JR Simplot Company Petition (14-093-01p) for Determination of Non-regulated Status for Innate™ Potatoes with Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot and Lowered Reducing Sugars: Russet Burbank Event W8.

OECD Unique Identifier: SPS-000W8-4

Plant Pest Risk Assessment

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A. Introduction

The JR Simplot Company (JR Simplot) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically-engineered (GE) potato (*Solanum tuberosum* L.) event W8 (OECD unique identifier SPS-00W8-4) with late blight resistance, low acrylamide potential, reduced black spot and lower reducing sugars is unlikely to pose a plant pest risk and, therefore, should no longer be regulated articles under the APHIS 7 Code of Federal Regulations (CFR) part 340 (JR Simplot Company, 2014). This petition was assigned the number 14-093-01p, and is hereafter referred to as JR Simplot, 2014. Throughout this document the event will be referred to as W8 potatoes. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000.[1] This plant pest risk assessment was conducted to determine if W8 is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under 7 CFR part 340 if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belong to any genera or taxa designated in 7 CFR §340.2 and meets the definition of plant pest, or is an unclassified organism and/or organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest.[2] The W8 potato event was produced by *Agrobacterium*-mediated transformation and include introduced genetic sequences that were designed based on sequences found in plant pest organisms listed in 7 CFR § 340.2 (i.e., border sequences were designed based on border sequences found in *Agrobacterium tumefaciens*; JR Simplot, 2014, Table 4-2, pp. 47-48 and Table 4-3, pp. 49). Therefore, the W8 potatoes are considered regulated articles under APHIS regulations at 7 CFR part 340. JR Simplot has conducted introductions of W8 potatoes under APHIS-authorized notifications since 2012 (JR Simplot, 2014, Table 11-14, page 180) in part, to gather information to support that W8 potatoes are unlikely to pose a plant pest risk.

[1] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[2] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

Potential impacts to be addressed in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with W8 potato and its progeny and their use in the absence of confinement relative to the unmodified recipient cultivars and/or other appropriate comparators. APHIS used data and information submitted by the applicant, in addition to current literature, to determine if the W8 potato event is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR §340.6(c) specify the information needed for consideration in a petition for non-regulated status. APHIS will assess information submitted by the applicant about the W8 potato event 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; weediness of the regulated articles; effects of the regulated articles on non-target organisms; impact on the weediness of any other plant with which they can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; indirect plant pest effects on other agricultural products; and transfer of genetic information to organisms with which they 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). 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 their characteristics, certain biotechnology-derived products are 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. According to Simplot's petition, an Experimental Use Permit (EUP) application was submitted to EPA on December 16, 2013, for field testing of Innate™ late blight resistant potatoes. An EUP, also for late blight resistant potatoes, with a Petition for Temporary Tolerance Exemption was submitted February 20, 2014. A Section 3 Registration will be filed after experiments are completed under the EUPs (JR Simplot, 2014, page 190).

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 genetically engineered 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 (FDA, 2006) and a more comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984, 1992). JR Simplot stated in their petition that they will complete a consultation process for food safety and nutrition with the Food and Drug Administration (FDA) prior to commercial distribution of potatoes from Russet Burbank W8 (JR Simplot, 2014, page 190).

B. Development of the W8 Potato Events

Potatoes (*Solanum tuberosum*) belong to the genus *Solanum*, section *petota*, subsection *potatoe*, and series *tuberosa*, which consists of approximately 54 species, of which only *S. tuberosum* is widely cultivated for food production (OECD, 1997). *S. tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is the cultivated potato widely in use as a crop plant in North America and Europe, and the subspecies *andigena* is also cultivated, but cultivation is largely restricted to Central and South America (OECD, 1997).

After China, India, Russia, and the Ukraine, the United States is the fifth largest potato producing country (FAO, 2013), with annual production over the last three years of between 404-467 million cwt (centum weight = 100 pounds), grown on 1.0 - 1.1M acres (USDA-NASS, 2013). Potatoes are grown across most of the continental US, with six States (Colorado, Idaho, Minnesota, North Dakota, Washington and Wisconsin) accounting for approximately 75% of annual production (USDA-NASS, 2013). The average American consumes about 115 pounds of potato annually, of which about two thirds is consumed as processed potato products (USDA-ERS, 2010). Different potato varieties have been selected for performance in the fresh and processed markets. The Russet Burbank cultivar is used for fries and table stock and in 2012 it comprised 20.3% of seed acreage in the US (NPC, 2013). The Russet Burbank cultivar is sterile and widely grown in the Northwest and Midwest, especially for the production of French fries with the added benefit of good long-term storage characteristics, and it represents the standard for excellent baking and processing quality (JR Simplot, 2014, pp. 36).

The tetraploid nature of commercial potato varieties is a significant impediment to potato breeding (Hoopes and Plaisted, 1987). Due to more complex chromosome segregation ratios, polyploid crops are inherently more difficult to breed. Furthermore, vegetatively propagated crops like potato are often poor seed producers due to partial or full sterility. For seed propagated crops, like corn or soybean, trait developers often create a single elite event and then backcross that elite event into a wide range of elite germplasm. This is not possible in potato. Each parent variety must be independently transformed to achieve the desired phenotype in that variety. Into the background of the commercial Russet Burbank potato cultivar, JR Simplot has used a genetic engineering approach to introduce four traits that are of interest to potato consumers, producers and processors:

late blight resistance; reduced acrylamide potential in certain processed or heated potato products; reduced black spot bruise and lowered reducing sugars. JR Simplot used two constructs pSIM1278 and pSIM1678 to transform the parental Russet Burbank potato cultivar and created the W8 event described in JR Simplot, 2014. The objective was to incorporate the new phenotypes into this important cultivar, while maintaining all of the desirable characteristics originally selected by potato breeders.

Potato late blight (*Phytophthora infestans*) is a significant problem in potato production. The infamous Irish potato famine of the 1840s was caused by late blight infections. When late blight infected tubers are discarded in fields at harvest time or dumped as waste near fields, the late blight oomycetes from one season can overwinter on the discarded tubers and infect the next season's seedlings and tubers (Schumann et al., 2000). The major movement pathways for the sporangia from infected tubers to healthy plants are through air currents and/or animal movements. Late blight of potato causes necrosis on leaves and stems which may be small at first and appear water-soaked or have chlorotic borders. Freshly cut seed tuber surfaces are especially susceptible to infections from airborne spores in contaminated storage facilities (Schumann et al.).

