Agrivida Petition (19-176-01p) for Determination of Non-regulated Status of Phytase Producing Maize Event PY203

OECD Unique Identifier:
AGV-PY203-4

Draft Plant Pest Risk Assessment

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TABLE OF CONTENTS

A. Introduction

Agrivida, Inc. has submitted a petition to Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) seeking a determination of nonregulated status for maize *(Zea mays*) event PY203 (OECD Unique Identifier AGV-PY203- 4) developed using engineering for phytase gene that it is unlikely to pose a plant pest risk and, therefore, should no longer be regulated under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 19-176-01p and is hereafter referenced as Agrivida 2019. Under the authority of the plant pest provisions of the Plant Protection Act (7 U.S.C. 7701 et seq.), the regulations in 7 CFR part 340, "Movement of Organisms Modified or Produced Through Genetic Engineering," regulate, among other things, the importation, interstate movement, or release into the environment of organisms modified or produced through genetic engineering that are plant pests or pose a plausible plant pest risk. This plant pest risk assessment (PPRA) was conducted to determine if PY203 maize is unlikely to pose a plant pest risk.

The petition for nonregulated status described in this PPRA is being evaluated under the version of the regulations effective at the time that it was received. Animal and Plant Health Inspection Service (APHIS) issued a final rule, published in the Federal Register on May 18, 2020 (85 FR 29790-29838, Docket No. APHIS-2018-0034)[1], revising 7 CFR part 340; however, the final rule is being implemented in phases. The new Regulatory Status Review (RSR) process, which replaces the petition for determination of nonregulated status process, became effective on April 5, 2021 for corn, soybean, cotton, potato, tomato, and alfalfa. The RSR process is effective for all crops as of October 1, 2021. However, "[u]ntil RSR is available for a particular crop APHIS will continue to receive petitions for determination of nonregulated status for the crop in accordance with the [legacy] regulations at 7 CFR § 340.6." (85 FR 29815). This petition for a determination of nonregulated status is being evaluated in accordance with the regulations at 7 CFR § 340.6 (2020) as it was received by APHIS on 6/25/2019.

PY203 maize was produced by the *Agrobacterium tumefaciens*- mediated transformation of immature embryos of the maize line High II B (Agrivida 2019), and six of the introduced genetic sequences come from plant pest organisms listed in 7 CFR 340.2, including the 25 base pairs of the right- and left-border T-DNA repeats as well as four terminators of the nopaline synthase (*nos*) gene from *A. tumefaciens*. Therefore, the PY203 maize is considered a regulated aticle under APHIS regulations at 7 CFR part 340. Agrivida has conducted introductions of PY203 maize as a regulated article under APHIS-authorized notifications since 2005 (Table 1, p. 15 in Agrivida 2019), in part, to gather information to support that PY203 maize is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with PY203 maize and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators.

[1] To view the final rule, go to www.regulations.gov and enter APHIS-2018-0034 in the Search field.

APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if PY203 maize is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about PY203 maize related to: plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302 1986; 57 FR 22984 1992; 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, https://usbiotechnologyregulation.mrp.usda.gov/2017_coordinated_framework_update.pdf). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived productsare subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq*) the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. EPA also sets tolerance limits for residues of pesticides on and in food and animal feed or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Other applicable EPA regulations include 40 CFR part 152 - Pesticide Registration and Classification Procedures, part 174 - Procedures and Requirements for Plant Incorporated Protectants (PIPs) and part 172 - Experimental Use Permits. No EPA reviews are relevant to PY203 maize.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from modified crops comply with their obligations, the FDA encourages them to participate in its voluntary early food safety evaluation for new non-pesticidal proteins produced by new plant varieties intended to be used as food (US-FDA 2006a, b) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984 1992). Agrivida has completed an Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use: Early Food Safety

Assessment for the phytase protein produced in PY203 maize (NPC 000015) on August 7, 2015. A Pre-market Biotechnology Notification (PBN) consultation for PY203 maize was submitted to the U.S. FDA in June 19, 2018 (BNF 000167). Data and information supporting the Generally Recognized as Safe (GRAS) nature of the phytase expressed in PY203 maize was reviewed by FDA's Center for Veterinary Medicine (CVM) in 2017, and FDA CVM had no further questions related to Agrivida's conclusion that the PY203 maize produced phytase enzyme is GRAS for usein poultry feed. Likewise, data and information supporting the GRAS nature of the phytase expressed in PY203 maize for use in swine feed has been submitted to FDA CVM for review in 2018. Based on the information Agrivida has presented to FDA, on January 27, 2021 FDA issued a letter to Agrivida that they have no further questions concerning human or animal food derived from PY203 corn at this time (BNF 000167, http://www.fda.gov/bioconinventory).

B. Development of Phytase Producing PY203 Maize

Maize (*Zea mays* L. ssp *mays*) belongs to genus *Zea*, which consists of five species including *Z. mays*, *Z. diploperennis*, *Z. luxurians*, Z. *nicaraguensis,* and *Z. perennis* (OECD 2003; OTGR 2008). Maize (*Z. mays* L. ssp *mays*) is the only cultivated subspecies, and all the other subspecies of *Z. mays* as well as other species in genus *Zea* are wild grasses and referred to as tesosintes (OTGR 2008). The closest known relative of genus *Zea* is genus *Tripsacum*. Maize can only be crossed experimentally with the genus *Tripsacum*, but it can easily cross with the species of its own genus under natural conditions (OECD 2003).

Maize is widely grown in the world from 58° North (e.g., Canada and Russia) to 40°South (e.g., Chile) (Farnham et al. 2003; OTGR 2008), and it is the largest grain crop in the world in total metric ton production as of 2017 (FAOSTAT 2019). The top five maize production countries in 2016/2017 include USA (385 million metric tons (MMT)), China (264 MMT), Brazil (99 MMT), the European Union (62 MMT), and Argentina (41 MMT) (USDA-FAS 2019). In United States, maize is grown in almost all the states (Figure 1, colored areas) (USDA-NASS 2019a). As shown in Figure 2, there exists a significant year-to-year variability in planted acreages, ranging from 68 to 97 million acres in the past 20 years (USDA-NASS 2019b). Maize yields (bushels/acre) also differ from year to year but show an apparent increase over the years (Figure 3) (USDA-NASS 2019c).

Maize has been being used as a basic food crop but its primary use in industrialized countries shifts more towards animal feed in the form of grain, forage or silage (Farnham et al. 2003; OECD 2003). In developed countries, more than 85% of the maize is used to feed animals (Farnham et al. 2003). Maize can also be processed for a range of uses as ingredients in food or drinks, or for industrial purposes, e.g., alcohol including fuel ethanol (OECD 2003; OTGR 2008).

