a] Acetochlor[a] Flumioxazin[a] Fomesafen[a] Lactofen[a] Glufosinate[a,4]	Fomesafen[c] Diuron[c] Lactofen[c] Pyroxasulfone[c] +MSMA[c] Glufosinate[c,4]	Mesotrione+ s-Metolachlor + Atrazine[a] Saflufenacil[a] Acetochlor[a] Pyrasulfotole+ Bromoxynil[a]	Carfentrazone+ 2,4-D[a] Triasulfuron+ Fluroxypyr[a] Prosulfuron+ 2,4-D[a] Pyrasulfotole+ Bromoxynil+ 2,4-D[a]

Table K-1. Representative Management Recommendations for Control of Common Weeds in Maize with Biotypes Resistant to the Herbicide(s) Tolerated by MON 87429 Maize and to Glyphosate (continued)

Weed Species[1,2]	Primary Crop Maize	Rotational Crops			
		Soybeans	Cotton	Sorghum	Wheat
Palmer amaranth	Acetochlor+ Atrazine[d] Thiencarbazone+ Isoxaflutole[d] Tembotrione+ Thiencarbazone[d] Bromoxynil+ Atrazine[d] Glufosinate[c,4]	Sulfentrazone+ Imazethapyr[d] Flumioxazin+ Pyroxasulfone[d] Lactofen[d] Glufosinate[c,4]	Fomesafen[c] Diuron[c] Pyroxasulfone + Carfentrazone[c] Glyphosate+ s-Metolachlor [c] Glufosinate[c,4]	s-Metolachlor + Mesotrione[d] Bromoxynil+ Atrazine[d] Carfentrazone[d]	Triasulfuron[d] Thifensulfuron+ Tribenuron+ Metsulfuron+ Dicamba[d] Bromoxynil+ MCPA[d]
Italian ryegrass	Carfentrazone+ Pyroxasulfone[c] Acetochlor+ Atrazine[c] Glyphosate[c] Pyroxasulfone[c]	Parquat[c] Glufosinate[c,4] Paraquat + Metribuzin[c] Paraquat + Oxyfluorfen[c]	Paraquat[c] Paraquat + Atrazine[c] Glyphosate + Saflufenacil[c] Glufosinate[c,4]	Paraquat[c] Paraquat+ Atrazine[c] Glyphosate + Saflufenacil[c, 3]	Thifensulfuron + Tribenuron[c] Pyroxasulfone[c] Mesosulfuron[c]

Table K-1. Representative Management Recommendations for Control of Common Weeds in Maize with Biotypes Resistant to the Herbicide(s) Tolerated by MON 87429 Maize and to Glyphosate (continued)

Weed Species[1,2]	Primary Crop	Rotational Crops			
	Maize	Soybeans	Cotton	Sorghum	Wheat
Johnsongrass	Glyphosate + s-Metolachlor + Mesotrione[c] Mesotrione + Rimsulfurone[c] Nicosulfurone[c] Glufosinate[c,4]	Glyphosate + s-Metolachlor [c] Glufosinate[c,4]	Trifloxysulfuron[c] Trifluralin[c] Glyphosate + s-Metolachlor [c] Glufosinate[c,4]	Glyphosate + Carfentrazone[c,3] Glyphosate + Flumioxazin[c,3] Paraquat + Atrazine[c]	Glyphosate + Carfentrazone[c,3] Glyphosate + Saflufenacil[c,3] Paraquat[c]

[1] Representative common weed species in maize in the U.S., chosen based on if a weed species has biotype(s) that is resistant to herbicide(s) tolerated by MON 87429 maize (dicamba, glufosinate, 2,4-D and/or "fops") and biotypes of these species reported to have resistance to glyphosate either as multiple resistance (kochia, Palmer amaranth, Italian ryegrass) or resistance to glyphosate exist separately in biotypes with high potential for biotypes with combined resistance development (waterhemp, johnsongrass) (Heap, 2019; see Table VIII-6 and Appendix K.8).
[2] Herbicide(s) represent a sub-set of recommendations only; unique case(s) of weed resistance in a state to be managed through local extension scientists and/or university weed control guides, if available.
[3] Glyphosate applied as burndown in non-glyphosate tolerant crops.
[4] Glufosinate applied on glufosinate-tolerant crops.
[a] University of Nebraska, 2019; [b] Take Action, 2016; [c] Mississippi State University, 2019; [d] Kansas State University, 2019;

K.11. Stewardship of Dicamba, Glufosinate, Quizalofop, 2,4-D and Glyphosate Use on MON 87429 Maize

To steward the use of agricultural herbicides and herbicide-tolerant cropping systems such as MON 87429 maize and the likely combined trait glyphosate-tolerant maize product, Monsanto has conducted investigations and worked extensively with academics and other herbicide registrants 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 single herbicide for weed control without the use of other herbicides or cultural control methods (Beckie, 2006; Norsworthy et al. 2012).

K.11.1. Weed Control Recommendations

The proposed label for dicamba, and the labels for glufosinate, quizalofop, 2,4-D and glyphosate use on MON 87429 maize combined with deregulated glyphosate tolerance traits, such as NK603, are based on the maximum allowable use rates and patterns. Prior to launch of, for example, MON 87429 × NK603 maize, Monsanto, in cooperation with academics, will conduct trials to confirm the optimum rate and timing for dicamba, glufosinate, quizalofop, 2,4-D 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, research conducted by Monsanto showed the value of multiple soil-active preplant herbicides followed by an early postemergence application of a mixture of herbicides with multiple sites-of-action, including a soil active herbicide, against broadleaf weeds that included species in the genus *Amaranthus* (Bayer CropScience, 2019b). Such a program would optimize the likelihood of using two or more effective sites-of-action against the targeted weeds. In areas with glyphosate-resistant and hard to control broadleaf weed populations, dicamba or 2,4-D may be applied pre-planting as a tank mix with preemergence herbicides and/or applied post-emergence in addition to glufosinate in MON 87429 maize. In some situations, a second in-crop application of either dicamba or 2,4-D tank-mixed with glyphosate or glufosinate, with or without a soil residual herbicide will be recommended with options to rotate additional herbicides with diverse sites-of action between preplant and postemergence applications and as per the herbicide's label. For postemergence grass control, quizalofop application recommended alone or in combination with glyphosate or in sequence with glyphosate or glufosinate. In cases of managing both grass and broadleaf weed species, a time interval is to be considered between quizalofop application and any postemergence broadleaf herbicides as per the label recommendation (see Section VIII.F.3. for additional details).

These recommendations will encourage the use of more than one site-of-action against the targeted weed species, which is a fundamental component of a good weed resistance management program. These management systems, which include the use of multiple

effective herbicide sites-of-action, will reduce the potential for further resistance development to dicamba, glufosinate, 2,4-D, quizalofop and glyphosate as well as other critical maize herbicides.

K.11.2. 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 (Monsanto, 2019) will set forth the requirements and best practices for cultivation of MON 87429 maize including recommendations on weed resistance management practices. Growers who purchase maize hybrids containing MON 87429 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 87429 and public websites[13]. 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, glufosinate, quizalofop, 2,4-D and glyphosate 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 non-performance of dicamba, glufosinate, quizalofop, 2,4-D or glyphosate on weeds in fields planted with MON 87429 maize 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 site-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) Notices 2017-1 and 2017-2 (U.S. EPA, 2017b; 2017c). The PR notice 2017-1 (U.S. EPA, 2017b) is a revised and updated version of an earlier PR notice 2001-5 (U.S. EPA, 2001), with much of its content being kept identical to the 2001-5 with the following changes: (1) provided additional guidance to registrants and a recommended format for resistance-management statements or information to place on labels; (2) included references to external technical resources on resistance management guidance; and (3) updated the instructions to submit changes to existing labels in order to enhance resistance-management language. The PR notice 2001-5 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

[13] http://tug.monsanto.com/, https://monsanto.com/products/product-stewardship/, http://www.roundupreadyplus.com/

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.

In summary, Monsanto will require and educate weed resistance management practices through the Material Transfer Service Agreement (MTSA) and TUG for its biotechnology-derived herbicide-tolerant products, including MON 87429 maize. Upon deregulation, MON 87429 will be combined, via traditional breeding methods, with other maize traits (e.g., deregulated glyphosate tolerance traits, such as NK603), 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, quizalofop, 2,4-D and glyphosate.

K.11.3. Weed Resistance Management Practices

Monsanto will provide information to growers and grower advisors on best management practices to delay development of additional resistance to dicamba, glufosinate, quizalofop and 2,4-D herbicides. Weed resistance management recommendations for the use of dicamba, glufosinate, quizalofop, 2,4-D and glyphosate herbicides in conjunction with maize hybrids containing MON 87429 will be consistent with the Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2015). These guidelines recommend an integrated approach to weed resistance management, including cultural, mechanical and chemical methods that include the use of multiple herbicide sites-of-action to manage a weed population.

In cases where resistance is confirmed for dicamba, glufosinate, quizalofop, 2,4-D or glyphosate in maize producing areas, Monsanto and University/Cooperative Extension Service (CES) personnel will provide recommendations for alternative herbicide control methods to growers. These recommendations can be made available through various channels including Monsanto supplemental labels, Monsanto and university publications, and internet sites to growers, consultants, retailers and distributors. For all existing cases of dicamba-, glufosinate-, quizalofop-, or 2,4-D- 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 alternative herbicides from University/CES personnel that are applicable to weed species known to be resistant to dicamba and other synthetic auxin herbicides, glufosinate and "fops" herbicides are found in Table K-1. However, these examples in Table K-1 are only a representative subset of product combinations of available maize herbicides.

K.12. 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 herbicide product performance. Monsanto has an extensive

technical, sales and marketing presence in maize markets where MON 87429 maize 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 new dicamba, glufosinate, quizalofop, 2,4-D or glyphosate 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.

K.13. Summary

Development of herbicide resistance in weeds 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, glufosinate, quizalofop, 2,4-D and glyphosate herbicides to continue to be used effectively. In cases where weed populations have evolved or developed resistance to dicamba, glufosinate, quizalofop, 2,4-D and/or glyphosate, effective management options are available, and experience has shown that growers will continue to find value in using dicamba, glufosinate, quizalofop, 2,4-D and glyphosate in their weed control programs.

The key principles for effective stewardship of dicamba, glufosinate, quizalofop, 2,4-D and glyphosate use comprise: 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 sites-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

Overall, there are multiple factors that reduce the potential for dicamba or 2,4-D resistant broadleaf weed populations to arise from the use of dicamba or 2,4-D applied to MON 87429 combined with, for example, NK603. These are as follows:

- Dicamba or 2,4-D 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 weed control and use of additional sites-of-action. These use patterns provide herbicides with multiple sites-of-action against major broadleaf weed species present in the maize production to delay the development of herbicide 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 two and five broadleaf weed species have been confirmed to be resistant to dicamba and 2,4-D, respectively, in the U.S., and relatively low numbers of broadleaf species have been confirmed to be resistant to synthetic auxin herbicides even though dicamba and 2,4-D have been widely in use for over 40 years.
- Known resistant broadleaf weed populations to dicamba, 2,4-D and other auxin herbicides are primarily found in the western U.S. and, have limited presence in the major maize geographies. Biotypes of problematic weeds resistant to 2,4-D were found in key maize growing states in the U.S. (for example, biotypes of waterhemp in Nebraska and Illinois and Palmer amaranth in Kansas). Known dicamba-resistant biotypes are not of major weed species present in the U.S. maize cropping systems. To delay resistance development in broadleaf weeds against dicamba or 2,4-D in the MON 87429 maize system, glufosinate can be used in combination and/or in sequence with 2,4-D or dicamba and in sequence with glyphosate, as allowed by the individual herbicide label. Residual herbicides will also be recommended and likely used in this cropping system.