The W8 potato event could have several positive effects in potato production. The use of fungicides to eradicate late blight in potatoes causes chemical residue in food, land and waste water. The W8 potato event could mean a reduction of 290 million pounds of fungicidal active ingredient in the production of potatoes (Context, 2014). It is estimated that $90 million is spent every year on fungicidal treatment of potato crops in the U.S. with the fungicidal treatment for late blight costing two-thirds ($60 million) of that amount (Context, 2014). The W8 potato event would reduce the need for fungicide applications, lower the fungicide residue content and lower the cost of treatment by approximately $29 million (Context, 2014).

In 2002, Swedish researchers demonstrated that acrylamide forms when starchy foods, such as potatoes and breads, are heated (Tareke et al., 2002). These scientists were looking for the source of acrylamide-induced blood adducts in individuals not known to be exposed to acrylamide. They demonstrated that acrylamide forms when starchy foods are heated, however it was not detected in unheated or boiled foods (Tareke et al., 2002). Therefore, even though dietary exposure to acrylamide is measurable, it is not a natural compositional component of unheated foods derived from plants.

The often desired non-enzymatic browning that occurs when food is cooked is referred to as the Maillard reaction (Martins et al., 2000). Maillard reaction products, which impact the flavor and texture of the cooked food, are formed by a chemical reaction between an amino acid and a reducing sugar. Stadler *et al* (2002) demonstrated that oxidation of the free amino acid asparagine is the main source of acrylamide when starchy foods are baked or fried. Asparagine is a major amino acid in potatoes and cereals (Mottram et al., 2002).

Because acrylamide is a known carcinogen of rodents and a probable carcinogen in people (WHO-IARC, 1994; NTP, 2011) the discovery of acrylamide in cooked potato

products raised concerns throughout the potato processing industry, as well as among consumers. The State of California listed acrylamide as a potential carcinogen under Proposition 65 in 1990 and established a No Significant Risk Level (NSRL) of 0.2 µg/day(CEPA-OEHHA, 2005). Subsequent to the discovery of acrylamide in cooked foods, this NSRL was revised to 1.0 µg/day (CEPA-OEHHA, 2005).

Proposition 65 requires that food manufacturers warn consumers about the dangers of acrylamide in their products. In 2005, the State of California sued Frito-Lay, Kettle Foods and Lance, Inc. for failing to provide such warnings. In the settlement, the potato chip manufacturers agreed to reduce the acrylamide in their products to 275 ppb, low enough to avoid the Proposition 65 warning. These three companies also agreed to pay close to $2M in penalties and court costs. The potato processing industry now has a strong financial incentive to reduce the levels of acrylamide in their retail products.

Black spot bruising is a post-harvest physiological disorder primarily resulting from the handling of potato tubers during harvest, transport and processing. If physical impact causes cell rupture and/or flesh injury, the injured cells release phenolic compounds, normally compartmentalized in the vacuoles, which are converted to *o*-phenols and *o*-quinones by the enzyme polyphenol oxidase (PPO). These quinoids auto-oxidize, forming melanin, leading to blackened tissue which is undesirable in processed potato product (Hunt et al., 1993).

High levels of reducing sugars (glucose and fructose) cause undesirable side effects such as bitter taste and dark colors in fries and chips. The levels may rise from any stress the potato plant encounters in the field but most of the increase in reducing sugars happens during the low temperature storage after harvest (Bethke et al, 2009). Currently potatoes that are meant for fry production are kept at 46^{o}-48^{o}F to maintain sugar levels and aid in the reduction of storage diseases of potatoes (Driskill et al., 2007). If potatoes could be stored at lower temperatures which would lower post-harvest diseases and keep the reducing sugars at decreased levels, the rejection of potatoes by fry and chip producers would be lowered and the quality of the products (fries and chips) would increase. It is theorized that the W8 would lower the levels of reducing sugars throughout the storage period and enable lower storage temperatures for longer storage periods (JR Simplot, 2014, p. 189-190).

The intended purpose of the W8 potato line is to provide the potato processing industry with new varieties with late blight resistance; reduced acrylamide potential in certain processed or heated potato products; reduced black spot bruise and lowered reducing sugars. All of these changes are intended to benefit potato consumers, producers, and processors. The late blight resistance will lower the need for fungicides for control of late blight disease and lessen the discards of potatoes that are infected which would lead increased yield and less chemical residues on potatoes, fields and waste water. The low acrylamide potential is intended to benefit consumers because of concerns about the health effects of ingesting acrylamide, and to benefit the industry relative to Proposition 65. The reduced black spot bruise is intended to benefit consumers by providing a higher quality product, to benefit producers by reducing culls at delivery, and to benefit

processors by reducing pick-outs. By lowering reducing sugars with the use of the W8 potato line, storage of potatoes for the fry and chip market could be accomplished at a lower temperature which would lower storage diseases and keep taste/color quality in the desired ranges.

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

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 GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of 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, suppression of existing genes and their products, or changes in plant metabolism or composition in W8 potatoes relative to their nontransgenic counterparts. The assessment encompasses a consideration of the expressed double stranded RNA (dsRNA) and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested potatoes derived from the GE crop event compared to those in the conventional counterpart 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 GE crop event; 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

The W8 potato event was produced by using *Agrobacterium*-mediated transformation of potato internode explants of the potato variety Russet Burbank, (JR Simplot, 2014, p. 40-41). The W8 potato event was constructed by first transforming Russet Burbank with plasmid, pSIM1278, followed by transformation with a second plasmid, pSIM1678 to produce the stacked traits. The binary plasmid vector pSIM1278 (JR Simplot, 2014, Figure 4.1 p.44), consists of the vector backbone (JR Simplot, 2014, Table 4.1, p.46) and the pSIM1278 DNA insert (JR Simplot, 2014, Table 4.2, p 47-48). The binary plasmid vector pSIM1678 (JR Simplot, 2014, Figure 4.2 , p.44), consists of the same vector backbone as pSIM1278 and the pSIM1678 DNA insert (JR Simplot, 2014, Table 4.3, p 49). Only the DNA insert portions were intended to be transferred to the recipient plants. After transformation, the explants were subjected to antibiotic treatment with 150 mg/L timentin, a concentration previously show to be effective to eliminate the *A. tumefaciens*

vector (Nauerby et al., 1997). Lack of *A. tumefaciens* was confirmed by incubating stem fragments on nutrient agar for 2 weeks at 28° C (repeated twice) with no outgrowth (JR Simplot, 2014, p. 40).

A marker-free selection system was used to eliminate plants in which the vector backbone was inserted into the genome due to inefficient cleavage at the Left Border site (Gelvin, 2003). To this end, JR Simplot inserted the isopentenyl transferase (*ipt*) gene from *A. tumefaciens* into the vector backbone just outside the Left Border (JR Simplot, 2014, Figure 4.1, 4.2 and Table 4.1). Transgenic plants expressing the *ipt* gene, which results in the production of the plant hormone cytokinin, display a stunted phenotype that can be visually identified, allowing the elimination of plants with inserted vector backbone. The remaining plants were then molecularly characterized by PCR and Southern blots to select those containing only the genes of interest, and thus do not contain the *ipt* gene.