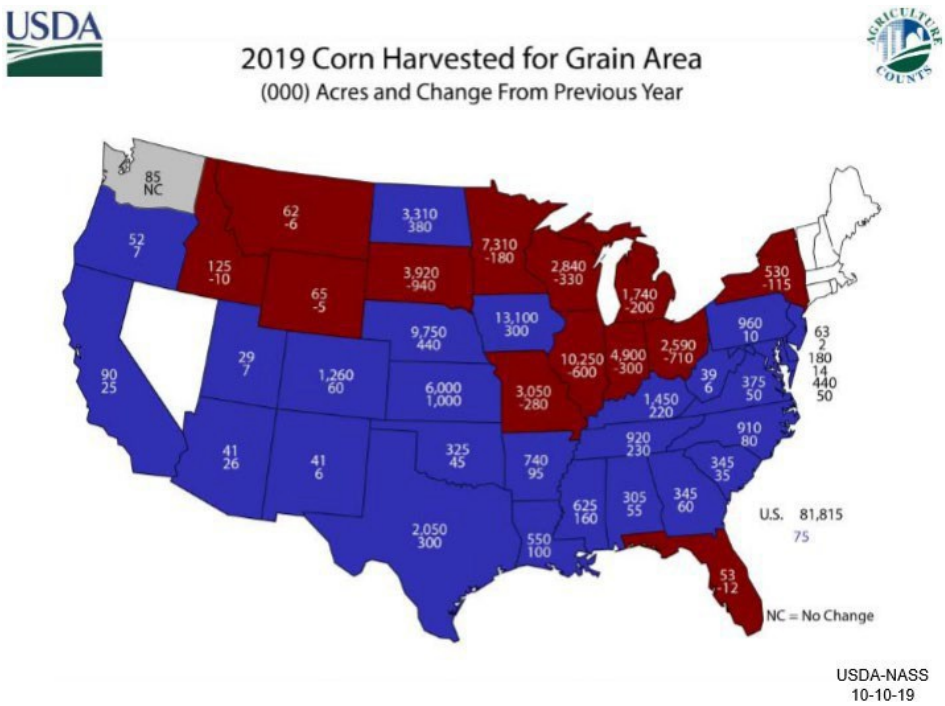


Figure 1. Maize production areas in the U.S. (USDA-NASS 2019a)

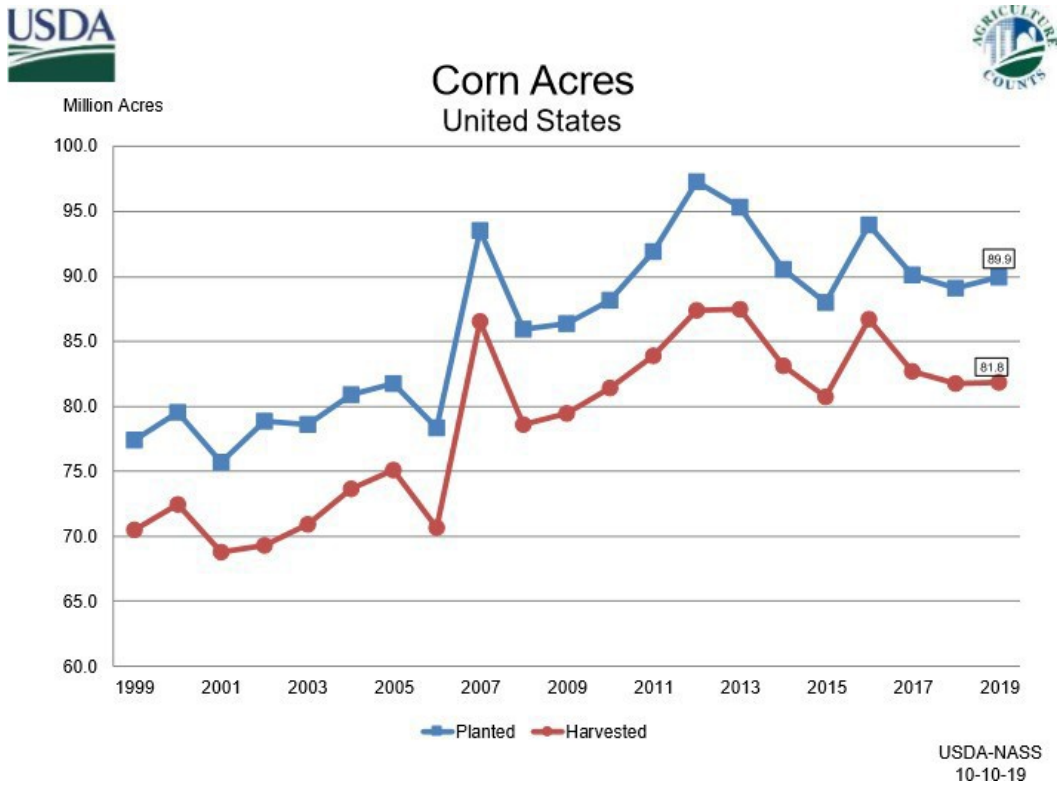


Figure 2. Maize acreage by year in the U.S. (USDA-NASS 2019b)

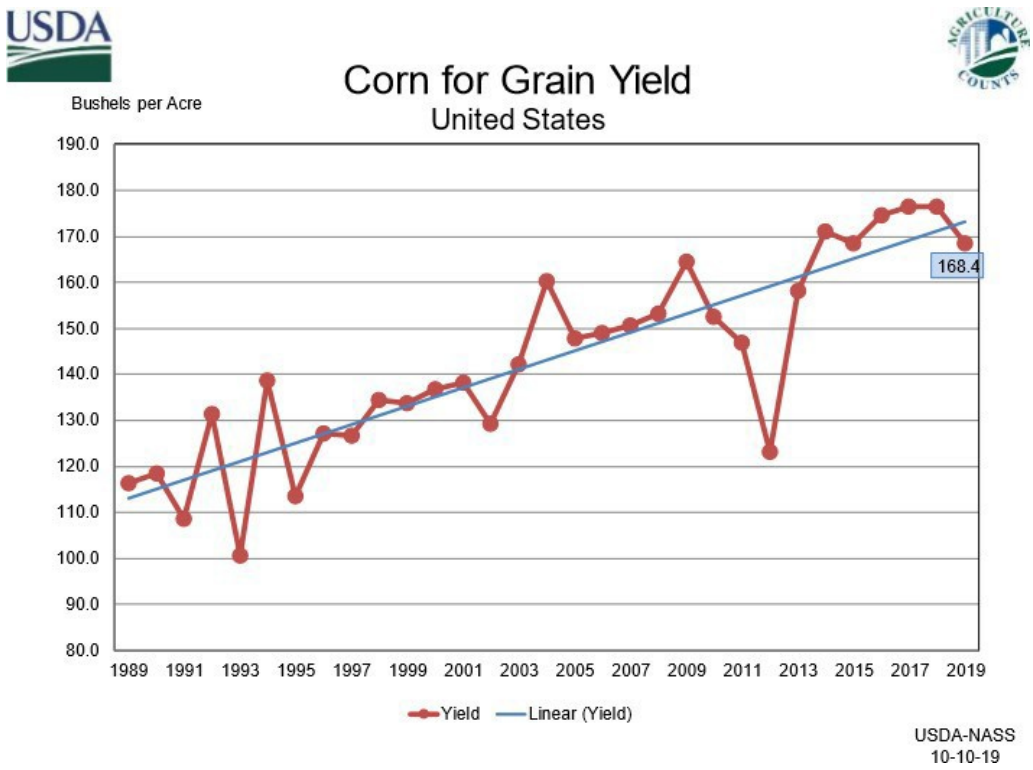


Figure 3. Maize yield by year in the U.S. (USDA-NASS 2019c)

Maize seeds contain large quantities of phytic acid (also referred to as phytate), with approximately 4.8 million metric tons of total phytic acid being produced annually around the globe (Lott et al. 2007). Phytic acid, however, is difficult for monogastric animals to digest and has a negative impact on animal nutrition and the environment (Jongbloed and Lenis 1998; Dersjant-Li et al. 2015). To make phosphorus (P) in phytic acid nutritionally available to monogastric animals, phytases as a class of acid phosphatase enzymes that hydrolyze phosphates from phytic acid to produce free phosphate and inositol are often added to animal feeds to improve feed and P utilization (Augspurger et al. 2003; Nyannor et al. 2007; Nyannor et al. 2009; Dersjant-Li et al. 2015). Phytases can also be used as human dietary supplements (Kumar et al. 2010). Phytases are ubiquitous in nature and are found in many microorganisms, plants, and some animals (Konietzny and Greiner 2002; Kumar et al. 2010). Nearly all the current phytase animal feed products are produced and purified from microorganisms (Pandey et al. 2001). The increased utilization efficiency of phosphorus and the reduction of phosphorus in animal manure through the addition of phytase into animal feed can assist concentrated animal feeding operations to meet EPA's National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitations Guidelines and Standards (EPA 2008).