Likewise, the probability for weed species to evolve resistance to glufosinate as a result of glufosinate use in the MON 87429 combined with glyphosate tolerance traits, such as NK603, is considered to be low because, only four species have been confirmed to be resistant to glufosinate worldwide and one (Italian ryegrass) in the U.S. This suggests that the frequency for resistant alleles in native weed populations is low. Known Italian ryegrass biotypes resistant to glufosinate herbicide within the U.S. are only found in Oregon and California, and thus, are not present in the major maize geographies. In the MON 87429 maize system, glufosinate can be used in combination and/or in sequence

with dicamba, quizalofop and/or 2,4-D and in sequence with glyphosate, as allowed by the individual herbicide label. Residual herbicides will also be recommended and likely used in this cropping system.

Resistance to quizalofop was reported in only five grass species in the U.S. Combining MON 87429 maize with glyphosate-tolerance traits, such as NK603, will also enable use of quizalofop to control a broad spectrum of grass weed species in addition to non-selective herbicides, glufosinate and glyphosate. Combination of these options will help delay or minimize potential evolution of resistance to quizalofop, glufosinate or glyphosate in grass species.

In the U.S., although resistance to glyphosate was reported in 17 weed species (11 broadleaf and 6 grass weed species), it is critical to maintain the efficacy of glyphosate and delay further development of glyphosate-resistant weed biotypes. MON 87429 maize when combined with other deregulated glyphosate-tolerant trait such as NK603, provides additional tools with multiple herbicide sites-of-action to manage glyphosate-tolerant weed biotypes and delay further glyphosate resistance development.

As noted above, MON 87429 maize when combined with other deregulated glyphosate tolerance traits, such as NK603, enable use of herbicides or herbicide mixtures with multiple sites-of-action to control broadleaf and grass weed species present in the maize production and can be used as part of an integrated weed management program with other SOAs (including residuals) and other weed control practices to delay development of herbicide resistant weed population.

References for Appendix K

Andrew, I.K., J. Storkey and D.L. Sparkes. 2015. A review of the potential for competitive cereal cultivars as a tool in integrated weed management. Weed Research 55:239-248.

Avila-Garcia, W.V. and C. Mallory-Smith. 2011. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. Weed Science 59:305-309.

Avila-Garcia, W.V., E. Sanchez-Olguin, A.G. Hulting and C. Mallory-Smith. 2012. Target-site mutation associated with glufosinate resistance in Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*). Pest Management Science 68:1248-1254.

Bayer CropScience. 2016. Liberty® herbicide label. Bayer CropScience LP, Research Triangle Park, North Carolina.

Bayer CropScience. 2018. XtendiMax with VaporGrip Technology herbicide label. Bayer CropScience LP, St. Louis, Missouri.

Bayer CropScience. 2019b. Spray Early with Confidence Program. Roundup Ready Xtend Crop System. Bayer CropScience, St. Louis, Missouri.

Beckie, H. 2006. Herbicide-resistant weeds: Management tactics and practices. Weed Technology 20:793-814.

Beckie, H. and N. Harker. 2017. Our top 10 herbicide-resistant weed management practices. Pest Management Science, Vol 73:1045-1052.

Beckie, H and X. Reboud. 2009. Selecting for weed resistance: Herbicide rotation and mixture. Weed Technology 23:363-370.

Beckie, H. and F. Tardif. 2012. Herbicide cross resistance in weeds. Crop Protection 35: 15-28.

Burke, I, Askew. S, Corbett. J and J. Wilcut. 2005. Glufosinate antagonizes clethodim control of goosegrass (*Eleusine indica*). Weed Technology 19:664–668.

Busi, R., D.E. Goggin, I.M. Heap, M.J. Horak, N.M. Jugulam, R.A. Masters, R.M. Napier, D.S. Riar, N.M. Satchivi, J. Torra, P. Westra and T.R. Wright. 2017. Weed resistance to synthetic auxin herbicides. Pest Management Science 74:2265-2276.

Christoffers, M.J., M.L. Berg and C.G. Messersmith. 2002. An isoleucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. Genome 45(6):1049-1056.

Cranston, H. J., A. J. Kern, J. L. Hackett, E.K. Miller, B. D. Maxwell, and W. E. Dyer. 2001. Dicamba resistance in kochia. Weed Science 49:164-170.

Cross, R.B., L.B. McCarty, N. Tharayil, J. S. McElroy, S. Chen, P. McCullough, B.A. Powell and W.C. Bridges, Jr. 2015. A Pro106 to Ala substitution is associated with resistance to glyphosate in annual bluegrass (*Poa annua*). Weed Science 63:613–622.

Delye, C., E. Calmes and A. Matejicek. 2002a. SNP markers for black-grass (*Alopecurus myosuroides* Huds.) genotypes resistant to acetyl CoA-carboxylase inhibiting herbicides. Theoretical and Applied Genetics 104:1114-1120.

Delye, C., A. Matejicek and J. Gasquez. 2002b. PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Management Science 58:474-478.

Delye, C., T. Wang and H. Darmency. 2002c. An isoleucine-leucine substitution in choroplastic acetyl-CoA carboxlase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta 214:421-427.

Delye, C., X-Q, Zhang, C. Chalopin, S. Michel and S.B. Powles. 2003a. An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-CoA carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. Plant Physiology 132:1716–1723.

Delye, C., C. Straub, A. Matejicek and S. Michel. 2003b. Multiple origins for black-grass (*Alopecurus myosuroides* Huds) target-site-based resistance to herbicides inhibiting acetyl-CoA carboxylase. Pest Management Science 60:35-41.

Devine, M.D. and A. Shukla. 2000. Altered target sites as a mechanism of herbicide resistance. Crop Protection 19:881-889.

Dow. 2017. Enlist One herbicide label. AgroSciences LLC, Indianapolis, Indiana.

Duke, S.O. 2018. The history and current status of glyphosate. Pest Management Science 74:1027-1034.

Evans, J.A., P.J. Tranel, A.G. Hager, B. Schutte, C. Wu, L.A. Chatham and A.S. Davis. 2016. Managing the evolution of herbicide resistance. Pest Management Science 72: 74–80.

Gaines, T.A., E.L. Patterson and P. Neve. 2019. Molecular mechanisms of adaptive evolution revealed by global selection for glyphosate resistance. New Phytologist 223:1770-1775.

Ganie, Z. and A. Jhala. 2017. Interaction of 2,4-D or dicamba with glufosinate for control of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in glufosinate-resistant maize (*Zea mays* L.). Frontiers in Plant Science 8:1207.

Gardner, A.P., A.C. York, D.L. Jordan and D.W. Monks. 2006. Glufosinate antagonizes postemergence graminicides applied to annual grasses and johnsongrass. Journal of Cotton Science 10:319-327.

Goss, G.A., and W. E. Dyer. 2003. Physiological characterization of auxinic herbicide-resistant biotypes of Kochia (*Kochia scoparia*). Weed Science 51:839-844.

Gressel, J. and L. A. Segel. 1990. Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. Weed Technology 4:186-198.

Grossmann, K. 2010. Auxin herbicides: Current status of mechanism and mode of action. Pest Management Science 66:113-120.

Heap, I. 2019. International survey of herbicide resistant weeds. Corvallis, Oregon. http://www.weedscience.org/in.asp (Accessed August 26, 2019).

Heap, I. and S.O. Duke. 2018. Overview of glyphosate-resistant weeds worldwide. Pest Management Sciences 74:1040-1049.

Hidayat I. and C. Preston. 1997. Enhanced metabolism of Fluazifop acid in a biotype of *Digitaria sanguinalis* resistant to the herbicide Fluazifop-P-Butyl. Pesticide Biochemistry and Physiology 57:137-146.

HRAC. 2015. Guideline to the management of herbicide resistance. Herbicide Resistance Action Committee. http://www.hracglobal.com.

Jalaludin, A., Yu, Q., Zoellner, P., Beffa, R. and Powles, S.B. 2017. Characterisation of glufosinate resistance mechanisms in *Eleusine indica*. Pest Management Science. 73(6):1091-1100.

Jasieniuk, M., I.N. Morrison, and A.L. Brûlé-Babel. 1995. Inheritance of dicamba resistance in wild mustard (*Brassica kaber*). Weed Science 43:192-195.

Jasieniuk, M., A.L. Brûlé-Babel, and I.N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Science 44:176-193.

Joseph, D.D., Marshall, M.W. and Sanders, C.H. 2018. Efficacy of 2,4-D, dicamba, glufosinate and glyphosate combinations on selected broadleaf weed heights. American Journal of Plant Sciences 9: 1321-1333

Jugulam, M., K. Niehues, A.S. Godar, D.H. Koo, T. Danilova, B. Friebe, S. Sehgal, V.K. Varanasi, A. Wiersma, P. Westra, P.W. Stahlman and B.S. Gill. 2014. Tandem amplification of a chromosomal segment harboring 5-*enolpyruvylshikimate*-3-*phosphate synthase* locus confers glyphosate resistance in *Kochia scoparia*. Plant Physiology 166:1200-1207.

Kansas State University. 2019. Chemical weed control for field crops, pastures, rangeland, and noncropland. Kansas State University, Kansas State, Kansas.

Koo, D.-H., W.T. Molin, C.A. Saski, J. Jiang, K. Putta, M. Jugulam, B. Friebe and B.S. Gill. 2018a. Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in crop weed *Amaranthus palmeri*. Proceedings of the National Academy of Sciences of the United States of America 115:3332-3337.

Koo, D.-H., M. Jugulam, K. Putta, I.B. Cuvaca, D.E. Peterson, R.S. Currie, B. Friebe and B.S. Gill. 2018b. Gene duplication and aneuploidy trigger rapid evolution of herbicide resistance in common waterhemp. Plant Physiology 176:1932-1938.

Kraehmer, H., Almsick, A., Beffa, R., Dietrich, H., Eckes, P., Hacker, E., Hain, R., Strek, H., Stuebler, H. and Willms, L. 2014. Herbicides as weed control agents: State of the art: II. Recent achievements. Plant Physiology 166:1132–1148.

Laforest, M., B. Soufiane, M.J. Simard, K. Obeid, E. Page and R.E. Nurse. 2017. Acetyl-CoA carboxylase overexpression in herbicide-resistant large crabgrass (*Digitaria sanguinalis*). Pest Management Science 73:2227-2235.

Lagator, M., T. Vogwill, A. Mead, N. Colegrave and P. Neve. 2013. Herbicide mixtures at high doses slow the evolution of resistance in experimentally evolving populations of *Chlamydomonas reinhardtii*. New Phytologist 198:938-945.

LeClere, S., C. Wu, P. Westra and R.D. Sammons. 2018. Cross-resistance to dicamba, 2,4-D, and fluroxypyr in *Kochia scoparia* is endowed by a mutation in an *AUX/IAA* gene. Proceedings of the National Academy of Sciences of the United States of America115:E2911-E2920.

Li, J., Q. Peng, H. Han, A. Nyporko, T. Kulynych, Q. Yu and S. Powles. 2018. Glyphosate resistance in *Tridax procumbens* via a novel EPSPS Thr-102-Ser substitution. Journal of Agricultural and Food Chemistry 66:7880-7888.

Lyon, D.J. and I.C. Burke. 2016. Integrated management of prickly lettuce in wheat production systems. P.N.E. Publication. Washington State University.

Mantzos, N., A. Karakitsou, S. Nikolaki, E. Leneti and I. Konstaninou. 2016. Dissipation and transport of quizalofop-*p*-ethyl herbicide in sunflower cultivation under field conditions. Enviornmental Science and Pollution Research 23:3481-3490.

Miller, T.W., S.L. Shinn, and D.C. Thill. 2001. Cross-resistance in and chemical control of auxinic herbicide-resistant yellow starthistle (*Centaurea solstitialis*). Weed Technology 15:293-299.

Mississippi State University. 2019. Weed control guidelines for Mississippi 2019. Mississippi State University, Mississippi.

Monsanto. 2017. Roundup Power Max II herbicide label. Monsanto Company, St. Louis, Missouri.

Monsanto. 2019. U.S. Technology use guide. St. Louis, Missouri.