Following the transformation and evaluation of the insert associated with the pSIM1278 vector, the plants were transformed using the same methods described above for a second plasmid, pSIM1678, to produce stacked events containing two independent inserts (JR Simplot, 2014, p. 41).

The DNA insert in plasmid pSIM1278 is designed to silence four different genes in the potato: asparagine synthetase-1 (*Asn1*), polyphenol oxidase-5 (*Ppo5*), potato phosphorylase L (*PhL*) and the starch-associated R1 gene (*R1*). For each gene, the expression cassettes are designed to produce dsRNA that functions through a RNA interference (RNAi) mechanism to degrade transcripts for the genes. The suppression of *Asn1* should result in potatoes with reduced free asparagine, and the suppression of *PhL* and *R1* should result in potatoes with a lower content of reducing sugars. Collectively, the silencing of these 3 genes should result in potato tubers with a reduced acrylamide potential. The suppression of *Ppo5* confers the W8 potatoes with a non-browning phenotype resulting in tubers with reduced black spot bruising. The DNA insert of pSIM1278 contains two expression cassettes, the first designed to silence *Asn1* and *Ppo5* and the second cassette designed to silence PHL and R1. With the exception of the left and right borders, all inserted DNA in the plasmid pSIM1278 were derived from *S. tuberosum* var. Ranger Russet or from *S. verrucosum.* Synthetic DNA designed to be similar to and function like *Agrobacterium* T-DNA borders was used to generate the left and right borders.

The first gene cassette of plasmid pSIM1278 consists of seven genetic elements designed to silence the *Asn1* and *Ppo5* genes:

- *pAgp*, the ADP glucose pyrophosphorylase promoter sequence from *S. tuberosum.* This promoter directs the silencing construct in the antisense orientation.
- *fAsn1*, a fragment of the protein coding region of the *Asn1* gene from *S. tuberosum*, arranged in antisense orientation (Chawla et al., 2012).
- *tPpo5*, the 3' untranslated leader sequence of the *Ppo5* gene from *S. verrucosum*, arranged in antisense orientation.

- Spacer-1, a 10 kb fragment derived from *S. tuberosum*, inserted between the two inverted repeats to create the hairpin which enhances gene silencing.
- *tPpo5*, the 3' untranslated leader sequence of the *Ppo5* gene from *S. verrucosum*, arranged in sense orientation.
- *fAsn1*, a fragment of the *Asn1* gene sequence from *S. tuberosum*, arranged in sense orientation.
- *pGbss*, the granule-bound starch synthase promoter sequence from *S. tuberosum.* This promoter directs the silencing construct in the sense direction, and is oriented convergently relative to the first *pAgp*.

The second gene cassette of plasmid pSIM1278 consists of seven genetic elements designed to silence the *PhL* and *R1* genes:

- *pAgp*, the ADP glucose pyrophosphorylase promoter sequence from *S. tuberosum.* This promoter directs the silencing construct in the antisense orientation.
- *pPhL*, a fragment of the *PhL* promoter from *S. tuberosum*, arranged in antisense orientation.
- *pR1*, a fragment of the *R1* promoter sequence from *S. tuberosum*, arranged in antisense orientation.
- Spacer-2, a 257 bp fragment derived from *S. tuberosum*, inserted between the two inverted repeats to create the hairpin which enhances gene silencing.
- *pR1*, a fragment of the *R1* promoter sequence from *S. tuberosum*, arranged in sense orientation.
- *pPhL*, a fragment of the *PhL* promoter from *S. tuberosum*, arranged in sense orientation.
- *pGbss*, the granule-bound starch synthase promoter sequence from *S. tuberosum.* This promoter directs the silencing construct in the sense direction, and is oriented convergently relative to the second *pAgp*.

The first cassette is preceded by the left border and the second cassette is followed by the right border. Table 4.2 in JR Simplot, 2014 (p.47-48) provides additional details for all of these genetic elements.

The DNA insert in plasmid pSIM1678 is designed to confer the late blight resistance gene (*Rpi-vnt1*) and a vacuolar invertase (*Vlnv*) silencing cassette. "The *Rpi-vnt1* gene cassette consists of the VNT1 protein coding region regulated by its native promoter and terminator sequences and the silencing cassette consists of an inverted repeat of sequence from the potato *Vlnv* gene flanked by opposing plant promoters, pGbss and pAgp" (JR Simplot, 2014, p.43). The expression of *Rpi-vnt1* should result in potatoes with late blight resistance and the silencing of *Vlnv* should prevent reducing sugar accumulation in cold-stored tubers (Wu et al., 2011). With the exception of the left and right borders, all inserted DNA in the plasmid pSIM1678 were derived from *S. tuberosum* var. Ranger Russet, *S. venturii* or from *S. verrucosum.* Synthetic DNA designed to be similar to and function like *Agrobacterium* T-DNA borders was used to generate the left and right borders.

The gene insertion from plasmid pSIM1678 consists of seven genetic elements designed to confer the late blight resistance gene (*Rpi-vnt1*) and include a vacuolar invertase (*Vlnv*) silencing cassette:

- *pVnt1*, the native promoter sequence from *S. venturii*. This promoter drives the expression of the late blight resistance gene *Rpi-vnt1*.
- *Rp1-vnt1, S. venturii* late blight resistance protein gene.
- *tVnt1*, the native terminator sequence from *S. venturii*. This promoter drives the expression of the late blight resistance gene *Rpi-vnt1*.
- *pAgp*, the ADP glucose pyrophosphorylase promoter sequence from *S. tuberosum*. This promoter directs the silencing construct in the antisense orientation.
- *Inv*, a fragment of the acid invertase sequence from *S. tuberosum* var. Ranger Russet, arranged in antisense orientation.
- *Inv*, a fragment of the acid invertase sequence from *S. tuberosum* var. Ranger Russet, arranged in sense orientation.
- *pGbss*, the granule-bound starch synthase promoter sequence from *S. tuberosum* var. Ranger Russet. This promoter directs the silencing construct in the sense direction, and is oriented convergently relative to *pAgp*.

The *Rpi-vnt1* gene is preceded by the left border and the vacuolar invertase (*Vlnv*) silencing cassette is followed by the right border. Table 4-3 in JR Simplot, 2014 provides additional detail for all of these genetic elements.