Agrivida developed the phytase producing PY203 maize by expressing in maize seeds the phytase gene (*phy02*) that was optimized based on *E. coli* phytase gene *appA*. Agrivida intends to grind PY203 maize grains with phytase into a meal and add to the feed of poultry and swine (nonruminants) to improve the nutritional availability of phosphorus in

the feed (Agrivida 2019). The genetic engineering and breeding steps for the development of PY203 maize are described in the petition (Section III, Agrivida 2019).

Based on maize biology (OECD 2003; OTGR 2008) and the data presented by Agrivida, APHIS concludes that PY203 maize was developed in a manner common to other modified maize and crops using *Agrobacterium*-mediated transformation (USDA-APHIS 2019a). APHIS believes the use of the unmodified near-isogenic non-transgenic control and other reference varieties as comparators is sufficient to determine that PY203 maize doesnot differ from the other maize varieties currently used in commercial production.

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to PlantMetabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the modified crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the locationof the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the PY203 maize relative to the near-isogenic non-transgenic control. The assessment encompasses a consideration of the expressed phytase enzyme, phosphomannose isomerase (PMI) enzyme and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seed/forage etc. derived from PY203 maize compared to those in the near-isogenic non-transgenic control and other comparators.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the Py203 maize; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

PY203 maize was developed through *Agrobacterium*-mediated transformation of the immature embryo of maize inbred line High II B using the disarmed pAG4758 binary vector (Agrivida 2019). The disarmed binary vector does not have the native T-DNA region from tumor-inducing (Ti) plasmids normally responsible for the incitation of crown gall tumors upon *A. tumefaciens* infection (Gelvin 2003).

Binary Plasmid Vector pAG4758

The disarmed pAG4758 binary vector is approximately 21.2 kb. It contains four gene expression cassettes, which are delineated by a right border (RB) and left border (LB) sequences of T-DNA as well as backbone vector sequences outside of the two T-DNA border sequences. Transgene elements within the T-DNA regions are shown in Figure 4 below (Agrivida 2019).

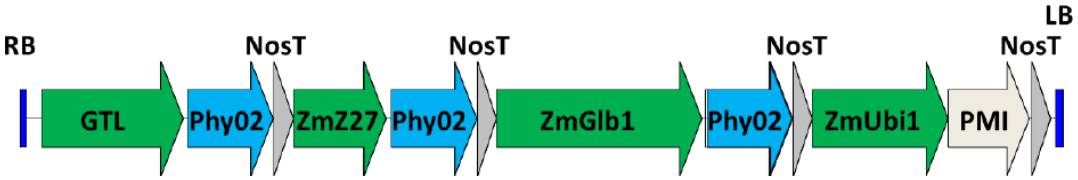


Figure 4. Transgene elements within the T-DNA regions (Figure 4, Agrivida 2019) The T-DNA contains tandemly arrayed three gene expression cassettes for the expression of the same modified Phy02 phytase gene derived from the *E. coli appA* phytase geneand a gene expression cassette for the expression of the *manA* gene encoding phosphomannose isomerase (PMI) derived from *E. coli.* All the three Phy02 phytase gene expression cassettes contain the same signal peptide sequence derived from the 27 kDa γ-zein seed storage protein of *Z. mays*, Phy02 phytase gene and terminator sequence of the nopaline synthase (*nos*) gene from *A. tumefaciens* but with a different monocot derived promoter, i.e., GTL promoter derived from the *Oryza sativa* Glutelin-1 gene, ZmZ27 promoter derived from the *Z. mays Zc2* gene, and ZmGlb1 promoter derived from the *Z. mays Glb1* gene. The PMI expression cassette contains ZmUbi1 promoter derived from the *Z. mays* ubiquitin 1 gene, *manA* gene encoding PMI and the terminator of the *nos* gene from *A. tumefaciens*.

Among the above transgene elements inserted into PY203 maize, six elements, including the T-DNA right- and left-border sequences and four *nos* gene terminators are derived from *A. tumefaciens* that is listed as a plant pathogen in 7 CFR 340.2. However, none of them is known to cause plant diseases.

Characteristics, Stability, and Inheritance of the Introduced DNA

Agrivida has provided data to characterize the inserted transgene DNAs in PY203 maize with a combination of techniques including Southern analyses, genome walking experiments, and DNA sequencing (Agrivida 2019). The data demonstrate that PY203 maize contains two independently-segregating T-DNA insertions. Southern blot and PCR tiling analyses demonstrate the absence of DNA fragments derived from the vector backbone of plasmid pAG4758 in the genome of PY203 maize.

Genome walking and sequencing analysis of both insertions including the flanking maize genomic DNA, demonstrates that one of the insertions, locus 3293, consists of the intact copy of T-DNA with the three Phy02 gene expression cassettes and *manA* gene expression cassette, and it is located on maize chromosome 8 and 308 bp downstream of

the stop codon of the annotated gene model GRMZM2G159344. The insertion resulted in a deletion of 24 bp nucleotide fragment in the non-genic region. The other insertion, locus 3507, consists of the truncated T-DNA with only two complete phytase genes lacking the third phytase gene and the *manA* selectable marker gene, and it is located on maize chromosome 2 in a genomic region containing a 99 bp unannotated open reading frame. The insertion resulted in the deletion of 40 bp of the maize genomic DNA at the insertion site (Agrivida 2019).

Both insertions were shown to be inherited in the expected Mendelian pattern and stable over multiple generations.

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

As described above, PY203 maize has two T-DNA insertions including loci 3293 and 3507. The T-DNA insertion locus 3293 contains all four of the tandemly arrayed expression cassettes that are present in the pAG4758 T-DNA (Figure 4). The first three expression cassettes are designed for the expression of the same *phy02* phytase gene with the only difference being that the promoters are from different monocot genes, i.e., GTL promoter from the *Oryza sativa* Glutelin-1 gene, ZmZ27 promoter from the *Z. mays Zc2* gene, and ZmGlb1 promoter from the *Z. mays Glb1* gene. The fourth expression cassette is for the expression of the *manA* gene encoding phosphomannose isomerase (PMI) under the regulation of *Z. mays* ubiquitin 1 gene promoter and *A. tumefaciens nos* gene terminator. The T-DNA insertion locus 3507contains only the first two expression cassettes for the expression of phytase (Figure 4).