Norsworthy, J.K., S.M. Ward, D.R. Shaw, R.S. Llewellyn, R.L. Nichols, T.M. Webster, K.W. Bradley, G. Frisvold, S. B. Powles, N. R. Burgos, W.W. Witt, and M. Barrett. 2012. Reducing the risks of herbicide resistance: Best management practices and recommendations. Weed Science: Special Issue: 31-62.

Perotti, V.E., A.S. Larran, V.E. Palmieri, A.K. Martinatto, C.E. Alvarez, D. Tuesca and H.R. Permingeat. 2019. A novel triple amino acid substitution in the EPSPS found in a high-level glyphosate-resistant *Amaranthus hybridus* population from Argentina. Pest Management Science 75:1242-1251.

Pettinga, D.J., J. Ou, E.L. Patterson, M. Jugulam, P. Westra and T.A. Gaines. 2017. Increased chalcone synthase (CHS) expression is associated with dicamba resistance in *Kochia scoparia*. Pest Management Science 74:2306-2315.

Powles, S.B. 2008. Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. Pest Management Science 64:360-365.

Powles, S.B. and Qin Yu. 2010. Evolution in action: Plants resistant to herbicides. Annual Review of Plant Biology 61:317-347.

Riar, D.S., I.C. Burke, J.P. Yenish, J. Bell and K. Gill. 2011. Inheritance and physiological basis for 2,4-D resistance in prickly lettuce (*Lactuca serriola* L.). Journal of Agricultural and Food Chemistry 59:9417-9423.

Sammons, R.D., D.C. Heering, N. DiNicola, H. Glick, and G.A. Elmore. 2007. Sustainability and stewardship of glyphosate and glyphosate-resistant crops. Weed Technology 21:347-354.

Sammons, R. D. and T. A. Gaines. 2014. Glyphosate resistance: state of knowledge. Pest Management Science 70: 1367-1377.

Seng, C.T., L. Van Lun, C.T. San and I. Bin Sahid. 2010. Initial report of glufosinate and paraquat multiple resistance that evolved in a biotype of goosegrass (*Eleusine indica*) in Malaysia. Weed Biology and Management 10:229-233.

Take Action, 2016. Kochia management in soybean. https://iwilltakeaction.com/.

Tal, A. and B. Rubin. 2004. Molecular characterization and inheritance of resistance to ACCase-inhibiting herbicides in *Lolium rigidum*. Pest Management Science 60:1013-1018.

Tan, X., L.I.A. Calderon-Villalobos, M. Sharon, C. Zheng, C.V. Robinson, M. Estelle and N. Zheng. 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446:640-645.

Tehranchian, P., J.K. Norsworthy, S.B. Powles, M.T. Bararpour, M.V. Bagavathianan, T. Barber and R.C. Scott. 2017. Recurrent sublethal-dose selection for reduced susceptibility of Palmer amaranth (*Amaranthus palmeri*) to dicamba. Weed Science 65:206-212.

Travlos, I.S., Cheimona, N., De Prado, R., Jhala, A.J., Chachalis, D. and Tani, E. 2018. First case of glufosinate-resistant rigid ryegrass (*Lolium rigidum* Gaud.) in Greece. Agronomy 8(4), 35.

University of Nebraska. 2019. 2019 Guide for weed, disease and insect management in Nebraska. University of Nebraska, Nebraska.

U.S. EPA. 1989. Glufosinate. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2001. PRN 2001-5: Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2005. Reregistration eligibility decision for 2,4-D. Case No. 0073. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2009. Reregistration eligibility decision for dicamba and associated salts. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

U.S. EPA, 2017b. PRN 2017-1: Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2017c. PRN 2017-2: Guidance for herbicide-resistance management, labeling, education, training, and stewardship. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2018. Federal Register, 83 FR 7111-7115, Quizalofop ethyl; Pesticide Tolerances. U.S. Environmental Protection Agency, Washington, D.C.

Walsh, T.A., R. Neal, A.O. Merlo, M. Honma, G.R. Hicks, K. Wolff, W. Matsumura, and J.P. Davies. 2006. Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2,4-dichlorophenoxyacetic acid or indole-3-acetic acid in arabidopsis. Plant Physiology 142:542-552.

Wright, T.R., G. Shan, T.A. Walsh, J.M. Lira, C. Cui, P. Song, M. Zhuang, D.M. Arnold, G. Lin, K. Yau, S.M. Russell, R.M. Cicchillo, M.A. Peterson, D.M. Simpson, N. Zhou, J. Ponsamuel and Z. Zhang. 2010. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proceedings of the National Academy of Sciences of the United States of America 107:20240-20245.

WSSA. 2018. WSSA herbicide site-of-action (SOA) classification list.. Weed Science Society of America, Westminster, CO. http://wssa.net/wssa/weed/herbicides/.

WSSA. 2019. WSSA lesson module: Herbicide resistant weeds. Weed Science Society of America, Champaign, Illinois. http://wssa.net/wssa/weed/resistance/ (Accessed August 24, 2019).

Yu, Q., A. Jalaludin, H. Han, M. Chen, R.D. Sammons and S.B. Powles. 2015. Evolution of a double amino acid substitution in the 5-Enolpyruvylshikimate-3-Phosphate Synthase in *Eleusine indica* conferring high-level glyphosate resistance. Plant Physiology 167:1440-1447.

Zagnitko, O., J. Jelenska, G. Tevzadze, R. Haselkorn and P. Gornicki. 2001. An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxphenoxypropionate and cyclohexanedione inhibitors. PNAS 98:6617-6622.

Zheng, H. and J.C. Hall. 2001. Understanding auxinic herbicide resistance in wild mustard: physiological, biochemical, and molecular genetic approaches. Weed Science 49:276-281.

Appendix L: MON 87429 Exogenous Substrate Specificity

L.1. Purpose

The purpose of this study was to determine if MON 87429 FT_T enzyme could potentially bind and catabolize various herbicides applied exogenously. The five herbicides tested (Table L-1) were selected based on mode of action class and agronomic importance for controlling grassy weeds and volunteer corn. MON 87429 and the near isogenic conventional control, LH244 × HCL617, were planted in pots and randomly placed in a greenhouse. Fourteen (14) days after herbicide application, individual plants were visually rated for herbicide induced injury using a scale from 0 percent (no visible injury) to 100 percent (plant death). The results showed that MON 87429 had no damage when 2,4-D and quizalofop were tested as compared to the conventional control, but injury ratings for the other herbicides were similar between MON 87429 and the conventional control. Therefore, FT_T was able to reduce 2,4-D and quizalofop injury through catabolization of this herbicide, but is unlikely to have catabolized any of the other herbicides.

L.2. Materials

This study was conducted in a greenhouse at the Monsanto research facility in Chesterfield, MO in 2016. The starting seed for this study consisted of test material, MON 87429 (lot IHW300000879549985106881), and conventional maize control material, LH244 × HCL617 (lot IHW300000879550160526685), which has a genetic background similar to the test material.

The herbicides used in this study (Table L-1) are commercially available and were selected based on mode of action class and agronomic importance for controlling grassy weeds and volunteer corn. For each of the five herbicides applied, two application rates were used that are within the labeled range for each herbicide or known to cause injury to conventional maize.

L.3. Methods

The study was conducted in a greenhouse maintained at approximately 28° C during the day and 20° C during the night with a 16-hour photoperiod. Relative humidity was maintained between 30 and 70% while watering was conducted as needed.

The test and control starting seed were planted in 3.5 inch square pots with Redi-Earth® potting soil medium. Ten replicates of test and control seed were planted for each herbicide and rate combination and randomly placed in the greenhouse. At the 3-leaf growth stage, herbicide applications were made with a track sprayer according to standard practice. Visual injury ratings were assessed 14 days after herbicide application. A visual rating scale of 0-100% injury was used to rate the overall herbicide induced injury to each plant. A rating of 0% = no herbicide induced injury and 100% = plant death. Ratings were based on visual assessment of chlorosis, necrosis, malformation,

stunting, and biomass reduction. The ratings were averaged across 10 replications per treatment.

L.4. Results and Discussion

MON 87429 demonstrated no injury for 2,4-D and quizalofop when compared to the conventional control. MON 87429 and the conventional control exhibited similar injury ratings and therefore similar levels of susceptibility for all other herbicides tested (Table L-1). Clethodim, sethoxydim, and paraquat are important herbicides for grassy weed and volunteer maize control and will be effective in controlling volunteer MON 87429.

L.5. Conclusions

These results demonstrate that MON 87429 FT_T has a high specificity for 2,4-D and quizalofop as a substrate. Applications of various herbicides to MON 87429 and the near isogenic conventional control demonstrated that the rate of injury was similar for all of the herbicides tested, except 2,4-D and quizalofop, demonstrating only 2,4-D and quizalofop served as substrates for the FT_T present in MON 87429. Therefore, MON 87429 FT_T is specific for its substrates and did not catabolize other herbicides tested.

Table L-1 Induced Herbicide Injury to MON 87429 and Control

					Herbicide Induced Injury Ratings (%)[2]	
Formulation	Manufacturer	Herbicide	Labeled Rate Range[1] (g a.i./ha)	Rate Applied (g a.i./ha)	Control Mean (Range)[3]	MON 87429 Mean (Range)
Assure II®	DuPont	quizalofop	30.8-92.4	45	100 (100-100)	0 (0-0)
Assure II®	DuPont	quizalofop	30.8-92.4	90	100 (100-100)	0 (0-0)
2,4-D Amine 4	Helena	2,4-D	280-4480	840	30.5 (25-35)	0 (0-0)
2,4-D Amine 4	Helena	2,4-D	280-4480	1680	76.5 (50-90)	0 (0-0)
Select Max®	Valent	clethodim	110-280	140	100 (100-100)	99.9 (99-100)
Select Max®	Valent	clethodim	110-280	280	100 (100-100)	100 (100-100)
Poast®	BASF	sethoxydim	112-448	224	98.5 (98-100)	97.8 (95-99)
Poast®	BASF	sethoxydim	112-448	448	99.2 (98-100)	99.1 (98-100)
Gramoxone®	Syngenta	paraquat	340-530	265	49.0 (40-60)	47.5 (40-55)
Gramoxone®	Syngenta	paraquat	340-530	530	93.9 (80-100)	81.0 (70-90)

[1] Labeled rate range based on U.S. herbicide EPA approved labeled rates.
[2] Rated on a 0-100% injury scale where 0% = no herbicide induced injury and 100% = plant death. Ratings based on visual assessment of chlorosis, necrosis, malformation, stunting, and biomass reduction.
[3] Means calculated based on 10 replications per treatment.

Appendix M: Materials and Methods for Compositional Analysis of Maize MON 87429 Grain and Forage

Compositional comparisons between MON 87429 and the conventional control maize hybrid were performed using the principles and analytes outlined in the OECD consensus document for maize composition (OECD, 2002). These principles are accepted globally and have been employed previously in assessments of maize products derived through biotechnology. The compositional assessment was conducted on grain and forage samples harvested from multiple U.S. field sites during 2017 grown under typical agronomic field conditions practices.

M.1. Materials

Harvested grain and forage from MON 87429 and a conventional control that has similar genetic background to that of MON 87429 hybrid were compositionally assessed.

M.2. Characterization of the Materials

The identities of MON 87429 and the conventional control were confirmed prior to use in the compositional assessment.

M.3. Field Production of the Samples

Grain and forage samples were harvested from MON 87429 and the conventional control grown in the United States during the 2017 season. The field production was conducted at five sites. The field sites were planted in a randomized complete block design with four replicates per site. MON 87429 and the conventional control were grown under normal agronomic field conditions for their respective growing regions. MON 87429 plots were treated with dicamba, glufosinate, quizalofop and 2,4-D to generate samples under conditions of the intended use of the product.

Grain was harvested at physiological maturity and shipped at ambient temperature from the field sites to Monsanto Company. Forage was harvested at R5 and shipped on dry ice from the field sites to Monsanto Company. A subsample for compositional analysis was obtained from each tissue sample collected. These subsamples were ground and stored in a freezer set to maintain –20°C until their shipment on dry ice to Covance Laboratories Inc. (Madison, Wisconsin) for analysis.