Through Southern Blot hybridizations, PCR and DNA sequencing, the characterizations of the inserts of W8 potato event were determined. It was determined that one insert of pSIM1278 and one insert of SIM1678 had been achieved (JR Simplot, 2014, pg. 54-56). One Southern Blot analysis showed that the pSIM1278 insert was more complex than expected when a larger fragment was found in the insert junctions following a BciVl restriction digestion

JR Simplot provided evidence demonstrating that:

- The *A. tumefaciens* strain AGL1 that was used to transform the 5 parental varieties is nonpathogenic, having been disarmed of sequences which lead to tumor formation in plants (Lazo et al., 1991).
- The W8 event did not contain vector backbone sequences as determined through Southern blot and Polymerase Chain Reaction assays (JR Simplot, 2014, p. 80-86).
- The genome of the W8 potato event contains one copy of full insert between the Left and Right borders of pSIM1278 and of pSIM1678 (JR Simplot, 2014, p. 54-56).
- The insertions in the W8 potato events were stably inherited through at least three generations of vegetative propagation, as confirmed by DNA gel blot hybridization and a phenotypic assay for the suppression of PPO (JR Simplot, 2013, p. 86-91). Since potatoes are not propagated by seed, stability through seed generations is not relevant to the present analysis.

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

APHIS assessed whether changes in plant metabolism or composition of W8 potatoes are likely to alter plant pest risk relative to the untransformed control. The assessment encompasses (1) characterization and safety of VNT1 protein (2) a consideration of the specific effects on plant metabolism due to silencing *Asn1*, *Ppo5*, *PhL*, *R1*and *Vlnv*; and (3) evaluation of whether the nutrient and antinutrient levels in harvested tubers of the W8 event are comparable to those in the parental variety. Host plant quality (including such components as carbon, nitrogen, amino acid sources, trace elements, and defensive metabolites) is known to affect herbivore performance and fecundity; and higher-trophic level interactions, such as the performance of predators and parasitoids, may also be affected (reviewed byAwmack and Leather, 2002). Similarly a vast array of secondary metabolites in plants is known to provide defense against microbes (Dixon, 2001). Thus changes in host plant quality and composition have the potential to affect the performance of W8 potatoes against pests and diseases and to affect the suitability of W8 tubers as a food source for non-target organisms.

Gene expression

JR Simplot inserted the gene *Rpi-vnt1* into the W8 event using the construct pSIM1678. *Rpi-vnt1* leads to the production of VNT1 protein which gives the host broad resistance spectrum against late blight (*Phytophthora infestans*) in potatoes. *Rpi-vnt1* is one of three genes found in the wild potato species *Solanum venturii* that confers late blight resistance and is identical to *Rpi-phul* gene in *Solanum phureja* (Sliwka et al. 2013). VNT1 represents only one of 68 distinct R-genes that have been described from wild *Solanum* species and it illustrates how important these genes could become to potato biology (Rodewald and Trognitz, 2013). A major advantage of using the type of protein that *Rpi-vnt1* encodes (VNT1) is that it does not produce a toxin or directly attack a pathogen but instead it activates an immune response in the plant which leads to necrosis of infected tissues.

- Levels of VNT1 protein in various tissues of W8 event potatoes were measured using Western Blots and the levels were so low (estimated at ~18 ppt) that that it was undetectable on the blots (JR Simplot 2014, p. 96-97).
- Quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) was subsequently used to measure *Rpi-vnt1* transcript expression (See JR Simplot, 2014, Appendix A for methods and normalization standards). Expression levels were low but were much higher than found in the parental variety (JR Simplot, 2014, Figure 6-4).
- RT-qPCR showed *Rpi-vnt1* transcript expression in roots, leaves, stems, flowers and tubers. All tissues showed low levels of expression and Vnt1 protein accumulation.
- Well-established bioinformatics tools were used that show that it is highly improbably that there is a toxin or allergen risk associated with the *Rpi-vnt1* gene (JR Simplot, 2014, p. 124-125).

There is only one other gene of possible concern in the W8 event. The *ipt* gene is used for negative selection but it resides in the vector backbone and is not expressed since there is no vector backbone present in the W8 event (JR Simplot, 2014, Figure 4.1, 4.2 and Table 4.1).

Gene Silencing

To assess the efficacy of the gene silencing constructs, JR Simplot examined *Asn1*, *Ppo5*, *PhL*, *R1*and *Vlnv* transcript levels in tubers, roots, stems, leaves, and flowers in the W8 events. In addition, to assess the efficacy of gene silencing and determine whether there are any unintended compositional changes to W8 potatoes, JR Simplot compared the tuber composition of the JRS06-W8 events with the tuber composition of its parental variety (Russet Burbank). JR Simplot selected locations for trials to represent the major potato production areas in the US. Trials were conducted in Idaho, Washington, North Dakota, Minnesota and Wisconsin; states that account for more than 60% of US potato production (USDA-NASS, 2013). The W8 event was sampled both years (2012 and 2013) and the data presented as combined analyses across locations and years.

The exact function of PPO in plants remains elusive (Bachem et al., 1994; Mayer, 2006). The potential role of PPO in plant defense against pests and pathogens is discussed in the next section. In potato, PPO is involved in black spot bruise formation, which reduces the quality of harvested tubers (Bachem et al., 1994; Corsini et al., 1999; Partington et al., 1999). PPO oxidizes monophenols and *o*-diphenols to *o*-quinones, which are then further oxidized non-enzymatically to polyphenols. These dark-pigmented polyphenols, also referred to as melanins, result in the darkening of potato tissue following mechanical bruising.

Black spot bruise can lead to economic losses as high as 20% (Partington et al., 1999). The potato industry therefore has a vested interest in minimizing these losses. Bachem et al. 1994 demonstrated that black spot bruise can be reduced by silencing *Ppo* genes in potatoes, and JR Simplot, 2014 has further developed this concept in the design of W8 potatoes.

PPO is not substrate specific and is capable of oxidizing a wide variety of monophenols and *o*-diphenols. Chlorogenic acid is the major phenolic component in potato tubers, making up 90% of the total phenolic content (Friedman, 1997), and is therefore the primary PPO substrate in potato tubers. There is a correlation between PPO activity and the rate of browning in different potato varieties, but browning is not correlated with content of chlorogenic acid. Internal blackspot in potatoes is caused by internal enzymatic browning type reactions initiated by PPO catalysis with tyrosine as the primary substrate (Friedman, 1997).