Phytase enzyme

Phytases are ubiquitous in nature and are produced by many microbes and plants. Phytases are a class of acid phosphatase enzymes that hydrolyze phosphates from phytic acid (also referred to as phytate) to produce free phosphate and inositol (Konietzny and Greiner 2002; Kumar et al. 2010). Phytate phosphorus is nutritionally unavailable to monogastric animals such as poultry and swine (Augspurger et al. 2003; Nyannor et al. 2007; Nyannor et al. 2009; Dersjant-Li et al. 2015). In addition, phytate also forms a complex salt called phytin with several mineral ions such as Fe^{+2}, K^{+}, Mg^{+2}, Ca^{+2} and Zn^{+2}, making these minerals nutritionally unavailable to monogastric animals, and thus, phytate is considered an anti-nutrient (Coulibaly et al. 2011). The addition of phytase to animal feeds has been shown to improve feed and phosphorus utilization and reduces phosphorus in animal wastes. (Augspurger et al. 2003; Nyannor et al. 2007; Nyannor et al. 2009; Dersjant-Li et al. 2015). Ruminant animals may gain the same dietary benefits from the addition of exogenous phytase to their diet as do monogastric animals.

The phytase gene *phy02* expressed in PY203 maize was designed from the modification of the native *E. coli appA* phytase gene using a combination of modeling and site-directed mutagenesis to make Phy02 phytase more thermos-tolerant and more susceptible to digestion in a gastric environment (Agrivida 2019). The mature Phy02 phytase protein

consists of 417 amino acids with a predicted molecular weight of 45,684 kDa. The native *E. coli* AppA phytase and recombinant Phy02 phytases are homologous with only 16 amino acid residue differences in the mature phytase protein (Agrivida 2019). Phy02 phytase was shown to exhibit the enzymatic properties common to phytases (Agrivida 2017), demonstrating that none of the amino acid changes have significantly changed its structure or biological function. Phy02 phytase is nearly identical and substantially equivalent to a commercial phytase (Quantum®, AB Vista) that has been used safely and effectively in poultry and swine diets for the past decade (EFSA 2008; US-FDA-CVM 2017). Both the altered thermotolerance and susceptibility to digestion are desirable characteristics for commercial feed enzymes because the increased thermotollerance can help Phy02 phytase prevent against heating-induced inactivation in a pelleting process of animal feed production, and the increased susceptibility of Phy02 phytase to gastric digestion can help reduce its potential to be allergenic.

Since the expression of the *phy02* genes is under the control of monocot derived seed specific promoters, nearly all expression of the *phy02* genes in PY203 maize will be in the grain with minimal expression in other tissues (Agrivida 2019). Phy02 protein was quantified from each individual tissue sample by enzyme-linked immunosorbent assay (ELISA). Overall the amount of Phy02 protein in grain ranged from 4548 to 9079 µg/g DW. The level of Phy02 protein in PY203 leaf, stem, root, and pollen was either below the limit of detection (LOD) or close to the limit of quantitation (LOQ) (Agrivida 2019).

PMI enzyme

The *manA* gene encoding the PMI enzyme as a plant selectable marker is expressed under the control of the *Z. mays* ubiquitin 1 gene pormoter in all maize tissues. The PMI enzyme enables maize tissue to grow on medium with mannose as a sole source of carbon nutrient (Negrotto et al. 2000). This selectable marker has been widely used in maize and other crop species that have been approved for food use by regulatory authorities in the United States, including maize Events 5307 and Mir604 maize with resistance to corn rootworm, lepidoptera resistant Mir162, and α-amylase expressing Event 3272 (USDA-APHIS 2019a).

Potential new ORFs

In addition to Phytase and PDI enzyme proteins expressed in PY203 maize, Agrivida analyzed the potential new open reading frames (ORFs) that are likely to result from the insertion of T-DNA (Agrivida 2019). It showed that the locus 3293 genomic region does not contain annotated genes or defined genetic elements whereas the locus 3507 genomic region contains a 99 bp unannotated ORF, that has been disrupted by the T-DNA insertion. However, no gene model was found to be associated with this ORF in the annotated B73 maize genome and a BLASTp analysis of the inferred amino acid sequence against the NCBI non-redundant protein sequences database

identified no proteins with significant similarity, suggesting that it is unlikely to correspond to a functional gene (Agrivida 2019).

Metabolism composition Analysis

To assess any potential metabolite alteration as a result of the expression of the above inserted genes, Agrivida analyzed the metabolism composition of PY203 maize grain and forage samples collected from replicated filed trials at five locations in 2016, including proximates, amino acids, fatty acids, minerals, vitamins, and other bioactive metabolites (phytic acid, trypsin inhibitor, p-coumaric acid, raffinose, and ferulic acid) (Agrivida 2019). The data demonstrate that there exist no significant differences for majority of the assayed compositional components between PY203 maize and the near-isogenic non-transgenic control. It is noteworthy that significant differences are shown in some measured compositional components of PY203 maize and the near-isogenic non-transgenic control, such as crude fat, carbohydrates and the contents of 14 amino acid contents in grain samples. However, the values of these measured compositional components are all within the range of the commercial maize as reported in the ILSI Crop Composition Database (Agrivida 2019). Thus, the nutrient composition of grain and forage of PY203 maize is substantially equivalent to that of other conventional maize varieties, suggesting that the expression of Phy02 protein in PY203 maize does not significantly affect the nutrient composition in the grain or forage of PY203 maize.

In summary, the expression of the inserted DNAs and the resulting phenotype in PY203 maize are consistent with the inheritance of the introduced genetic material. The sequence analysis showed no evidence supporting any potential creation of new ORFs or any unintended effects resulting from the insertion of the genetic materials (Agrivida 2019). The compositional analyses demonstrated that introduction of the pAG4758 T-DNA in PY203 maize achieved the intended expression of Phy02 phytase enzyme in seeds while maintaining the equivalent metabolism composition of gain and forage tissues in comparison to the near-isogenic non-transgenic control as well as other conventional maize varieties. (Section 5.6, p. 70; Bayer 2017) (Section 5.6, p. 70; Bayer 2017) (Section 5.6, p. 70; Bayer 2017) (Section 5.6, p. 70; Bayer 2017)

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences of plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in PY203 maize that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether PY203 maize is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new modified crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2)result

in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist including the programs for grasshoppers (Order Orthoptera) on rangelands, light brown apple moth (*Epiphyas postvittana*) in California, and of more relevance, Japanese beetle (*Popillia japonica*), Old World bollworm (*Helicoverpa armigera*), and witchweed (*Striga asiatica*) that can affect maize (USDA-APHIS 2019b).

The grasshoppers are normally natural components of the rangeland ecosystem (Figure 5), but they can invade adjacent cropland and cause serious economic losses when their populations reach outbreak levels, especially when accompanied by a drought.