M.4. Summary of Analytical Methods

Nutrients analyzed in this study included protein, total fat, ash, amino acids, linoleic acid, carbohydrates by calculation, fiber (acid detergent fiber (ADF), neutral detergent fiber (NDF)) in the grain, and protein, total fat, ash, carbohydrates by calculation and fiber (ADF and NDF) in the forage. The anti-nutrients assessed in grain included phytic acid and raffinose.

All compositional analyses were performed at Covance Laboratories, Inc. (Madison, Wisconsin). Methods for analysis were based on internationally-recognized procedures and literature publications. Brief descriptions of the methods utilized for the analyses are described below.

M.5. Acid Detergent Fiber

Sample aliquots were weighed into pre-weighed filter bags. Samples were placed in an ANKOM Fiber Analyzer and treated with an acid detergent solution containing sulfuric acid with cetyl trimethylammonium bromide, then filtered to remove proteins, starches, simple sugars, pectins, and ash. Fats and pigments were removed via an acetone wash leaving cellulose and lignin fractions. The remaining residue was the acid detergent fiber and was determined gravimetrically. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 1.00%.

M.6. Amino Acids

The following 10 amino acids were analyzed:

Total arginine	Total methionine
Total histidine	Total phenylalanine
Total isoleucine	Total threonine
Total leucine	Total tryptophan
Total lysine	Total valine

The samples were hydrolyzed in 6N hydrochloric acid for approximately 24 hours at approximately 106-118ºC. Tryptophan was hydrolyzed from proteins by heating at approximately 110ºC in 4.2N sodium hydroxide for approximately 20 hours.

The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were derivatized with o-phthalaldehyde (OPA) and the secondary amino acids were derivatized with fluorenylmethyl chloroformate (FMOC) before injection. The amino acids were quantified using external standards. The results are reported on a fresh weight basis. The limit of quantitation for this study was 0.100 mg/g.

Reference Standards:

Component	**Manufacturer**	**Lot No.**	**Purity** (%)
L-Arginine Monohydrochloride	Sigma-Aldrich	SLBP2435V	100
L-Histidine Monohydrochloride Monohydrate	Sigma-Aldrich	SLBQ0900V	100
L-Isoleucine	Sigma-Aldrich	SLBF7068V	100
L-Leucine	Sigma-Aldrich	SLBQ0257V	99
L-Lysine Monohydrochloride	Sigma-Aldrich	SLBL2974V	99
L-Methionine	Sigma-Aldrich	SLBL7822V	100
L-Phenylalanine	Sigma-Aldrich	MKBQ7887V	99
L-Threonine	Sigma-Aldrich	SLBR2767V	100
L-Valine	Sigma-Aldrich	SLBS4748	100
L-Tryptophan	Sigma-Aldrich	SLBR5743V	100

M.7. Ash

All organic matter was driven off when the samples were ignited at approximately 550ºC in a muffle furnace for at least 5 hours. The remaining inorganic material was determined gravimetrically and referred to as ash. The results are reported on a fresh weight basis. The limit of quantitation was 0.100%.

M.8. Carbohydrate

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

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

The limit of quantitation was calculated as 0.100% on a fresh weight basis.

M.9. Fat by Acid Hydrolysis

The samples were hydrolyzed with hydrochloric acid. The fat was extracted using ether and hexane. The extracts were dried down and filtered through a sodium sulfate column. The remaining extracts were then evaporated, dried, and weighed. The limit of quantitation was calculated as 0.100% on a fresh weight basis.

M.10. Fat by Soxhlet Extraction

The samples were weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the samples to remove the fat. The extract was then evaporated, dried, and weighed. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 0.100%.

M.11. Linoleic Acid

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The results were converted to their triglyceride equivalent and reported on a fresh weight basis. The Methyl Linoleate analytical reference standard was purchased from Nu-Chek Prep. The lot numbers were N25-Z, AU31-A, F1-B and AU31A with a purity of 99.8%. The limit of quantitation was calculated as 0.00220%.

M.12. Moisture

The samples were dried in a vacuum oven at approximately 100°C. The moisture weight loss was determined and converted to percent moisture. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 0.100%.

M.13. Neutral Detergent Fiber

Sample aliquots were weighed into pre-weighed filter bags. Samples were placed in an ANKOM Fiber analyzer and treated with a neutral detergent solution containing EDTA. Samples were then filtered to remove proteins, simple sugars, pectins, and ash. Fats and pigments were removed via an acetone wash leaving hemicellulose, cellulose, and lignin fractions. Starches were removed with a heat stable alpha amylase soak. The remaining residue was the neutral detergent fiber and was determined gravimetrically. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 1.00%.

M.14. Phytic Acid

The samples were extracted using hydrochloric acid and sonication, purified using a silica-based anion exchange column, concentrated and injected onto a high-performance liquid chromatography (HPLC) system with a refractive index detector. The Phytic Acid Sodium Salt Hydrate analytical reference standard was purchased from Sigma-Aldrich. The lot number was BCBQ7037V with a purity of 78.280%. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 0.125%.

M.15. Protein

The protein and other organic nitrogen in the samples were converted to ammonia by digesting the samples with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. Instrumentation was used to automate the digestion, distillation and titration processes. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 0.100%.

M.16. Raffinose

Sugars in the samples were extracted with a 50:50 water: methanol solution. Aliquots were taken, dried under inert gas, and then reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucopyranoside as the internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid treatment, and then analyzed by gas chromatography using a flame ionization detector. The D-(+)-Raffinose pentahydrate analytical reference standard was purchased from Sigma-Aldrich. The lot number was WXBB7085V with a purity of 99.7%. The results are reported on a fresh weight basis. The limit of quantitation was calculated as 0.0500%.

M.17. 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 the Monsanto Regulatory Statistics Team 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 M-1):

Table M-1. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates, Fiber, Phytic Acid, Raffinose	% fw	% dw	X/d
Amino Acids (AA)	mg/g fw	% dw	X/(10d)
Fatty Acids (FA), Linoleic Acid	% fw	% 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). No analytes with more than 50% of observations below the assay LOQ were observed.

The following linear mixed model was used for the combined-site analysis.

$$Y_{ijk} = \mu + S_i + R(S)_{j(i)} + M_k + (SM)_{ik} + \varepsilon_{ijk} \quad (1)$$

where:

Y_{ijk} is the observed response for the k_{th} substance in the j_{th} replicate of the i_{th} site;

μ is the overall mean;

S_i is the random effect of the i_{th} site;

$R(S)_{j(i)}$ is the random effect of the j_{th} replicate nested with the i_{th} site;

M_k is the fixed effect of the k_{th} substance;

$(SM)_{ik}$ is the random effect of the interaction between the i_{th} site and k_{th} substance;

ε_{ijk} is the residual error.

SAS PROC MIXED was used to fit model (1) separately for each component to conduct the statistical analysis. Studentized residuals were obtained to detect potential outliers in the dataset. Studentized residuals tend to have a standard normal distribution when

outliers are absent. Thus, most values are expected to be between ± 3. Data points that are outside of the ± 6 studentized residual ranges are considered as potential outliers. No value had a studentized residual outside of the ± 6 range for this study.

The linear mixed model (1) assumes that the experimental errors, ε_{ijk}, are independent, normally-distributed, and have a common variance. In this analysis, independence of the errors was controlled by the randomized complete block design. The normality and common variance assumptions were checked by visual examination of residual plots and histograms. No extreme violations were observed for any characteristic. Comparisons between MON 87429 and conventional control were defined within the model (1) and tested using t-tests.

References for Appendix M

OECD. 2002b. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients and secondary plant metabolites. OECD ENV/JM/MONO (2002)25. Organisation of Economic Co-Operation and Development, Paris, France.

Appendix N: Digestive Fate of the FT_T Protein

N.1. Digestive Fate of the FT_T Protein

As part of a comprehensive evaluation, introduced proteins in biotechnology-derived crops are evaluated for their safety for human and animal consumption. Proteins are an essential dietary component for humans and animals, and most are rapidly degraded to the component amino acids for nutritional purposes (Hammond and Jez, 2011). Although the vast majority of ingested proteins are non-allergenic, a small set of proteins or their fragments have been associated with a variety of gastrointestinal and systemic manifestations of immune-mediated allergy. One characteristic of several food allergens is their ability to withstand proteolytic digestion by enzymes present in the gastrointestinal tract (Astwood et al., 1996; Moreno et al., 2005; Vassilopoulou et al., 2006; Vieths et al., 1999), although exceptions to the correlation can be identified (Fu et al., 2002). The enzymatic degradation of an ingested protein by exposure to gastric pepsin and/or intestinal pancreatic proteases (e.g., pancreatin) makes it highly unlikely that either the intact protein or protein fragment(s) will reach the absorptive epithelial cells of the small intestine where antigen processing cells reside (Moreno et al., 2005). Therefore, the susceptibility of FT_T to the presence of pepsin was evaluated using an assay protocol that has been standardized based on results obtained from an international, multi-laboratory ring study (Thomas et al., 2004).The susceptibility of proteins in the presence of pancreatin has also been used as a separate test system to assess the digestibility of food components (Okunuki et al., 2002; Yagami et al., 2000). The relationship between protein allergenicity and protein susceptibility to pancreatin degradation is limited, because the protein has not been first exposed to the acidic, denaturing conditions simulating the stomach, as would be the case with in vivo digestion (FAO-WHO, 2001).

N.1.1. Degradation of FT_T Protein in the Presence of Pepsin

Degradation of the FT_T protein by pepsin was evaluated over time by analyzing digestion mixtures incubated for targeted time intervals following a standardized protocol validated in an international, multi-laboratory ring study (Thomas et al., 2004) collected at targeted incubation time points. The specific methods used to assess the digestive fate/degradation of the FT_T protein in pepsin are summarized below and detailed in Appendix N.2. The study showed that the results of *in vitro* pepsin digestion assays using this protocol were reproducible and consistent for determining the digestive susceptibility of a protein. This standardized *in vitro* pepsin digestion protocol utilized a physiologically relevant acidic buffer appropriate for pepsin activity. The susceptibility of FT_T protein to pepsin degradation was assessed by visual analysis of a Brilliant Blue G Colloidal stained SDS-PAGE gel and by visual analysis of a western blot probed with an anti-FT_T polyclonal antibody. Both visualization methods were run concurrently with separate SDS-PAGE and western blot analyses to estimate the limit of detection (LOD) of the FT_T protein for each method.

For SDS-PAGE analysis of the digestibility of the FT_T protein in pepsin, the gel was loaded with 1 μg of total test protein (based on pre-digestion protein concentrations) for each of the digestion samples (Figure N-1, Panel A). The SDS-PAGE gel for the digestibility assessment was run concurrently with a separate SDS-PAGE gel to estimate the LOD of the FT_T protein (Figure N-1, Panel B). The LOD of intact FT_T protein was approximately 3.1 ng (Figure N-1, Panel B, lane 8). Visual examination of SDS-PAGE data showed that the intact FT_T protein was digested within 0.5 min of incubation in pepsin (Figure N-1, Panel A, lane 5). Therefore, based on the LOD, more than 99.7% (100% - 0.3% = 99.7%) of the intact FT_T protein was digested within 0.5 -min of incubation in pepsin. Transiently-stable peptide fragments at ~4-kDa were observed throughout the course of the digestion. There is a slight decrease in the intact FT_T protein band intensity and generation of fragments greater than 20 kDa observed in the 60 min No Pepsin Control compared to the 0 min No Pepsin Control (Figure N-1, Panel A, lanes 12 and 3, respectively). This likely indicates that a small portion of FT_T protein is instable possibly due to either the acidic conditions or minor contaminants in the FT_T solution; however, the rapid degradation of the intact FT_T protein and the subsequent fragments in the pepsin degradation samples was due to the proteolytic activity of pepsin.