In potato, PPO comprises a multigene family with at least six genes differing in temporal and tissue-specific expression (Thygesen et al., 1995). Thygesen and colleagues reported that one member of the PPO gene family, *Ppo5* (called *POT32* in Thygesen *et al* 1995) is the primary form found in potato tubers, and is the primary message detected in older tubers. While also expressed in roots, no expression was detected in photosynthetic

tissues. *NOR333* (also called *P2*), another member of the potato PPO gene family, was detected in young leaves and tissue near the tuber skin, but was highly expressed in flowers (Thygesen et al., 1995). Other *PPO* genes are expressed in tubers in lower amounts: *POT33* is expressed mainly in the tissue near the skin and *POT72* is expressed in developing tubers; expression patterns for *POT41* and *P1*, which are 95% and 97% homologous with *Ppo5* and *NOR333* respectively, were not reported (Thygesen et al., 1995). W8 potatoes are engineered to specifically reduce expression of *Ppo5*.

JR Simplot provides evidence demonstrating that:

- Based on qualitative RNA (northern) blot analyses of various tissues of the W8 event and non-transgenic control varieties, *Ppo5* transcripts were down-regulated in tubers, but not in stems, roots, leaves and flowers (JR Simplot, 2014, Figures 7-2 through 7-6, p. 104-106).
- Using a catechol assay, there was a lack of functional PPO activity in tubers of the W8 event (see Figure 10-3; JR Simplot, 2014, p. 143-144) and thus lessened black spot potential. In contrast, Russet Burbank are more susceptible to black spot bruise (Potato Association of America, 2009).

W8 potatoes were designed to have reduced levels of free asparagine by silencing *Asn1* as well as reduced levels of reducing sugars by silencing *PhL, R1*and *Vlnv*. Together, the silencing of these three genes was expected to reduce the acrylamide content in cooked potato products.

JR Simplot qualitatively examined the *Asn1*, *R1*, *PhL* and *Vlnv* transcript levels in tubers from field and greenhouse grown plants and in roots, stems, leaves, and flowers of greenhouse grown plants (JR Simplot, 2014, JR Simplot, 2014, Figures 7-2 through 7-6, p. 104-106). This data are summarized below. Free glutamine, free glutamic acid, free asparagine, free aspartic acid and reducing sugar levels in tubers were also examined (JR Simplot, 2014) and tubers were processed into French fries and potato chips in order to analyze acrylamide content (JR Simplot, 2014, p. 142). JR Simplot provides evidence demonstrating that:

- Based on qualitative RNA (northern) blot analyses of various tissues of the W8 event and non-transgenic control varieties, *Asn1* transcripts were down-regulated in tubers, but not in stems, roots, and leaves. (JR Simplot, 2014, Figures 7-2 through 7-5, p. 104-106). There was some evidence of *Asn1* silencing in flowers but the down-regulation was minimal compared in tubers (JR Simplot, 2014, Figures 7-6, p. 106).
- The W8 event displayed a statistically significant reduced content of free asparagine.
- The W8 event showed higher levels of free glutamine, free glutamic acid and free aspartic acid compared to the parental variety control. Figure 10-1 shows how reducing the content of free asparagine can increase the amount of these other amino acids (JR Simplot, 2014, p.138).
- *R1* and *Phl* transcripts were minimally down-regulated in the W8 event (JR Simplot, 2014, Figures 7-2 through 7-5, p. 104-106). This minimal effect has

been observed with other events that contain the pSIM1278 construct (Collinge and Clark, 2013).

- Based on qualitative RNA (northern) blot analyses, *Vlnv* transcripts were down-regulated in tubers. (JR Simplot, 2014, Figures 7-2, p. 104).
- Invertase activity related to the *Vlnv* silencing cassette was measured using tubers stored at 39^{o}F for one month. Three replicates were used of the control and W8 potatoes. The W8 event showed an 85% reduction in vacuolar invertase activity over the control (JR Simplot, 2014, p139-140).
- Reducing sugar levels in control and W8 potatoes, during long-term storage were also measured that show that the *Vlnv* transcripts' down-regulation did lead to a reduction in the reducing sugars. JR Simplot ran long-term storage experiments in order to measure reducing sugars (glucose and fructose) and non-reducing sugar (sucrose) levels of the W8 event and its parental control. The experiments were carried out at 46^{o}F (the usual storage temperature for tubers destined for the fry and chip industry) and at 38^{o}F (the storage temperature that down-regulation of *Vlnv* would allow for the same type of tubers). Sugar levels were taken at harvest, 3 months, 6 months and 9 months. At all the time points and the two temperatures, the reducing sugars of the W8 event were lower than the control (JR Simplot, 2014, Tables 10-2 and 10-3, p. 141). This lowering of the reducing sugars could be attributed to the silencing of the *Vlnv* gene. All W8 tubers had higher sucrose levels because silencing of the *Vlnv* gene inhibited the ability of sucrose to convert to glucose and fructose. The levels of the reducing sugars did rise over time but remained consistent with the levels measured in the control tubers.
- Cooked potato products from fresh tubers, as well from tubers after up to 9 months in storage (46^{o}F and 38^{o}F), had statistically significant reductions in acrylamide content. (JR Simplot, 2014, Table 10-4 & 10-5, p. 142-143).

The silencing of two of the genes *Asn1* and *Vlnv* was achieved. Reductions in free asparagine, and increases in free glutamine in the -W8 event suggests that the level of silencing of *Asn1* was sufficient to achieve the desired low acrylamide phenotype. Considering the significant differences in reducing sugar content in the W8 tubers relative to their parent variety (JR Simplot, 2014, Tables 10-2 and 10-3, p. 141), the efficacy of silencing *Vlnv* conferred by pSIM1678 conferred the desired phenotype of lower levels of reducing sugars and contributed to lower acrylamide.

JR Simplot also analyzed tubers of the W8 event and its respective parent for the following constituents: protein, fat, ash, crude fiber, carbohydrates, calories, moisture, vitamin B_3, vitamin B_6, vitamin C, Cu, Mg, K, amino acids, and glycoalkaloids (JR Simplot, 2014, Section 8, p. 109-120). This compositional analysis of tubers confirmed the following:

- For key proximates, vitamins, and minerals, there were no statistically significant differences between W8 event and its parent variety for content of protein, fat, ash, crude fiber, carbohydrates, calories, moisture, niacin (vitamin B3), copper,

magnesium or potassium. However, there was a statistically significant decrease in pyridoxine (vitamin B6) and a statistically significant increase in vitamin C content (JR Simplot, 2014, Table 8-1, p. 110). The increase of vitamin C content and the decrease of pyridoxine (vitamin B6) were within the tolerance intervals and combined literature ranges (JR Simplot, 2014).