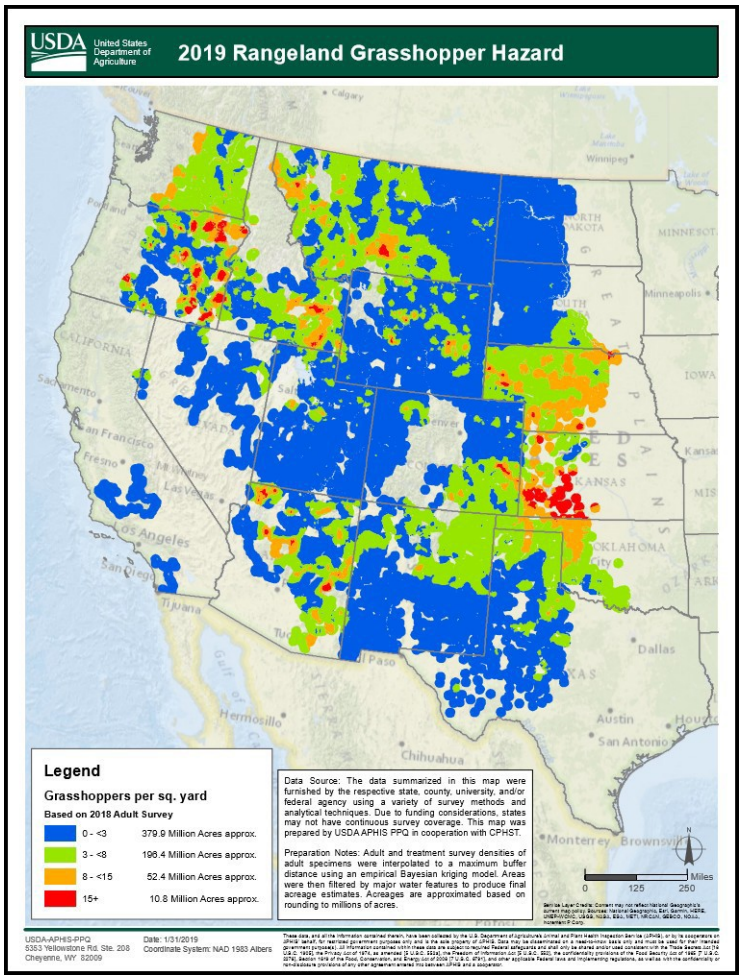
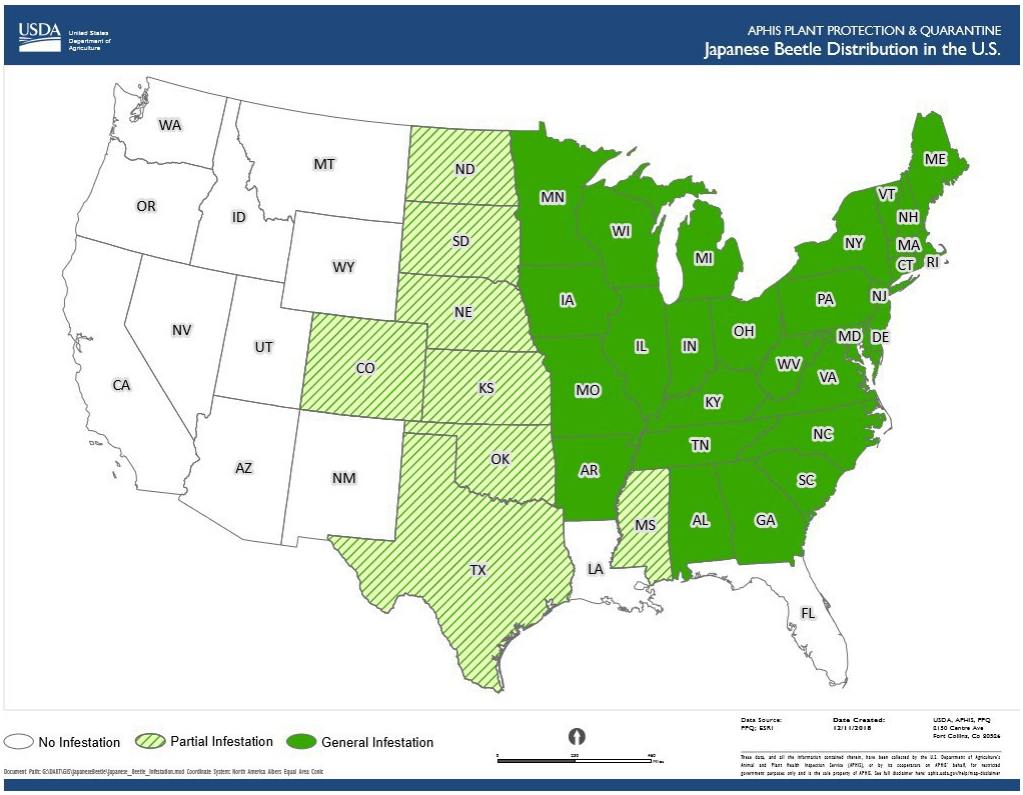


Figure 5. 2019 U.S. rangeland grasshopper hazard

The light brown apple moth (LBAM) can damage a wide range of crops and other plants. The LBAM was found in California in 2007, and some areas have been designated as quarantined areas (Figure 6).

Figure 6. 2018 Quarantined areas for light brown apple moth in California

The Japanese beetle is a highly destructive plant pest that can be very difficult and expensive to control. Japanese beetle adults attack the foliage, flowers, or fruits of more than 300 different ornamental and agricultural plants. Japanese beetles have spread throughout many states of the U.S. (Figure 7). APHIS maintains the Japanese Beetle Quarantine and Regulations that can be found in 7 CFR 301.48 with the objective to protect the agriculture of the Western United States and prevent the human-assisted spread of the beetle from the Eastern U.S.



The Old-World bollworm can affect 180 species of plants, with maize listed as one of its preferred hosts. It is closely related to the corn earworm (*Helicoverpa zeae*). Old World bollworm was first detected in western Puerto Rico in September 2014, and APHIS is conducting a variety of activities to protect the continental U.S from this pest (USDA-APHIS 2019b).

Witchweed (*Striga asiatica*) is a parasitic plant listed as a Federal Noxious Weed that affects maize and several other crops. Infested areas are found in North and South Carolina, and APHIS and state collaborators aim to stop the spread from infested areas and eradicate the pest (USDA-NRCS 2019).

Maize itself is not considered a plant pest in the United States (7 CFR 340.2). The Agrobacterium strain *A. tumefaciens* used in the generation of PY203 maize were disarmed and also were already removed with antibiotics during the transformation process (Negrotto et al. 2000). The inserted DNA elements derived from plant pests do not result in the production of infectious agents or disease symptoms in plants. The genetic modifications of PY203 maize including genetic elements, expression of the gene products and their functions have been summarized above and are not expected to impart any new plant pest or disease risk than non-modified maize.

Agrivida evaluated the differences between PY203 maize and null control plants in the incidence of insect predation (e.g., aphids, *Colaspis* spp., cutworms, grasshoppers, sap beetles, woolybear caterpillars, stink bugs, and thrips) plant disease (e.g., anthracnose, *Cercospora zeae-maydis*, *Colletotrichum graminicola*, dumping off, ear rot,

Helminthosporium maydis, *Helminthosporium turcicum*, Mal de Río IV virus, *Pseudomonas alboprecipitans*, rust, stalk rot, and *Ustilago maydis*) and other biota (e.g., bees, butterflies, ladybeetles, grasshoppers and other insects, birds and mammals) at all growth stages up to maturity (Agrivida 2019). There were no significant differences between PY203 maize and the near-isogenic null comparator line for all the observed insect predation, plant disease and other biota (pp 71-75, Agrivida 2019). These data demonstrate that integration of the T-DNA of plasmid pAG4758 and accumulation of Phy02 phytase and PMI enzyme in PY203 maize did not significantly alter the insect predation, disease occurrence, or rendering PY203 maize more susceptible to pests and diseases over its control or reference maize varieties. Also, as discussed earlier, there were no observed or anticipated unintended metabolic composition changes in PY203 maize that could impart any new plant pest or disease risk than non-modified maize (Agrivida 2019). Thus, PY203 maize is unlikely to be more susceptible to plant pathogens and insect pests than conventional maize. For this reason, PY203 maize is unlikely to differ from conventional maize in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