The 0 min No Test Protein Control and 60 min No Test Protein Control (Figure N-1, Panel A, lanes 2 and 13) demonstrated that the pepsin is stable throughout the experimental phase.

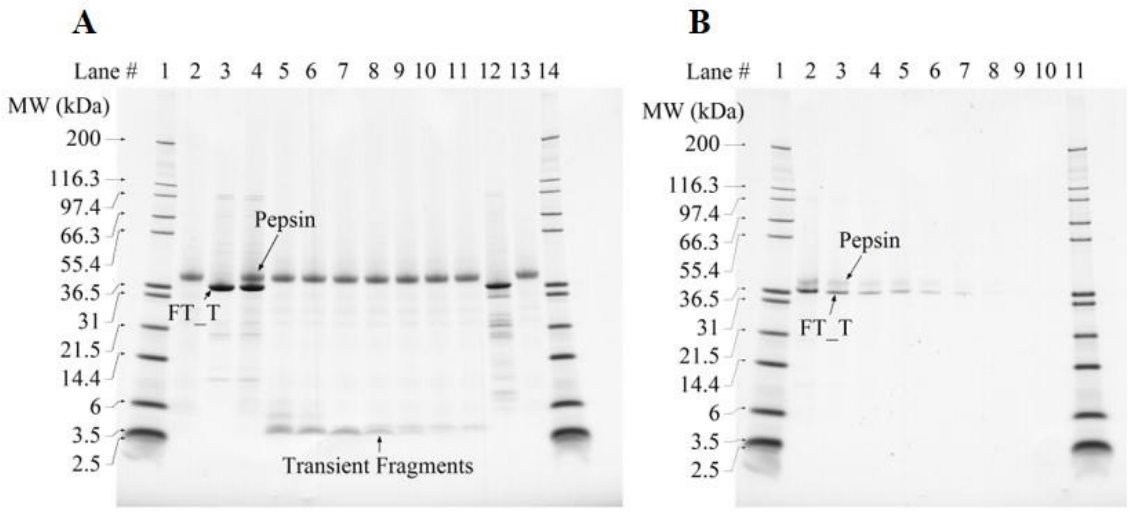


Figure N-1. SDS-PAGE Analysis of the Degradation of FT_T Protein by Pepsin

Colloidal Brilliant Blue G stained SDS-PAGE gels were used to assess the degradation of FT_T protein by pepsin. Molecular weights (kDa) are shown on the left of each gel and correspond to the markers loaded. In each gel, the FT_T protein migrated to approximately 35 kDa and pepsin to approximately 38 kDa. Empty lanes and molecular weight markers that were not visible on the film were cropped from the images.

A: FT_T protein degradation in the presence of pepsin. Based on pre-reaction protein concentrations, 1 µg of test protein was loaded in each lane containing FT_T protein.

B: LOD determination. Indicated amounts of the test protein from the Pepsin Treated T0 sample were loaded to estimate the LOD of the FT_T protein.

Lane	Sample	Incubation Time (min)
1	Mark12 MWM	-
2	0 min No Test Protein Control	0
3	0 min No Pepsin Control	0
4	Pepsin Treated T0	0
5	Pepsin Treated T1	0.5
6	Pepsin Treated T2	2
7	Pepsin Treated T3	5
8	Pepsin Treated T4	10
9	Pepsin Treated T5	20
10	Pepsin Treated T6	30
11	Pepsin Treated T7	60
12	60 min No Pepsin Control	60
13	60 min No Test Protein Control	60
14	Mark12 MWM	-
15	Empty	-

Lane	Sample	Amount (ng)
1	Mark12 MWM	-
2	Pepsin Treated T0	200
3	Pepsin Treated T0	100
4	Pepsin Treated T0	50
5	Pepsin Treated T0	50
6	Pepsin Treated T0	25
7	Pepsin Treated T0	12.5
8	Pepsin Treated T0	3.1
9	Pepsin Treated T0	1.6
10	Pepsin Treated T0	0.8
11	Mark12 MWM	-
12	Empty	-
13	Empty	-
14	Empty	-
15	Empty	-

For western blot analysis of FT_T pepsin susceptibility, the FT_T protein was loaded with approximately 40 ng per lane of total protein (based on pre-reaction total protein concentrations) for each reaction time point examined. The western blot used to assess FT_T protein degradation (Figure N-2, Panel A) was run concurrently with the western blot used to estimate the LOD (Figure N-2, Panel B). The LOD of the FT_T protein was approximately 0.31 ng (Figure N-2, Panel B, Lane 10). Western blot analysis demonstrated that the intact FT_T protein was degraded below the LOD within 0.5 min of incubation in the presence of pepsin (Figure N-2, Panel A, Lane 6). Based on the western blot LOD for the FT_T protein, more than 99.2% (100% - 0.8% = 99.2%) of the intact FT_T protein was degraded within 0.5 min. No peptide fragments were detected at the 0.5 min and beyond time points in the western blot analysis.

No change in the intact FT_T protein band intensity were observed in the 0 min No Pepsin Control and the 60 min No Pepsin Control. There were three antibody-recognized fragments greater than 20 kDa observed in the 60 min No Pepsin Control that were not observed in the 0 min No Pepsin Control (Figure N-2, Panel A, lanes 13 and 4, respectively). This indicates that small portion of the FT_T protein is slightly instable possibly due to either the acidic conditions or minor contaminants in the FT_T solution; however, a majority of the degradation of the intact FT_T protein and the subsequent fragments in the pepsin degradation samples was due to the proteolytic activity of pepsin. The transiently-stable fragments at ~4 kDa that were observed by SDS-PAGE were not recognized by the antibody used in this western blot.

No immunoreactive bands were observed in 0 min No Protein Control and 60 min No Protein Control (Figure N-2, Panel A, lanes 3 and 14). This result indicates that there was no non-specific interaction between the pepsin solution and the FT_T-specific antibody under these experimental conditions.

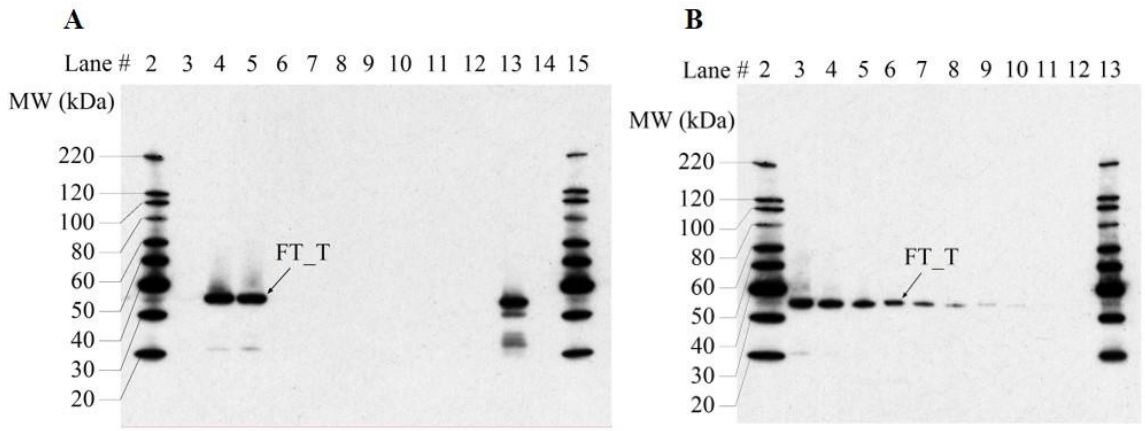


Figure N-2. Western Blot Analysis of the Degradation of FT_T Protein by Pepsin

Western blots probed with an anti-FT_T antibody were used to assess the degradation of FT_T by pepsin. Molecular weights (kDa) are shown on the left of each gel and correspond to the MagicMark™ molecular weight marker loaded in two lanes of each gel. Empty lanes and molecular weight markers that were not visible on the film were cropped from the images. A 30 sec exposure is shown.

A: FT_T protein degradation by pepsin. Based on pre-reaction protein concentrations, 40 ng of test protein was loaded in each lane containing FT_T protein.

B: LOD determination. Indicated amounts of the test protein from the Pepsin Treated T0 sample were loaded to estimate the LOD of the FT_T protein.

Lane	Sample	Incubation Time (min)
1	Precision Plus MWM	-
2	MagicMark MWM	-
3	0 min No Test Protein Control	0
4	0 min No Pepsin Control	0
5	Pepsin Treated T0	0
6	Pepsin Treated T1	0.5
7	Pepsin Treated T2	2
8	Pepsin Treated T3	5
9	Pepsin Treated T4	10
10	Pepsin Treated T5	20
11	Pepsin Treated T6	30
12	Pepsin Treated T7	60
13	60 min No Pepsin Control	60
14	60 min No Test Protein Control	60
15	MagicMark MWM	-

Lane	Sample	Amount (ng)
1	Precision Plus MWM	-
2	MagicMark MWM	-
3	Pepsin Treated T0	40
4	Pepsin Treated T0	20
5	Pepsin Treated T0	10
6	Pepsin Treated T0	5
7	Pepsin Treated T0	2.5
8	Pepsin Treated T0	1.25
9	Pepsin Treated T0	0.63
10	Pepsin Treated T0	0.31
11	Pepsin Treated T0	0.16
12	Pepsin Treated T0	0.08
13	MagicMark MWM	-
14	Precision Plus MWM	-
15	Empty	-

N.1.2. Degradation of FT_T Protein in the Presence of Pancreatin

The degradation of the FT_T protein by pancreatin was assessed by western blot analysis (Figure N-3). The western blot used to assess the FT_T protein degradation (Figure N-3, Panel A) was run concurrently with the western blot used to estimate the LOD (Figure N-3, Panel B) of the FT_T protein. The LOD of the FT_T protein was observed at approximate 0.31 ng protein loading (Figure N-3, Panel B, lane 10). The LOD was used to calculate the maximum relative amount of FT_T protein that could remain visually undetected after digestion, which corresponded to approximately 0.8% of the total protein loaded.

The gel used to assess degradation of the FT_T protein by western blot was loaded with approximately 40 ng per lane of total protein (based on pre-reaction protein concentrations) for each reaction time point examined. Western blot analysis demonstrated that a band corresponding to the FT_T protein was degraded to a level below the LOD within 5 minutes of incubation with pancreatin (Figure N-3, Panel A, lane 5), the first time point assessed. Therefore, based on the LOD, more than 99.2% (100% - 0.8% = 99.2%) of the FT_T protein was digested within 5 minutes. No peptide fragments were detected at the 5 min and beyond time points in the western blot analysis.

No obvious change in the intact FT_T (~35.5 kDa) band intensity was observed in the absence of pancreatin in the 0 min No Pancreatin Control and 24 hour No Pancreatin Control (Figure N-3, Panel A, lanes 3 and 13). This indicates that the degradation of all immunoreactive forms of the FT_T protein was due to the proteolytic activity of pancreatin and not due to instability of the protein when incubated in 50 mM KH_2PO_4, pH 7.5 over the course of the experiment.

No immunoreactive bands were observed in the 0 min No Test Protein Control and 24 hour No Test Protein Control (Figure N-3, Panel A, lanes 2 and 14), demonstrating the absence of non-specific antibody interactions with the pancreatin solution.