- For amino acids, the W8 event had no statistically significant changes in 8 of the 17 amino acids when compared to its parental variety. There were increases in alanine, glycine, leucine, proline, serine, threonine, and tyrosine compared to the parental variety. These increases were within tolerance intervals and combined literature ranges (JR Simplot, 2014, Table 8-2, p. 111-112).
- There were decreases in aspartic acid compared to the parental variety but this was expected based on the intended reduction of asparagine.
- There were increases in glutamic acid compared to the parental variety but this was expected since the reduction of asparagine leads to increases in glutamic acid.
- In all cases, the tolerance intervals were generated from six non-GE commercial varieties grown concurrently at the same field sites (JR Simplot, 2014, Table 8-4, p. 115).
- There was no significant difference between the W8 event and its parental variety for mean glycoalkaloid toxin content (JR Simplot, 2014, Table 8-3, p. 114. It was concluded that the glycoalkaloids content was therefore no different between the W8 event and the parental variety, Russet Burbank.

Based on the compositional analyses presented in JR Simplot, 2014 (Section 8, p. 109-120), APHIS concludes that although raw tubers had some statistically significant differences in nutritional components (vitamins C and B6; certain amino acids) compared to the parental variety (Russet Burbank), they are within the normal range for commercial potato varieties. For the few cases when significant differences were detected between the W8 event and its parental variety, the mean values for the W8 event all fell within the tolerance interval and within the combined literature range for potatoes. The primary *Ppo* gene expressed in roots and tubers, is suppressed in the W8 event, and reduction in PPO activity in W8 event tubers has been demonstrated. As discussed in the next section, the W8 potatoes were not observed to consistently exhibit any increase in susceptibility to plant pathogens or pests as a result of the reduction in PPO activity or the expression of the late blight resistance gene *Rpi-vnt1*.

APHIS therefore concludes that the W8 potatoes pose no more of a plant pest risk from the introduction of the *Rpi-vnt1* gene, changes to plant metabolism or composition than its respective parental variety or other conventional potato varieties.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, DNA sequences from plant pests, or from any other expression products such as new enzymes, proteins, changes in plant metabolism or composition in the W8 potatoes. These impacts are known to or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as

identified from the previous section). APHIS also assessed whether the W8 potatoes are likely to have significantly increased disease and pest susceptibility based on data and observations from field trials and laboratory experiments 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 GE 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 (USDA-APHIS, 2013a).

Currently, PPQ has active pest management programs for the golden nematode (*Globodera rostochiensis*) and the pale cyst nematode (*Globodera pallida*), both of which can be very destructive pests of potato (USDA-APHIS, 2013a) . Golden nematode is a major potato pest in Europe and is currently limited to Long Island and six counties in western New York. Pale cyst nematode is widely distributed throughout the world, and was first detected in the United States in Idaho in 2006. It is currently limited to a five mile radius within two counties in Idaho. PPQ also has an active pest management program for potato tuber necrotic ringspot disease (PTNRD) caused by necrotic strains of Potato Virus Y (PVYN) (Bamberg to Doley_Wild Potatoes_03-14-12_a/b). PVYN is common in Europe and found in Canada and was first reported in the U.S. Pacific Northwest in 2002. In the early 1990's, Canada and the U.S. implemented a Management Plan with the objective of controlling its spread. Two additional potato pathogens, *Synchytrium endobioticum* (the cause of potato wart) and *Ralstonia solanacearum* race 3 biovar 2 (the cause of brown rot) are APHIS quarantine pests and are listed as Select Agents in the U.S. (US-FSAP, 2014). *S. endobioticum* is the most important world-wide quarantine pest of potato. In 2007, USDA established a quarantine plan for *S. endobioticum*, to be used in the event of its detection in the U.S. (USDA, 2007). *R. solanacearum* race 3 biovar 2 is found worldwide except the U.S. and Canada. There have been sporadic introductions into the U.S. via imports of geranium plants. These introductions have been limited to greenhouses. In 2008, USDA-APHIS established New Pest Response Guidelines for this pathogen, to be used in the event of its detection in the U.S. (USDA-APHIS, 2008b). Additional information on all of these programs can be found on the PPQ website (http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml)

The potato crop is intensively managed with integrated pest management (IPM) to control a variety of insect and disease (Johnson, 2007). In particular, infestations of Colorado potato beetle (*Leptinotarsa decemlineata*) and the late blight fungus (*Phytophthora infestans*) are often countered by spraying insecticides and fungicides, respectively. Other economically important potato insect and disease pests managed in the IPM programs include: the green peach aphid (*Myzus persicae*); wireworms (*Limonius californicus, L. canus, Ctenicera pruinina*), which damage tubers and shoots; the potato leafhopper (*Emposasca fabae*); and the early blight fungus (*Alternaria solani*). Potatoes can also become infected with a number of viruses (e.g. potato leafroll virus, and potato viruses A, M, X and Y); however, IPM options for virus pests are limited to insecticidal control of their insect vectors and planting of resistant varieties (Arif et al., 2012).

JR Simplot presented pest and disease response data from the W8 event from at least two distinct sources: (1) They took observational data on any insects or diseases present in the regulated trials conducted in 2012 and 2013 and (2) they conducted laboratory assays and field disease assays to generate response data to one important disease of potato: late blight caused by *P. infestans* (laboratory and field assays) and one important symptom of potato: black spot caused by a leakage of polyphenol oxidase (laboratory assays only) (JR Simplot, 2014).

In 11 field trials conducted in 2012 and 2013, JR Simplot collected data on insect, disease and abiotic stressors. Field trial sites were selected to represent the major production areas for potatoes, including Russet Burbank, in the US. All field trials were conducted in Idaho, Washington, North Dakota, Minnesota and Wisconsin. The agronomic practices and pest control measures employed were location-specific, recommended by experts, and typical for potato cultivation. The events and untransformed varieties received identical inputs and treatments within each site.

Stressor data were collected early, mid, and late season on insect populations, disease symptoms, and abiotic stressor symptoms. A list of common insect pests and potato diseases is presented (JR Simplot, 2014, Table 11-5). Stressors were rated on a 0-3 scale (0=stressor not present, 1=present, 2=moderate, 3=severe). The range of ratings for the W8 event and the Russet Burbank control were compared for each observation. An observed difference occurred when the ratings range of W8 did not overlap with the ratings range of the Russet Burbank control.

During 92 individual observations of 7 insect stressors, three differences were noted between the W8 event and the Russet Burbank control. W8 had greater stress from Colorado potato beetles during 2 of 32 observations (1 in Minidoka County, ID and 1 in Adams County, WI) and greater stress from loopers during 1 of 7 observations (1 in Minidoka, County, ID).

During 95 individual observations of disease stressors, 8 differences were noted between the W8 event and the Russet Burbank control. The control had greater disease stress from Early Blight during 2 observations out of 29 observations (2 in Grant County, WA).