The PY203 maize is not engineered for pest resistance other than for the expression of phytase enzyme, thus there are no 'target' species nor 'nontarget' species. APHIS assessed whether exposure or consumption of the PY203 maize would have a direct or indirect adverse impact on species beneficial to agriculture. Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on PY203 maize compared to the non-modified counterpart for any biologically relevant changes in the phenotype or substances (e.g., proteins, nutrients, or anti-nutrients) produced whichmay be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

As described above in Section C "*Expression of inserted DNA, changes in gene expression, new proteins or metabolism*", the inserted T-DNA in PY203 maize encodes only two proteins novel to maize, Phy02 phytase enzyme and phosphomannose isomerase enzyme (PMI). PMI catalyzes the reversible inter-conversion of mannose-6-phospate and fructose-6-phosphate, and it is expressed as a plant selectable marker under the control of the promoter from the *Z. mays* ubiquitin 1 gene that provides expression in all maize tissues. The gene encoding PMI and associated regulatory sequences introduced into PY203 maize are identical to the genetic sequences inserted in Syngenta Seeds, which have been granted an exemption for the requirement of a tolerance in all plants by EPA (EPA 2004). This weight of evidence and history of safe use supports Agrivida's conclusion that the PMI protein expressed in maize Event PY203 is safe for food use and the environment.

The Phy02 phytase protein expressed in Py203 maize does not present safety concerns for humans and animals as well as for non-target orgasms beneficial to agriculture based on multiple lines of evidence, including the protein's safety profile with a long history of safe use, its expression level and specificity in maize, and its potential routes of exposure in the environment. Phytases are ubiquitous in nature and have a long history of safe use in human food from natural sources and in dietary supplements (Konietzny and Greiner 2002; Lott et al. 2007; Kumar et al. 2010). Likewise, phytases have been added safely to the feed of monogastric animals for decades to improve phosphorus digestibility (EFSA 2008; Agrivida 2017; US-FDA-CVM 2017). Phytase is the most widely used feed enzyme and globally it is included in approximately 90% of poultry and 70% of swine diets (Agrivida 2019). With the Phy02 amino acid sequence as the query sequence, Agrivida conducted a sequence similarity search against the NCBI Protein dataset and the database of known or suspected allergenic proteins, and no similarities to known protein toxins or known or putative protein allergens were discovered (Agrivida 2019). The rapid degradation of Phy02 phytase protein in simulated gastric fluid containing pepsin further confirmed that it is unlikely to be an allergenic protein (Agrivida 2019).

A detailed assessment of human and animal safety of the Phy02 phytase protein has been provided to the FDA as part of a food and feed safety and nutritional assessment for maize Event PY203 (Agrivida 2017). Agrivida has completed an Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use: Early Food Safety Assessment for the Phy02 Phytase Protein (NPC 000015) in 2015. A Pre-market Biotechnology Notification (PBN) consultation for Event PY203 was submitted to the U.S. FDA in 2018 (BNF 000167). In addition, the safety and efficacy of Phy02 as a feed additive in poultry has been reviewed by the FDA Center for Veterinary Medicine with no questions concerning Agrivida's conclusion that the Phy02 phytase is GRAS for this purpose (US-FDA-CVM 2017).

The exposure levels of Phy02 phytase to wildlife including nontarget organisms beneficial to agriculture are also taken into account to assess the potential impacts of Phy02 phytase on nontarget organisms. As described earlier, the Phy02 phytase is predominantly present in maize seeds but little in other tissues such as leaf, stem, pollen and roots due to the use of seed-specific promoters for the expression of Phy02 phytase in Py203 maize. The highest level of Phy02 phytase detected in kernels was 9079.5 µg/g (Agrivida 2019). A variety of insects would feed on maize in theenvironment. Insects that consume nonseed tissues including leaf and/or stalk tissue at any developmental stage, pollen and roots are not expected to be adversely impacted by Phy02 phytase because there is little to no production of the Phy02 phytase protein in those tissues. Insects that consume developing or mature seed of PY203 maize would be exposed to higher dietary levels of Phy02 phytase than insects consuming nonseed tissues. However, Phy02 phytase would not be expected to have significant enzymatic activity in the digestive tracts of insects that have digestive systems with a more basic pH than that in maize seed (pH 2 to 7). In this case, the Phy02 phytase protein in the digestive tracts of insects would be digested into its constituent amino acids and no adverse impact to the insect is expected. Even if the Phy02 phytase

may maintain some enzymatic activity in the digestive process of consuming insects, this activity is known not to be toxic or otherwise harmful because seeds of many plants have been demonstrated to contain phytase enzymes and consumption of these seeds by insects has not been known to be harmful. To assess the potential impact of phytase on wild animals, Agrivida conducted two tolerance studies in which animals were dosed with high levels of the Phy02 phytase, and the data demonstrated that high doses of phytase are well tolerated by animals, indicating that the consumption of grain from PY203 maize by wildlife in the environment is unlikely to have any adverse effects or negative impacts on the consuming animals (Agrivida 2019). Furthermore, in the event of the consumption by wildlife of tissues of PY203 maize that contain the Phy02 phytase, wildlife would derive similar benefits related to increased phosphorus and mineral availability as do poultry and swine that consume phytase routinely in their diets. Taken together, APHIS conclude that Phy02 phytase would have no adverse environmental or health impacts on the consuming individuals or their populations.

Therefore, based upon the above analysis of the safety profile, the long history of use in human food and animal feed, and the expression profiling of the Phy02 phytase and PMI as well as the compositional analysis of Py203 maize, APHIS concludes that exposure to and/or consumption of PY203 maize is unlikely to have any adverse impacts to organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of PY203 Maize

APHIS assessed whether the PY203 maize is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of PY203 maize compared to the its near-isogenic control and other reference maize hybrids for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include seed dormancy and germination, agronomic and phenotypic traits, disease and pest susceptibility, and ecological characteristics.

In the United States, maize is not listed as a weed in the major weed references (Crockett 1977) and it is not designated as a noxious weed by the federal government (USDA-APHIS 2019b). Further, maize does not possess weedy characteristics such as high level of seed dormancy, ease of seed shattering, and strong growth competitiveness (OECD 2003b; OTGR 2008). Maize seeds do not exhibit dormancy and are well retained on the cob and covered by multiple layers of husk leaves. Also, maize are sensitive to low temperatures, and the germinating seedlings and plants do not survive freezing winter conditions (OECD 2003; OTGR 2008) (Andersson and de Vicente 2010). Although maize seed does not shatter, harvest process or foraging wildlife can result in seed disperse, and some may overwinter and germinate when conditions are ideal, anddevelop

into volunteer plants the following year. However, maize has not been reported to be able to establish self-sustaining populations outside of cultivation (OECD 2003; OTGR 2008). This is further supported by data from controlled experiments where maize plants were left unharvested but no feral plants were discovered within a year or two after planting (Raybould et al. 2012; Sammons et al. 2014). Similar to conventional maize volunteers, PY203 maize volunteers can be managed by employing mechanical cultivation, crop rotation, and the careful selection of the modes of action for pre-emergent and post-emergent herbicides to balance competing herbicide sensitivities between volunteers and the rotational crop (Vencill et al. 2012).