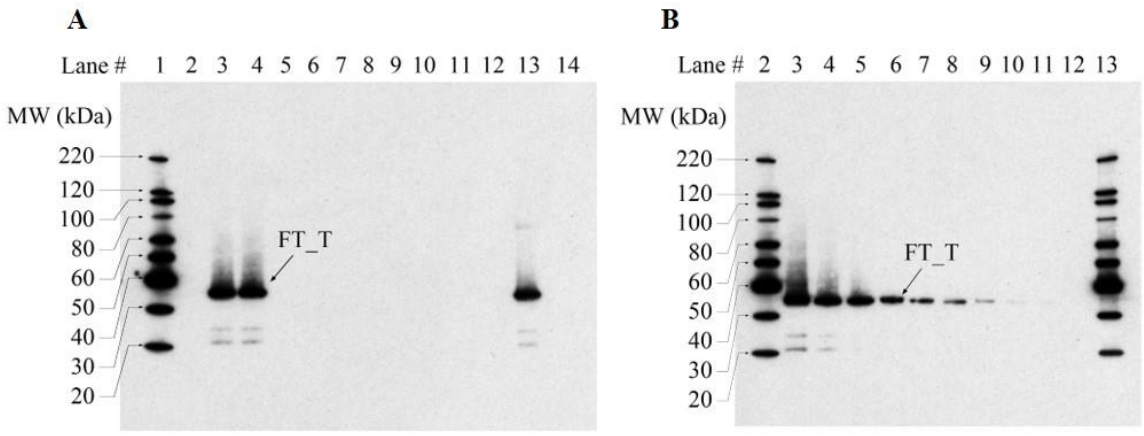


Figure N-3. Western Blot Analysis of the Degradation of FT_T Protein by Pancreatin

Western blots probed with an anti-FT_T antibody were used to assess the degradation of FT_T by pancreatin. Molecular weights (kDa) are shown on the left of each gel and correspond to the MagicMark™ molecular weight marker loaded in each gel. Empty lanes and molecular weight markers that were not visible on the film were cropped from the images. A 60 sec exposure is shown.

A: FT_T protein degradation by pancreatin. Based on pre-reaction protein concentrations, 40 ng of test protein was loaded in each lane containing FT_T protein.

B: LOD determination. Indicated amounts of the test protein from the Pancreatin Treated T0 sample were loaded to estimate the LOD of the FT_T protein.

Lane	Sample	Incubation Time
1	MagicMark MWM	-
2	0 min No Test Protein Control	0
3	0 min No Pancreatin Control	0
4	Pancreatin Treated T0	0
5	Pancreatin Treated T1	5 min
6	Pancreatin Treated T2	15 min
7	Pancreatin Treated T3	30 min
8	Pancreatin Treated T4	1 h
9	Pancreatin Treated T5	2 h
10	Pancreatin Treated T6	4 h
11	Pancreatin Treated T7	8 h
12	Pancreatin Treated T8	24 h
13	24 h No Pancreatin Control	24 h
14	24 h No Test Protein Control	24 h
15	Precision Plus MWM	-

Lane	Sample	Amount (ng)
1	Precision Plus MWM	-
2	MagicMark MWM	-
3	Pancreatin Treated T0	40
4	Pancreatin Treated T0	20
5	Pancreatin Treated T0	10
6	Pancreatin Treated T0	5
7	Pancreatin Treated T0	2.5
8	Pancreatin Treated T0	1.25
9	Pancreatin Treated T0	0.63
10	Pancreatin Treated T0	0.31
11	Pancreatin Treated T0	0.16
12	Pancreatin Treated T0	0.08
13	MagicMark MWM	-
14	Precision Plus MWM	-
15	Empty	-

N.1.3. Degradation of FT_T Protein by Pepsin Followed by Pancreatin

To better understand the fate of the transiently-stable peptide fragments at ~4 -kDa that were observed in the reaction mixtures throughout the course of the pepsin digestion of FT_T, sequential digestibility of the FT_T protein was conducted. This sequential digestibility was assessed both by visual analysis of a Colloidal Brilliant Blue G stained SDS-PAGE gel, and visual analysis of a western blot probed with an anti FT_T-polyclonal antibody. Methods for the sequential digestibility are provided in Appendix E.

For the sequential degradation assay, the FT_T protein was incubated with pepsin for 2 min, followed by incubation with pancreatin. For the Colloidal Brilliant Blue G stained SDS-PAGE assessment, the gel was loaded with 1 µg of FT_T protein (based on pre-digestion protein concentrations) for each of the digestion samples. Examination of SDS-PAGE data showed that the intact FT_T protein was digested within 2 min of incubation in pepsin (Figure N-4, Panel A, lane 3) and the small transient fragments at ~4 kDa was completely digested within 0.5 min of pancreatin exposure (Figure N-4, Panel A, lane 7).

No change in the fragment band intensities was observed in the absence of pancreatin in the SEQ 0 min No Pancreatin Control and SEQ 2 hour No Pancreatin Control (Figure N-4, Panel A, lanes 5 and 14). This indicates that the digestion of the fragments was due to the proteolytic activity of pancreatin and not due to instability of the fragment when incubated in 50 mM KH_2PO_4 at pH 7.5 over the course of the experiment.

The SEQ 0 min No Test Protein Control and SEQ 2 hour No Test Protein Control (Figure N-4, Panel A, lanes 4 and 15) demonstrated the integrity of the pancreatin over the course of the experiment. The intensity of some pancreatin bands decreased somewhat during the course of the experiment, most likely due to auto-digestion. This is not expected to adversely impact the pancreatin degradation results, as the transiently stable fragments were digested within 0.5 min of exposure to pancreatin.

The sequential digestion of the FT_T protein was also assessed by western blot (Figure N-4, Panel B), with 40 ng of the test protein (based on total protein pre-digestion concentrations) loaded per lane. No bands were detected in the 2 min Pepsin Treated sample (Figure N-4, Panel B, lane 3).

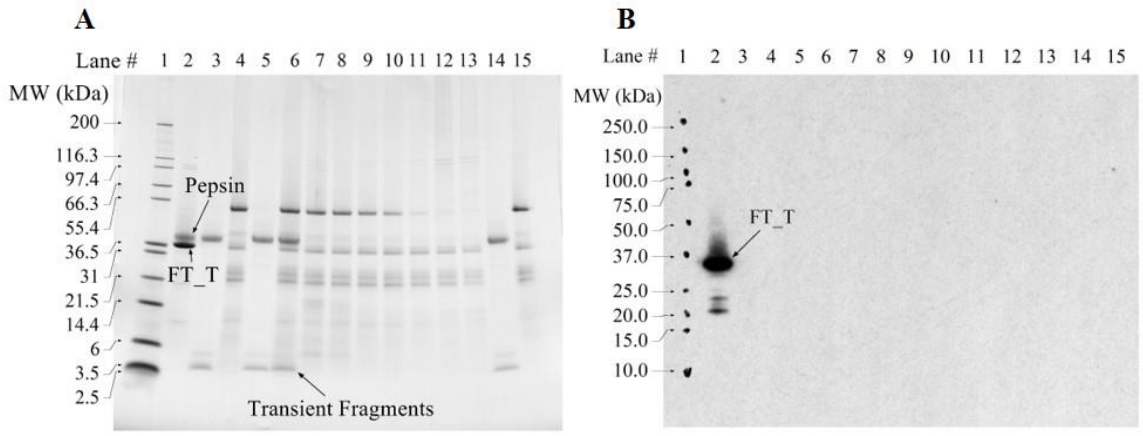


Figure N-4. SDS-PAGE and Western Blot Analysis of the Degradation of FT_T Protein by Sequential Digestion

SDS-PAGE and western blot analysis were used to assess the degradation of FT_T in sequential digestion. Molecular weights (kDa) are shown on the left of each gel and correspond to the markers loaded (cropped in panel B).

A: Colloidal Brilliant Blue G stained SDS-PAGE gel analysis of FT_T in sequential digestion. Based on pre-digestion protein concentrations, 1 µg of test protein was loaded in each lane containing FT_T protein.

B: Western blot analysis of FT_T in sequential digestion. Based on pre-digestion protein concentrations, 40 ng of test protein was loaded in each lane containing FT_T protein. A 60 sec exposure is shown.

Lane	Sample	Incubation Time
1	Mark12 MWM	-
	Pepsin Degradation	
2	0 min Pepsin Treated	0 min
3	2 min Pepsin Treated	2 min
	Pancreatin Degradation	
4	SEQ 0 min No Test Protein Control	0 min
5	SEQ 0 min No Pancreatin Control	0 min
6	SEQ T0	0 min
7	SEQ T1	0.5 min
8	SEQ T2	2 min
9	SEQ T3	5 min
10	SEQ T4	10 min
11	SEQ T5	30 min
12	SEQ T6	1 h
13	SEQ T7	2 h
14	SEQ 2 h No Pancreatin Control	2 h
15	SEQ 2 h No Test Protein Control	2 h

Lane	Sample	Incubation Time
1	Precision Plus MWM	-
	Pepsin Degradation	
2	0 min Pepsin Treated	0 min
3	2 min Pepsin Treated	2 min
	Pancreatin Degradation	
4	SEQ 0 min No Test Protein Control	0 min
5	SEQ 0 min No Pancreatin Control	0 min
6	SEQ T0	0 min
7	SEQ T1	0.5 min
8	SEQ T2	2 min
9	SEQ T3	5 min
10	SEQ T4	10 min
11	SEQ T5	30 min
12	SEQ T6	1 h
13	SEQ T7	2 h
14	SEQ 2 h No Pancreatin Control	2 h
15	SEQ 2 h No Test Protein Control	2 h

N.1.4. Degradation of FT_T Protein Conclusion

The ability of FT_T protein to be degraded by pepsin and by pancreatin was evaluated in this study. The results showed that at least 99.7% of the intact FT_T protein was degraded by pepsin within 0.5 min when analyzed by SDS-PAGE and at least 99.2% of the intact FT_T was degraded by pepsin within 0.5 min when analyzed by western blot using a FT_T specific antibody. SDS-PAGE analysis showed that transient peptide fragments at ~4 kDa were observed throughout the course of the pepsin digestion. At least 99.2% of the intact FT_T protein was degraded by pancreatin within 5 min when analyzed by western blot. These results show that the full-length FT_T is rapidly degraded by pepsin and pancreatin. The transient fragments at ~4 kDa were rapidly degraded by sequential digestion, indicating that gastrointestinal digestion is sufficient to degrade the intact FT_T protein and any fragments thereof. Rapid and complete degradation of the FT_T protein by pancreatin alone and pepsin followed by pancreatin indicates that the FT_T protein poses no meaningful risk to human or animal health.

N.2. Materials and Methods Used in Assessing Stability of FT_T Proteins in Pepsin and Pancreatin

N.2.1. Test Substance

The test substance was the FT_T protein (lot # 11478164) purified from *Escherichia coli* (*E. coli*) transformed with the pMON374248 plasmid (*E. coli*-produced FT_T, referred to in this document as FT_T protein). The FT_T protein is stored in a -80 °C freezer in a buffer solution containing 40 mM Tris, pH 8, 0.2 M NaCl (storage buffer).

N.2.2. Characterization of Test Substance

The test protein (lot 11478164) had a purity of 92%, a total protein concentration of 4.8 mg/ml, and an apparent molecular weight of 35.5 kDa.

N.3. Experimental Design

Assays designed to assess the degradation of FT_T protein by pepsin and by pancreatin were performed independently. Because protein fragments attributable to the FT_T protein were identified after 10 min of pepsin degradation, a sequential digestion of the FT_T protein with pepsin followed by pancreatin was also performed.

N.3.1. Preparation of the Pepsin Stock Solution

High purity pepsin (Sigma catalog number P6887; specific activity of 3546 U/mg, 96% protein, purity-corrected specific activity of 3404 U/mg) was dissolved in 10 mM HCl, 2 mg/ml NaCl, pH ~1.2 to a concentration of ~2,632 U/ml. The amount of powder used to prepare the pepsin stock solution was calculated by using the purity-corrected pepsin specific activity value of 3404 U/mg. Activity was assessed using a pepsin activity assay (Section N.4.1).