Similarly, the W8 event had greater disease stress from Early Blight during 2 out of 29 observations (1 in Jerome County, ID and 1 in Adams County, WA). The W8 event also had greater disease stress from Late Blight during 2 out of 21 observations (1 in Jerome County, ID and 1 in Adams County, MI). Multiple replicated field trials with inoculated strains of late blight confirmed significant late blight resistance in the W8 event compared with the Russet Burbank control as tested by expert plant pathologists at Michigan State University (JR Simplot, 2014, Chapter 10: Trait Efficacy). The W8 event had greater disease stress from *Verticillium* during 2 out of 8 observations (1 in Canyon County, ID and 1 in Grant County, WA).

During 69 observations of the eight abiotic stressors, three differences between the W8 event and the Russet Burbank control were noted. In 2 out of 25 observations, the W8 event had greater heat stress (1 in Canyon County, ID and 1 in Minidoka County, ID). W8 also had greater nutrient imbalance in 1 out of 6 observations (Adams County, WI).

Table 1. Field Trial Insect and Disease Stressor Observations for W8 event in 2012-2013

Stressor	Total # Observations for each Event (W8 observations outside the range of control)
Insects	**W8**
Aphids	30
Colorado Potato Beetle	32 (2)
Grasshopper	2
Leaf Hopper	8
Loopers	7 (1)
Psyllids	11
Stink Bugs	2
Insect Totals	92
Diseases	**W8**
Black Dot	2
Black leg	5
Early Blight	29 (2)
Fusarium	1
Grey Mold	2
Late Blight	21 (2)
Leaf Roll virus	3
Psyllids	2
Rhizoctonia	3
Sclerotina	3
Stem Rot	3
Verticillium	8 (2)
White Mold	13
Disease Totals	95

Adapted from JR Simplot, 2014, Table 11-5

JR Simplot evaluated resistance to late blight *(P. infestans)* in both laboratory and field experiments. Tests with foliage and tubers were conducted as quantitative trials to demonstrate the efficacy of late blight resistance in the W8 event to common strains in the U.S. (JR Simplot, 2014, Table 10-6). Tests were conducted by Pennsylvania State University, Michigan State University, and by an independent potato researcher in northern Idaho. A set amount of inoculum was applied to the foliage or tubers so that W8 potatoes and the Russet Burbank control potatoes would be treated equally. The inoculum was applied in late July or August in the foliar tests and the plants were evaluated based on percent foliar infection.

Tubers supplied by JR Simplot to scientists at Michigan State University were inoculated and evaluated by comparing percent tuber infection in W8 and the Russet Burbank control after inoculation.

Table 2. Late Blight Inoculation Study Information

Year	Study	State	Trial Type	Material Tested	Inoculation Date	Inoculum Genotype
2012	Tubers	WA	Lab	W8, Control	Lab Assay	US22, US8
2013	Foliar	MI	Field	W8, Control, References	7/26/2013	US22, US23
2013	Foliar	PA	Field	W8, Control, References	8/8/2013	US23
2013	Foliar	ID	Field	W8, Control, References	8/1/2013	US8

Adapted from JR Simplot, 2014, Table 10-6

Results from both the foliar and tuber late blight tests are summarized below (JR Simplot, 2014, Table 10-7, Table 10-8). A significant reduction in percent foliar late blight infection and tuber late blight infection was detected for the W8 event compared to the Russet Burbank control. For the foliar tests, each trial site had different strain isolates depending on the strains that were found in that area and included US8, US22, or US23. At the end of the field trial period, the last rating from each site is summarized to illustrate the effect of the potato late blight resistance gene on foliar resistance in the W8 event. The tuber tests included US8 and US22 strain isolates.

Table 3. Percent Foliar Late Blight Infection at Last Rating

Variety	Mean Percent Foliar Late Blight Infection
Control	58.3
W8	0.50

Adapted from JR Simplot, 2014, Table 10-7

Table 4. Percent Tuber Late Blight Infection

Isolate	Variety	Mean Percent Late Blight Tuber Infection
US-22	Control	100.0
US-22	W8	51.0
US-8	Control	67.0
US-8	W8	21.1

Adapted from JR Simplot, 2014, Table 11-8

The late blight stressor observations reported more late blight in the W8 event than in the controls. However, the quantitative test results with late blight inoculum on tubers and foliage support the conclusion that the late blight resistance gene confers strong resistance in potatoes. The stressor observations are subjective rather than quantitative data dependent on the principal investigators professional opinion.

The Russet Burbank W8 event also contains a silencing cassette to reduce the polyphenol enzyme (PPO) enzyme in tubers, resulting in a reduced incidence of black spot. Black spot is a discoloration of potato tubers caused by the leakage of PPO from damaged plastids into the cytoplasm. Dark precipitants are formed in the cytoplasm when the enzyme oxidizes polyphenol. In order to improve potato quality by reducing expression of the enzyme responsible for black

spot, the potato *Ppo5* gene was silenced in the W8 event. A catechol assay was used to demonstrate trait efficacy. An indirect method based on the application of catechol to the cut surfaces of tubers was used to test for black spot tolerance. In this assay, two slices each from 10 tubers of W8 and the Russet Burbank control (JR Simplot, 2014, Table 10-9) were tested by the application of 1ml catechol to the cut surfaces of randomly chosen W8 and Russet Burbank control tubers. All -W8 tubers remained unchanged while all the Russet Burbank control tubers turned darker in color within 20 minutes. These results indicate black spot resistance in the W8 event as compared to the Russet Burbank control tubers supporting the efficacy of the reduced black spot trait. Additional enzymatic assays were conducted to measure PPO activity. These assays showed that the W8 event had a 90% decrease in PPO activity compared to the Russet Burbank control. The reduced PPO activity in the W8 tubers was intentional. It is associated with reduced black spot through silencing of the *Ppo5* gene.

Table 5. Origin of Tubers from 2013 Field Trials for PPO Assay

Site Code	Material Tested	Trial Design[1]
Minidoka County, ID	W8, Control	RCB, 4 reps
Grant County, WA	W8, Control	RCB, 4 reps
Grand Forks County, ND	W8, Control	RCB, 4 reps

[1]RCB=Randomized Complete Block design. Number of blocks was equal to the number of reps.
Adapted from JR Simplot, 2014, Table 10-9

As part of the agronomic assessment, JR Simplot also conducted a disease susceptibility assessment. Quantitative assays demonstrated that W8 confers resistance to late blight by expressing the VNT1 protein without effecting susceptibility or resistance to other diseases. The VNT1 protein induces resistance against late blight with a broad spectrum against *P. infestans* isolates (Foster et al. 2009). Several researchers have also investigated the relationship between polyphenoloxidase (PPO) and disease resistance (Hakimi et al. 2006; Li and Steffens 2002; Valentines et al. 2005) with some proposing that enhanced PPO may increase resistance to disease, while others claim that reduced PPO could increase resistance. Due to the evidence that a relationship exists between PPO and diseases and the direct relationship between the VNT1 and disease, JR Simplot tested W8 and the Russet Burbank control for trait specificity by testing for resistance to 3 common potato foliar diseases, one common potato stem/stolon disease, and 6 common potato tuber diseases.