To assess the seed germination and dormancy potential of PY203 maize seed, Agrivida conducted laboratory seed germination experiments under two temperature regimes, 10°C and 25°C. At the lower temperature of 10 °C, PY203 maize seed is not able to germinate, demonstrating that similar to the seed of other maize varieties, the seed of PY203 maize is not capable of germination under cool conditions. In contrast, at the higher temperature of 25 °C, PY203 maize seed showed a 92% germination rate with no statistical significant difference compared to that of the control. These results demonstrate that the seed germination characteristics and dormancy potential of PY203 maize are no different than those of other cultivated maize varieties (Agrivida 2019).

Agrivida also evaluated the phenotypic and agronomic characteristics of PY203 maize by comparing with near-isogenic non-transgenic lines as controls under field conditions at six locations representing major U.S. maize growing regions in 2017 and at two locations in Argentina in the 2016/2017 growing season (Agrivida, 2019). The field evaluation results showed no statistically significant differences in ear height, plant height, stay green, root lodging, days to 50% pollen shed, days to 50% silking, or barren plants between PY203 maize and the near-isogenic, non-transgenic control lines. While a statistically significant difference was observed in both emergent and final stand counts, stalk lodging, dropped ears, grain weight, grain test weight and grain moisture between PY203 maize and the control lines, the measured values for each of these characteristics except grain moisture were slightly lower for PY203 maize compared to the control. Furthermore, the lower emergent stand count resulted in a lower final stand count and then further resulted in lower grain weight for PY203 maize. In addition, lower grain test weights that were seen for PY203 maize compared to the control line are expected because the potential deposition of heterologous Phy02 phytase in protein bodies in endosperm led to the formation of floury or opaque maize kernels and floury kernels are often associated with lower grain test weights (Gerde et al. 2016). Therefore, the differences seen in some agronomic characteristics between PY203 maize and the non-transgenic control lines would not be expected to significantly impact the ability of PY203 maize to survive or to be more persistent in the environment compared to conventional maize varieties. These data support the conclusion that PY203 maize is unlikely to develop into feral persistent populations or to be more weedy or invasive in the environment compared to conventional maize varieties.

Additionally, the impacts of environmental abiotic stresses such as wind, hail and high temperature on the growth and vitality of PY203 maize were also evaluated, and no substantial differences in abiotic stress responses were observed between PY203 maize and its near-isogenic control and other reference maize hybrids (Table 18, Agrivida 2019). Also, as previously examined, PY203 maize responded to biotic stressors in a similar manner as its conventional maize comparators. Collectively, the data described herein demonstrate that PY203 maize interacts with the biotic and abiotic aspects of the environment in a manner that is identical to that of conventional varieties of maize.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, PY203 maize is unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, extensive post-harvest monitoring of field trial plots planted with PY203 maize under USDA-APHIS notifications did not reveal any differences in survivability or persistence relative toother varieties of the same crop currently being grown. These data suggest that PY203 maize is no more likely to become a weed than conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants withwhich PY203 maize Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant 1981; Rieseberg and Wendel 1993; Soltis et al. 1993; Hegde et al. 2006), and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace 1987; Rieseberg and Wendel 1993; Peterson et al. 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury et al. 2013a; Khoury et al. 2013b). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (see Table 1 in (Ellstrand et al. 1999)). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the PY203 maize to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa based on the phenotypic changes that have been observed in PY203 maize.

Potential for gene flow, hybridization, and gene introgression

Maize is a wind pollinated species with plant morphology and reproductive biology that facilitates cross pollination, leading to relatively high levels of pollen-mediated gene flow occurrence in this species. However, for pollen-mediated gene flow to occur between maize and its allied species and subspecies, certain conditions must be satisfied such as sexual compatibility, flowering synchrony and sufficient proximity to each other.

Cultivated maize (*Zea mays* L. ssp. *Mays*) is a member of the grass family Graminae (Poaceae). The genus *Zea* consists of five species: 1) *Z. diploperennis*, perennial diploid (2n=20); 2) *Z. luxurians*, annual diploid (2n=20); 3) *Z. nicaraguensis*, annual diploid (2n=20); 4) *Z. perennis*, perennial tetraploid (2n=40); and the *Z. mays*, annual diploid (2n=20). *Z.* The latter encompasses four annual diploid (2n=20) subspecies: ssp. *mays*, ssp. *huehuetenangensis,* ssp. *mexicana* and ssp. *parviglumis* (Hufford et al. 2012). Within genus *Zea*, *Z. mays* ssp. *mays* is the only domesticated maize and all the other species and subspecies are the wild relatives of the maize and are collectively named as teosintes (OTGR 2008; Andersson and de Vicente 2010).

Hybridization with Teosinte

All species in teosintes except the tetraploid perennial *Z. perennis* can cross with cultivated maize to produce fertile hybrids, but typically occur at very low rate (Doebley 1990; Baltazar et al. 2005; OTGR 2008). It is reported that hybridization between maize and *Z. mays* ssp. *mexicana* occurs sporadically and at very low rates (Baltazar et al. 2005; Ellstrand et al. 2007) but maize can hybridize with *Z. mays* ssp. *parviglumis* readily at higher rates (Ellstrand et al. 2007). However, while these hybridizations occurred in the direction of maize as female and teosinte as male, hybridizations in the opposite direction rarely occurred (Ellstrand et al. 2007; Mauricio et al. 2013). Gene flow from maize to teosinte most probably results from crosses where teosinte first pollinates maize (Baltazar et al. 2005). This limited and asymmetric gene flow, favoring teosinte introgression into maize may be attributed to the genetic barrier between teosinte and maize that is controlled by a gene called the *'Teosinte crossing barrier*' (*Tcb*)(Evans and Kermicle 2001). Gene flow and introgression between maize and teosintes is also limited by their geographical distribution, flowering synchrony and proximity.

Teosinte is not native to the U.S. but the annual teosinte (*Z. mays* ssp. *mexicana*) is reported to have feral populations in Florida, Alabama, and Maryland (USDA-NRCS 2019a); *Z. perennis* is listed in Texas and South Carolina (USDA-NRCS 2019b). For *Z. diploperennis* and *Z. luxurians,* there are no reported information about their location and status in U.S.(USDA-NRCS 2019c, 2019d). Experts familiar with the teosinte collections in the United States have been previously consulted and are not aware of the presence of any naturalized or native populations of teosintes in the United States (USDA-APHIS 2013).

Taken together, the genetic barrier, differences in developmental and morphological factors, potential flowering asynchrony and insufficient proximity between maize and teosinte as well as the limited geographical distribution of teosinte make natural crosses and gene introgression from PY203 maize into teosinte unlikely in the United States.

Hybridization with *Tripsacum*

Tripsacum is the genus that is the closet known relative of *Zea* and it consists of 16 recognized species (OECD 2003). *Tripsacum* species has a base chrome number of x=18 compared to the base chromosome of maize (x=10) and can be represented by diploid (2n=36), triploid (2n=54), tetraploid (2n=72), pentaploid (2n=90) and hexaploid

(2n=108). There are five species of *Tripsacum* that are present in the U.S., including three species native to the U.S.: *T. floridanum* (Florida gamagrass), *T. lanceolatum* (Mexican gamagrass), and *T. dactyloides* (Eastern gamagrass); two species introduced in Puerto Rico: *T. latifolium* (wideleaf gamagrass) and *T. fasciculatum* (Guatemalan gamagrass) (USDA-NRCS 2019e, 2019f, 2019g, 2019h, 2019i).