N.3.1.1. Degradation of the FT_T Protein by Pepsin

Degradation of the FT_T protein by pepsin was evaluated over time by analyzing samples collected at targeted incubation time points. A numerical code using the numbers 0 through 7 was used to distinguish incubation time points as follows:

Targeted Incubation Time Point	Sample Code
0 min	0 min No Test Protein Control
0 min	0 min No Pepsin Control
0 min	Pepsin Treated T0
0.5 min	Pepsin Treated T1
2 min	Pepsin Treated T2
5 min	Pepsin Treated T3
10 min	Pepsin Treated T4
20 min	Pepsin Treated T5
30 min	Pepsin Treated T6
60 min	Pepsin Treated T7
60 min	60 min No Pepsin Control
60 min	60 min No Test Protein Control

The reaction mixture was prepared by adding 911.9 µl of pre-heated (37.2ºC, 10 min) pepsin stock solution to a tube containing 50 µl of FT_T protein, which corresponded to 240 µg of FT_T protein and 2400 U of pepsin. The tube contents were mixed and immediately returned to the water bath (37.1ºC). Samples (96.2 µl) were removed at 0.5, 2, 5, 10, 20, 30 and 60 min (corresponding to Pepsin Treated T1 through Pepsin Treated T7). Each 96.2 µl sample was placed immediately in a tube containing quenching mixture consisting of 33.7 µl of 0.7 M sodium carbonate buffer and 32.5 µl of 5× loading buffer (5× LB; ~310 mM Tris-HCl, 25% (v/v) 2-mercaptoethanol, 10% (w/v) sodium dodecyl sulfate, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8).

The Pepsin Treated T0 sample was prepared in a separate tube. The pepsin stock solution (91.2 µl) was quenched by the addition of 0.7 M sodium carbonate buffer (33.7 µl) and 5× LB (32.5 µl) and heated at 95-100 ºC for 5-10 min prior to the addition of the FT_T protein (5.0 µl).

All quenched samples were frozen on dry ice and stored in a 80 ºC freezer until analysis.

N.3.1.2. Pepsin Degradation Experimental Controls

Experimental control samples were prepared to determine the stability of the FT_T protein in 10 mM HCl, 2 mg/ml NaCl, pH ~1.2, the solution used to prepare the pepsin stock solution. The 0 min No Pepsin Control was prepared in a similar manner as described in Section N.4.1 for Pepsin Treated T0, except that 10 mM HCl, 2 mg/ml NaCl, pH 1.2 was added instead of the pepsin stock solution. The 60 min No Pepsin Control was prepared in a manner similar to 0 min No Pepsin Control, except the protein and

10 mM HCl, 2 mg/ml NaCl, pH 1.2 were incubated for 60 min at 37.1ºC before quenching with 0.7 M sodium carbonate buffer and 5× LB.

Experimental control samples were also prepared to determine the stability of the pepsin stock solution lacking the FT_T protein. The 0 min No Test Protein Control was prepared in a similar manner as described in Section N.4.1 for Pepsin Treated T0, except that protein storage buffer (40 mM Tris, pH 8, 0.2 M NaCl) was added in place of the FT_T protein. The 60 min No Test Protein Control was prepared in a manner similar to 0 min No Test Protein Control, except that protein storage buffer and the pepsin stock solution were incubated for 60 min at 37.1ºC before quenching with 0.7 M sodium carbonate buffer and 5× LB.

All quenched samples were frozen on dry ice and stored in a 80 ºC freezer until analysis.

N.3.2. Preparation of the Pancreatin Stock Solution

Pancreatin contains a mixture of proteolytic enzymes and was prepared based on the method described in The United States Pharmacopoeia (USP, 1995). The pancreatin was obtained from Alfa Aesar (catalog number J62162) and was dissolved in 50 mM potassium phosphate buffer (pH 7.5) to a concentration of 10 mg of pancreatin powder/ml. Activity was assessed using a pancreatin activity assay (Section N.4.2)

N.3.2.1. Degradation of the FT_T Protein by Pancreatin

Degradation of the intact FT_T protein by pancreatin was evaluated over time by analyzing samples at multiple incubation time points. A numerical code using the numbers 0 through 8 was used to distinguish incubation time points as follows:

Targeted Incubation Time Point	Sample Code
0 min	0 min No Test Protein Control
0 min	0 min No Pancreatin Control
0 min	Pancreatin Treated T0
5 min	Pancreatin Treated T1
15 min	Pancreatin Treated T2
30 min	Pancreatin Treated T3
1 h	Pancreatin Treated T4
2 h	Pancreatin Treated T5
4 h	Pancreatin Treated T6
8 h	Pancreatin Treated T7
24 h	Pancreatin Treated T8
24 h	24 h No Pancreatin Control
24 h	24 h No Test Protein Control

The reaction mixture was prepared by adding 929 µl of pre-heated (37.0ºC, 10 min) pancreatin stock solution to a tube containing 35 µl of the FT_T protein, corresponding to 168 µg of FT_T protein and 9,290.4 µg of pancreatin. The tube contents were vortex

mixed and immediately returned to the water bath (37.0ºC). Digestion samples (96.4 µl) were removed at 5, 15, 30 min, 1, 2, 4, 8, and 24 h (corresponding to time points Pancreatin Treated T1 through Pancreatin Treated T8) and immediately placed in a tube containing 24.1 µl of 5× LB, heated to 95-100 ºC for 5 10 min, and frozen on dry ice for complete quenching.

The Pancreatin Treated T0 sample was prepared in a separate tube by first quenching 92.9 µl of pancreatin stock solution with 24.1 µl of 5× LB buffer and heating to 95-100 ºC for 5 10 min prior to the addition of 3.5 µl of the FT_T protein.

All quenched samples were frozen on dry ice and stored in a 80 ºC freezer until analysis.

N.3.2.2. Pancreatin Degradation Experimental Controls

Experimental control samples were prepared to determine the stability of the FT_T protein in 50 mM KH2PO4, pH 7.5, the buffer used to prepare pancreatin stock solution. The 0 min No Pancreatin Control was prepared in a similar manner as described in Section N.4.2 for Pancreatin Treated T0, except that 50 mM KH2PO4, pH 7.5 was added instead of the pancreatin stock solution. The 24 hour No Pancreatin Control was also prepared in a similar manner, except the protein and 50 mM KH2PO4, pH 7.5 were incubated for 24 hour at 37.0ºC before quenching with 5× LB and heating.

Experimental control samples were also prepared to characterize the test system lacking the FT_T protein. The 0 min No Test Protein Control was prepared in a similar manner as described in Section N.4.2 for Pancreatin Treated T0, except that protein storage buffer (40 mM Tris, pH 8, 0.2 M NaCl) was added instead of FT_T protein. The 24 hour No Test Protein Control was also prepared in a similar manner, except the protein storage buffer and pancreatin stock solution were incubated for 24 hour at 37.0ºC before quenching with 5× LB and heating.

All quenched samples were frozen on dry ice and stored in a 80 ºC freezer until analyzed.

N.3.3. Degradation of the FT_T Protein by Pepsin Followed by Pancreatin

Degradation of the FT_T protein by sequential digestion was evaluated by first incubating the FT_T protein with pepsin for 2 min, quenching the pepsin degradation reaction, and then incubating the reaction mixture with pancreatin. The sequential digestion of the FT_T protein was evaluated over time by analyzing samples at multiple incubation time points with a numerical code using the number 0 through 7 to distinguish incubation time points as follows:

Targeted Incubation Time Point	Sample Code
Pepsin Degradation	
0 min	0 min Pepsin Treated
2 min	2 min Pepsin Treated
Pancreatin Degradation	
0 min	SEQ 0 min No Test Protein Control
0 min	SEQ 0 min No Pancreatin Control
0 min	SEQ T0
0.5 min	SEQ T1
2 min	SEQ T2
5 min	SEQ T3
10 min	SEQ T4
30 min	SEQ T5
1 h	SEQ T6
2 h	SEQ T7
2 h	SEQ 2 h No Pancreatin Control
2 h	SEQ 2 h No Test Protein Control

In the pepsin degradation phase, 729.5 µl of pre-heated (37.4ºC, 10 min) pepsin solution (2632 units of pepsin activity/ml) was added to 40 µl of the FT_T protein, corresponding to 192 µg of FT_T protein and 1920 U of pepsin. The tube contents were mixed by vortexing and immediately returned to the water bath (37.3ºC). The tube was removed at a targeted time of 2 min, and immediately quenched by adding 269.3 µl of 0.7 M sodium carbonate buffer. The 2 min Pepsin Treated sample was prepared by mixing 120 µl of the quenched sample with 30 µl of 5× LB, and heating to 95-100 ºC for 5-10 min. The sample was then frozen on dry ice and stored in a -80 °C freezer until analyzed.

In the pancreatin degradation phase, the quenched 2 min pepsin treated sample was digested in pancreatin. For this phase, 525.4 µl of pre-heated (37.3ºC) pancreatin solution was added to 500 µl of quenched 2 min pepsin treated sample, corresponding to 95 µg total FT_T protein (based on predigested concentration) and 5,253.5 µg of pancreatin. The tube contents were mixed by vortexing and immediately returned to the water bath (37.2ºC). Digestion samples (102.5 µl) were removed from the tube at targeted times of 30 sec, 2, 5, 10, 30 min, 1, and 2 h (corresponding to specimen codes SEQ T1 through SEQ T7) and immediately quenched by placing in a tube containing 25.6 µl of 5× LB, heated to 95-100 ºC for 5-10 min, and frozen on dry ice for complete quenching.

The zero incubation time point for the pepsin degradation phase (0 min Pepsin Treated) was prepared in a separate tube by first quenching 73 µl of pepsin solution with 26.9 µl of sodium carbonate buffer and 26 µl of 5× LB, then heating to 95-100 ºC for 5-10 min prior to the addition of 4 µl of the FT_T protein.

The zero incubation time point for the pancreatin digestion phase (SEQ T0) was prepared in a separate tube by first quenching 52.5 µl of pancreatin solution with 25.6 µl of 5× LB

buffer and heating to 95-100 ºC for 5-10 min prior to the addition of 50 µl of the quenched 2 min pepsin treated FT_T.

All quenched samples were frozen on dry ice and stored in a -80 ºC freezer until analyzed.

N.3.3.1. Pepsin Followed by Pancreatin Degradation Experimental Controls

The SEQ 0 min No Pancreatin Control was prepared in a similar manner as described for SEQ T0, except that 50 mM KH_2PO_4, pH 7.5 was added instead of pancreatin solution. The SEQ 2 hour No Pancreatin Control was also prepared in a similar manner, except the protein and 50 mM KH_2PO_4 were incubated for 2 hour at 37.2°C before quenching with 5× LB and heating.

The SEQ 0 min No Test Protein Control was prepared in a similar manner as described in for SEQ T0, except that protein storage buffer (40 mM Tris, pH 8, 0.2 M NaCl) was added instead of FT_T protein. The SEQ 2 hour No Test Protein Control was also prepared in a similar manner, except the protein storage buffer and pancreatin solution were incubated for 2 hour at 37.2ºC before quenching with 5× LB and heating.

All quenched samples were frozen on dry ice and stored in a -80 ºC freezer until analyzed.

N.4. Analytical Methods

N.4.1. Pepsin Activity Assay

Pepsin activity was determined by measuring the degradation of denatured hemoglobin. To conduct the assay, the pepsin stock solution (Section 4.1), was diluted to 0.03 mg of powder per ml with 10 mM HCl, 2 mg/ml NaCl, pH ~1.2 (dilution factor (DF) = 25.67). Five ml of acidified hemoglobin (2% (w/v)) was added to 3 test sample tubes and 3 blank tubes and all were pre-warmed for 8-10 min in a water bath at 37.0-37.1°C. Diluted pepsin stock solution (1 ml) was added to the 3 test sample tubes (test samples) and all 6 tubes were incubated for 10 min in the water bath. Ten ml of 5% (v/v) chilled trichloroacetic acid (TCA) was added to each of the 6 tubes and 1 ml diluted pepsin stock solution was then added to the 3 blank samples. Samples were mixed and then incubated for another 5-10 min in the water bath. Precipitated protein was removed from each sample using 0.45 µm syringe filters and the absorbance of the test and blank sample filtrates at 280 nm were measured using a SpectraMax M2 plate reader (Molecular Devices). One unit of pepsin produces a change in absorbance at 280 nm of 0.001 per min at 37 ± 2 ºC, pH 1.2. The units of pepsin in 1 ml of the stock solution were calculated using the following equation:

$$\frac{Mean\ Test_{A280nm} - Mean\ Blank_{A280nm}}{0.001\ \times\ 10\,\text{min}\ \times\ 1\,ml} \times DF$$

where 0.001 is the change in the absorbance at 280 nm per min produced by one unit of pepsin activity at 37 ± 2 ºC, pH 1.2; 10 min is the reaction time; 1 ml is the volume of the diluted pepsin stock solution added to the reaction; and DF is the dilution factor for the

pepsin stock solution. The activity of pepsin was converted from units/ml to units/mg. Acceptable specific activity (units/mg pepsin powder) for the pepsin solution was equal to the specific activity determined by the manufacturer ± 1,000 units/mg.

N.4.2. Pancreatin Activity Assay

The pancreatin activity was determined by measuring the degradation of resorufin-labeled casein (Roche Life Science). To conduct the assay, 50 µl of 0.4% (w/v) resorufin-labeled casein and 50 µl of incubation buffer (200 mM Tris, pH 7.8, 20 mM CaCl2) were added to 3 test sample tubes and 3 blank tubes and all were pre-warmed for 3 min in a water bath at 37.0-37.3°C. To initiate the reaction, 100 µl of 0.05× pancreatin stock solution (pancreatin stock solution was diluted to 0.05× with 50 mM KH2PO4, pH 7.5 before the activity assay was initiated) was added to each of the 3 test sample tubes while 100 µl of 50 mM KH2PO4, pH 7.5 was added to each of the 3 blank tubes. All 6 tubes were incubated for 15 min in the water bath. Reactions were quenched by adding 480 µl of 5% (w/v) TCA to each tube, followed by vortex mixing. The samples were incubated in the water bath for an additional 10 min. The supernatants recovered after centrifugation (400 µl) were neutralized by the addition of 600 µl assay buffer (500 mM Tris-HCl, pH 8.8), and the absorbance of the test and blank supernatants was read at 574 nm using a SpectraMax M2 plate reader (Molecular Devices). One unit of pancreatin produces a change in the absorbance at 574 nm of 0.001 per min at 37 ± 2 ºC. The units of pancreatin in the stock solution were calculated using the following equation:

$$\frac{Mean\ Activity_{A574nm} - Mean\ Blank_{A574nm}}{0.001\ \times 15\,\text{min} \times\ 0.005\ ml}$$

where 0.001 is the change in the absorbance at 574 nm per min at 37 ± 2 ºC produced by one unit of pancreatin activity, 15 min is the reaction time, 0.1 ml is the volume of diluted pancreatin stock solution added to the reaction, and 0.005 ml is the volume of the pancreatin stock solution used in the assay (0.05× Pancreatin solution, 0.1 ml). An acceptable specific activity for the pancreatin stock solution was defined as 11,000 ± 3,000 U/ml.

N.4.3. SDS-PAGE and Colloidal Brilliant Blue G Staining

Pepsin-treated samples and associated control samples were subjected to SDS-PAGE using pre-cast Tricine 10-20% (w/v) polyacrylamide gradient mini-gels and Tricine SDS running buffer (Invitrogen). The FT_T protein was loaded at 1 µg per lane based on total pre-digestion protein concentration. The experimental controls were loaded at the same volumes as those containing FT_T protein so that they would be comparable. Mark12™ molecular weight marker (Invitrogen) was loaded to estimate the relative molecular weight of proteins and peptides. Electrophoresis was performed at a constant voltage of 165 volts for 60-61 minutes. After electrophoresis, gels were fixed in a solution containing 7% (v/v) acetic acid and 40% (v/v) methanol for 30 min and stained for 17-20 hours in 1× Brilliant Blue G-Colloidal stain solution (Sigma), destained for 30 sec in 10% (v/v) acetic acid, 25% (v/v) methanol and then destained for 4.25-6 hours in a 25% (v/v)

methanol solution. The gels were scanned using a Bio Rad GS 900 densitometer (Bio Rad) to produce digitized images to be used as figures for reporting purposes.

To estimate the LOD of the FT_T protein, dilutions of the Pepsin Treated T0 samples were loaded on a second SDS-PAGE gel and the gel was run and processed exactly as the gel used to assess FT_T protein degradation in the presence of pepsin. Loads per lane were approximately 200 to 0.8 ng of the FT_T. The LOD was determined as the lowest amount of the FT_T protein that was visible on the gel with Colloidal staining.

Sequential digestion samples were also analyzed by SDS-PAGE as described above for pepsin degradation samples.

N.4.4. Western Blot Analysis

Pepsin-treated, pancreatin-treated, and sequential digestion samples, and the associated control samples, were subjected to SDS-PAGE using pre-cast Tricine 10-20% (w/v) polyacrylamide gradient mini-gels. The FT_T protein was loaded at approximately 40 ng per lane based on total pre-digestion protein concentration. The experimental controls were loaded to equal the digestion samples. Electrophoresis was performed at a constant voltage of 165 volts for 60-70 minutes. After electrophoresis, the proteins were electrotransferred to PVDF membranes (Bio Rad). Pre-stained molecular weight markers (Precision Plus Protein™ Standards Dual Color Standard, Bio Rad; MagicMark™ XP, Invitrogen) were used to verify electrotransfer of the proteins to the membranes and estimate the relative molecular weight of proteins.

Proteins transferred to PVDF membranes were analyzed by western blot. The western blotting procedure was performed using an iBind™ western System apparatus (Life Technologies). The membranes were blocked with 1× iBind™ Solution (Life Technologies) and incubated with goat anti FT_T antibody (lot 27003) at a dilution of 1:1,000 in 1× iBind™ Solution. After washing with 1× iBind™ Solution, the membrane was next incubated with horseradish peroxidase (HRP)-conjugated horse anti goat IgG (Vector Laboratories) at a dilution of 1:1,500 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). The films were scanned using a Bio Rad GS 900 densitometer to produce electronic images to be used as figures for reporting purposes.

To estimate the LOD of the FT_T protein, dilutions of either the Pepsin Treated T0 or the Pancreatin Treated T0 samples were subjected to western blot analysis run and processed exactly as the western blot used to assess FT_T protein degradation in the presence of pepsin or pancreatin, respectively. Loads per lane were approximately 40 to 0.08 ng of the FT_T protein. The LOD was determined as the lowest amount of the FT_T protein that was visible on the film.

References for Appendix N

Astwood, J.D., J.N. Leach and R.L. Fuchs. 1996. Stability of food allergens to digestion in vitro. Nature Biotechnology 14:1269-1273.

FAO-WHO. 2001. Evaluation of allergenicity of genetically modified foods. Report of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fu, T.-J., U.R. Abbott and C. Hatzos. 2002. Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid - A comparative study. Journal of Agricultural and Food Chemistry 50:7154-7160.

Hammond, B.G. and J.M. Jez. 2011. Impact of food processing on the safety assessment for proteins introduced into biotechnology-derived soybean and corn crops. Food and Chemical Toxicology 49:711-721.

Moreno, F.J., F.A. Mellon, M.S.J. Wickham, A.R. Bottrill and E.N.C. Mills. 2005. Stability of the major allergen Brazil nut 2S albumin (Ber e 1) to physciologically relevant in vitro gastrointestinal digestion. FEBS Journal 272:341-352.

Okunuki, H., R. Techima, T. Shigeta, J. Sakushima, H. Akiyama, Y. Goda, M. Toyoda and J. Sawada. 2002. Increased digestibility of two products in genetically modified food (CP4 EPSPS and Cry1Ab) after preheating. Journal of the Food Hygienic Society of Japan 43:68-73.

Thomas, K., M. Aalbers, G.A. Bannon, M. Bartels, R.J. Dearman, D.J. Esdaile, T.J. Fu, C.M. Glatt, N. Hadfield, C. Hatzos, S.L. Hefle, J.R. Heylings, R.E. Goodman, B. Henry, C. Herouet, M. Holsapple, G.S. Ladics, T.D. Landry, S.C. MacIntosh, E.A. Rice, L.S. Privalle, H.Y. Steiner, R. Teshima, R. van Ree, M. Woolhiser and J. Zawodny. 2004. A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regulatory Toxicology and Pharmacology 39:87-98.

USP. 1995. Test solutions. Page 2053 in The National Formulary. United States Pharmacopeia, Washington, D.C.

Vassilopoulou, E., N. Rigby, F.J. Moreno, L. Zuidmeer, J. Akkerdaas, I. Tassios, N.G. Papadopoulos, P. Saxoni-Papageorgiou, R. van Ree and C. Mills. 2006. Effect of in vitro gastric and duodenal digestion on the allergenicity of grape lipid transfer protein. Journal of Allergy and Clinical Immunology 118:473-480.

Vieths, S., J. Reindl, U. Müller, A. Hoffmann and D. Haustein. 1999. Digestibility of peanut and hazelnut allergens investigated by a simple in vitro procedure. European Food Research and Technology 209:379-388.

Yagami, T., Y. Haishima, A. Nakamura, H. Osuna and Z. Ikezawa. 2000. Digestibility of allergens extracted from natural rubber latex and vegetable foods. Journal of Allergy and Clinical Immunology 106:752-762.

RECEIVED
By USDA APHIS BRS Document Control Officer at 2:20 pm, Dec 11, 2019

Supplemental Information for Technical Completeness of Petition 19-316-01p (Monsanto Petition Number CR279-19U4; revised December 10, 2019)

Monsanto[1] is providing the following information as a supplement to Petition Number 19-316-01p (Monsanto Petition Number CR279-19U4) requesting the Determination of Nonregulated Status for Dicamba, Glufosinate, Quizalofop and 2,4-Dichlorophenoxyacetic Acid Tolerant MON 87429 Maize with Tissue Specific Glyphosate Tolerance Facilitating the Production of Hybrid Maize Seed (OECD Unique Identifier: MON 87429-9). This information also supports the clarifications requested in the Clarification Letter from USDA-APHIS dated November 20, 2019.

1. The cited 2018 reference has been marked as CBI in the three locations on pages 131, 136 and 181.

   A brief description of the methodology and production of the data cited in Table VIII-3 has been added to the PDF reference, included with this submission.

   The discrepancy between the percent of maize acres treated with the herbicide glyphosate in Table VIII-3 in the petition and Table 2 in the USDA-NASS 2019b reference is due to reporting of a sub-set of glyphosate use data in the USDA-NASS 2019b summary report. Table 2 from the USDA-NASS 2019b only reports a single glyphosate formulation (isopropylamine salt) use in maize (34% acres treated) while the total maize acres treated with herbicide glyphosate irrespective of its formulations was approximately 76% as per the pesticide use data available at USDA-NASS survey and is summarized below. Table VIII-3 in the petition includes total glyphosate use in maize irrespective of its formulations.

Glyphosate formulations	**Percent of maize acres treated**
CHEMICAL, HERBICIDE: (GLYPHOSATE = Code: 417300)	12%
CHEMICAL, HERBICIDE: (GLYPHOSATE AMM. SALT = Code: 103604)	Data not available
CHEMICAL, HERBICIDE: (GLYPHOSATE DIM. SALT = Code: 103608)	4%
CHEMICAL, HERBICIDE: (GLYPHOSATE ISO. SALT = Code: 103601)	34%*
CHEMICAL, HERBICIDE: (GLYPHOSATE POT. SALT = Code: 103613)	26%
Total per USDA NASS Survey	**~76%**

*Published in USDA-NASS 2019b.

[1] Monsanto Company remains a legal entity holding registrations, permits and other approvals, but it is wholly owned by Bayer. We will let you know about any reorganization of legal entities, including transfer of authorizations/registrations from Monsanto and/or address updates. The names of entities holding registrations should not have any bearing on our approvals. These changes are for legal administrative reasons and have no influence on the quality of the end-use products, grain or seeds and they have no consequences for existing safety evaluations.

2. Typo has been corrected to chloroacetamide.
3. Buckhorn plantain has been used throughout.
4. Delye et al., 2003b has been added as a citation.
5. Hidayat and Preston 1997 PDF is provided with this submission.
6. "+MCPA" has been added to the recommendation for kochia control in wheat in Table K-1.