Unlike teosinte that its member species can hybridize with maize under natural conditions, out-crossing between maize and *Tripsacum* species is not known to occur in the wild and can only be made experimentally with extreme difficulty(OECD 2003). *Tripsacum* species (*T. dactyloides*, *T. floridanum*, *T. lanceolatum*, and *T. pilosum*) have been crossed with maize under experimental conditions, however, the resultant hybrids have a high degree of sterility and are genetically unstable (Galinat 1988; OTGR 2008; Andersson and de Vicente 2010). Thus, *Tripsacum* species are unlikely to form viable hybrid progeny with maize under natural conditions.

The introduced genes encoding Phy02 phytase and PMI in PY203 maize are not expected to change the ability of the plant to interbreed with other plant species. Indeed, the agronomic and phenotypic data of PY203 maize provided by Agrivida indicated no unintended changes likely to affect the potential gene flow from PY203 maize to sexually compatible species.

Based on all the above information, the genetic modification in PY203 maize is not expected to increase the potential for gene flow, hybridization and/or introgression to sexually-compatible taxa compared to the other maize varieties. Gene flow, hybridization and/or introgression of genes from PY203 maize to other sexually-compatible relatives with which it can interbreed is not likely to occur in the United States and its territories.

Potential for enhanced weediness of recipients after hybridization and/or introgression

Based on the data presented in the petition, PY203 maize does not exhibit characteristics that may cause it to be any weedier than other cultivated maize based on the data presented in the petition (Agrivida 2019). Furthermore, none of the sexually compatible-relatives of maize in the United States are considered to be weeds in the United States (Holm et al. 1979). Therefore, even in the extremely unlikely event of successful hybrids and/or introgression between PY203 maize and its wild relatives, the inserted transgenes of PY203 maize are unlikely to transform its wild relatives into more weedy species. Moreover, its potential impact due to the extremely limited potential for gene introgression into teosinte and *Tripsacum* species is not expected to be any different than that of other cultivated maize varieties. Based on the above considerations, PY203 maize is unlikely to adversely impact sexually-compatible wild relatives or their weediness characters.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions. The genetic modification in PY203 maize is not expected to increase the potential for gene flow, hybridization, and/or introgression to occur to sexually-compatible taxa compared to the non-transgenic recipient or other varieties of maize that are commonly grown. Gene flow, hybridization, and/or

introgression of genes from PY203 maize to other sexually-compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories is not likely to occur. Furthermore, both the maize and its sexually compatible relative species are not considered weedy or invasive, and the phytase expression conferred by genetic engineering is not likely to increase the weediness of these species. The modified phenotype is not expected to affect the current ability to control these species in situations where they are considered weedy or invasive; the following measures are still available for their control: herbicides, tillage and other methods. Therefore, PY203 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the United States and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from adoption of the PY203 maize are likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

Information contained within the Agrivia petition demonstrates that the cultivation practices needed for growing PY293 maize are similar to that used to grow conventional maize. Additionally, no biologically significant differences in insect abundance, insect and disease damage were observed in field trials or targeted studies of PY203 maize and its near-isogenic non-transgenic control or reference maize hybrid comparators (see Section D. Potential Plant Pest and Disease Impacts). Furthermore, PY203 maize exhibits growth and developmental characteristic that are similar to conventional maize (see Section F. Potential for Enhanced Weediness of PY203 maize). As a result, APHIS does not foresee changes in either insects or disease damage or control measures employed due to agricultural or cultivation practices with PY203 maize. Additionally, 38 modified maizevarieties have been previously evaluated and determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act, in part due to an absence of these introduced traits to substantially alter maize cultivation practices (USDA-APHIS 2019a).

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of PY203 maize; therefore, no impact on plant diseases or pests, or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which PY203 Maize Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into PY203 maize to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic

plants. The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from organisms developed using genetic engineering to another organism without reproduction or human intervention were recentlyreviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombinationand rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in thelong run, to major transitions in evolution (Brown 2003; Keeling and Palmer 2008; Keese2008).

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

PY203 maize contains protein-coding genes (phytase gene *phy02* and *manA*) derived from the *E. coli* and non-coding regulatory elements (nos terminator and T-DNA borders) derived from *A. tumefaciens*. HGT and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al. 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al. 2011; Acuna et al. 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the modified plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al. 2001; Brown 2003; EFSA 2009). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus, even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the US-FDA (US-FDA 1998) and the European Food Safety Authority (EFSA 2009) have evaluated horizontal gene transfer from the use of antibiotic resistance marker genes and concluded that the likelihood of transfer of

antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is very rare or remote.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from the modified plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. PY203 maize contains no sequences from plant viruses. Nevertheless, this issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar torecombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants andno increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al. 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al. 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses withnew properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al. 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant (Yoshida et al. 2010). According to this study, the incorporation of the specific genetic

sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. Furthermore, *S. hermonthica* is not found in the U.S. and *S. asiatica*, another related parasite of cereal crops, is only present in North Carolina and South Carolina (USDA-NRCS 2019j). More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al. 2012) and 24 –41% of mitochondrial (Xi et al. 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore, in PY203 maize, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome (Agrivida 2019).

If PY203 maize becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from PY203 maize. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis, APHIS therefore concludes that HGT of the new genetic material inserted into PY203 maize to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, public comments in response to Federal Register notices concerning this petition, and other relevant information to assess the plant pest risk of PY203 maize compared to the unmodified variety from which it was derived. APHIS concludes that the PY203 maize is unlikely to pose an increased plant pest risk compared to the unmodified variety from which it was derived based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in PY203 maize because the *A. tumefaciens* transformation vector was disarmed, the transformed material was treated with an antibiotic to kill the bacterium, and the inserted plant pest sequences do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified in PY203 maize from expression of the inserted genetic material, the Phy02 phytase and PMI enzyme, or changes in metabolism or composition because there were no significant changes in agronomic, ecological and compositional characteristics that would render PY203 maize more susceptible to pests and diseases over its control or reference maize varieties.

- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in PY203 maize compared to the nontransgenic counterpart or other comparators in field trials conducted in growing regions representative of where PY203 maize is expected to be grown. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that PY203 maize is more susceptible to pests or diseases. Therefore, no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of PY203 maize is unlikely to have any adverse impacts on organisms beneficial to agriculture based on the analysis of studies on PY203 maize food and feed safety and composition.
- PY203 maize is no more likely to become a weed or become weedier than conventional varieties of the crop based on its observed agronomic characteristics, weediness potential of the crop and current management practices available to control PY203 maize as a weed. Volunteers and feral populations of PY203 maize can be managed using a variety of currently available methods and herbicides.
- PY203 maize is not expected to increase the weed risk potential of other species with which it can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from PY203 maize to other sexually compatible relatives with which it can interbreed is not likely to occur. The sexual compatible relatives of maize are not considered weedy or invasive, and the new phenotype conferred by genetic engineering is not likely to increase the weediness of these sexually compatible relatives or affect the current ability to control these relatives in situations where they are considered weedy or invasive.
- Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of PY203 maize were not identified and are not likely to increase plant diseases or pests or compromise their management.
- Horizontal gene transfer of the new genetic material inserted into PY203 maize to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

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