The disease susceptibility assessment evaluated whether W8 demonstrated altered disease susceptibility other than the intended increased resistance to late blight. Quantitative assays were performed by inoculating the most relevant susceptible parts of the plant with common potato diseases. Tests on foliage were conducted for *Alternaria alternata* (early blight), *Alternaria solani* (brown leaf spot) and *Botrytis cinerea* (botrytis leaf spot). Stolons and stems from field grown potato plants were tested for resistance to *Rhizoctonia solani* (black scurf). Field grown tubers were also tested for *Rhizoctonia solani*, *Fusarium sambucinum* (dry rot), *Phytophthora erythroseptica* (pink rot), *Pythium ultimum* (phythium leak), *Pectobacterium carotovora* (soft rot), and *Streptomyces scabies* (common scab). The assays were conducted by expert pathologists and results were statistically analyzed. Details of each disease specificity trial are summarized in JR Simplot 2014, Table 11-11 (2012 and 2013 Disease Study Details).

Overall, W8 and the Russet Burbank control showed no difference in susceptibility out of 3 foliar diseases, 1 stem/stolon disease and 6 tuber diseases analyzed with the exception of a reduction in tuber coverage and severity of common scab caused by *S. scabies* (JR Simplot 2014, Table 11-12. Disease Susceptibility Assessment Results).

W8 had less scab than the control as measured by mean percent coverage and the lesions were less severe than the control. *S. scabies* susceptibility will be monitored in future field and storage studies to determine if this pattern of resistance persists in commercial production. Not only would *S. scabies* resistance be considered a positive finding, but also *S. scabies* resistance would not enhance the plant pest potential of W8.

No biologically meaningful differences were observed for W8 compared to the Russet Burbank control in susceptibility or resistance to insect pests or potato diseases, other than increased resistance to some strains of the late blight fungus. These observations, along with APHIS assessment of the transformation process, DNA sequences from plant pests, expressed products, and changes in plant metabolism or composition support a conclusion that the plant pest potential for W8 is no different than the Russet Burbank control.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

W8 potatoes are not engineered for insect resistance, thus there are no "target" or "nontarget" insect species. APHIS assessed whether exposure to or consumption of W8 potato plants would have a direct or indirect adverse effect 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 the W8 potatoes compared to the non-GE Russet Burbank control for many substances (proteins, RNAi, nutrients, anti-nutrients, metabolites, etc.) produced by the GE plant that may be novel or expressed at significantly altered amounts, changes in the phenotype, and/or any reported impacts on organisms beneficial to agriculture.

W8 potato tubers are nutritionally and compositionally similar to the respective parental control and other commercial potato varieties with the exception of the intentional changes conferred by the introduced or silenced genes. These intentional changes include late blight resistance, low acrylamide potential, reduced black spot, and lowered reducing sugars. The altered composition of W8 potato tubers is unlikely to alter the suitability of potato tubers as a foodstuff for beneficial organisms.

JR Simplot collected 92 total observations of insect stressors during field trials of the W8 potatoes, of which only 3 were reported with minor differences from the Russet Burbank control indicating that W8 potatoes do not cause harm to insect pests that feed on potato leaves. By extension, W8 potatoes are unlikely to adversely affect other insects that may feed on potato leaves. None of the insect stressor observations included insects that damage or feed on flowers. Beneficial organisms associated with potatoes in the field

include pollinators, particularly bumblebees (OECD, 1997). *Asn1* transcripts were down-regulated in W8 flowers suggesting that asparagine levels might be reduced in the flowers of the W8 event. However, asparagine is not an essential amino acid for honey bees, which are in the same family as bumblebees (Cook et al., 2003), or many other insects (Boudko, 2012). Thus, it is unlikely that there will be a negative effect on bumblebees as a result of potential altered composition of -W8 flowers. Moreover, since potatoes are propagated vegetatively from seed potato tuber pieces, pollination by bumblebees is not important to potato tuber production. In addition to observations on insect stressors, there were no trends observed with respect to significant differences in W8 potato responses to disease stressors in the 95 observations made, including bacterial and fungal pathogens. The Potato Late Blight Resistance Gene (*Rpi-vnt1*) expresses a protein that halts the spread of the infection by the pathogen. The mechanism of action for the expressed effector protein is very specific and extremely unlikely to impact other organisms such as insects and other non-target organisms (JR Simplot, 2014, page 16-17). Considering the high specificity of the *Rpi-vnt1* protein in W8 potatoes and the observed results stated above, the W8 event is highly unlikely to adversely impact soil or plant-associated fungi or bacteria.

The results of the beneficial arthropod and pest arthropod abundance monitoring produced no significant differences between the W8 event and the control for any of the arthropods collected. There were no damsel bugs, a beneficial insect, present in the control while the presence of damsel bugs in the W8 control was very low. The overall lack of differences in 15 of the 16 arthropod types supports a conclusion that the environmental interactions of the W8 potatoes were not altered as a result of the introduced GE traits compared to the control potatoes. The inserted traits do not have an impact on arthropod abundance within the potato agroecoystem.

RNAi mediated gene expression generally requires sequence homology of at least 90% between the silencing construct and the target sequence to be successful and even higher degrees of homology over 21-23 nucleotide stretches (Sharp, 2001). It is not likely that the genetic construct components responsible for gene silencing in the W8 potatoes would contribute to silencing genes in other non-target organisms through direct consumption of pollen by pollinators or through secondary exposure of beneficial predator or parasitic arthropods or other potential biological control agents for potato pests (Lacey et al., 2001) since sequences from arthropods, bacteria, fungi and viruses are expected to be highly divergent from the sequences used to silence genes in the W8 potatoes. Furthermore, indirect exposure scenarios are unlikely to lead to impacts to non-target predators and parasitic arthropods since 1) they may not receive effective doses, 2) intracellular amplification of siRNA, the active gene silencing component derived from dsRNA, is not widely found in insects, 3) environmental and physiological conditions in the gut may destroy RNA, 4) and they may not have the appropriate enzyme to direct RNAi silencing (e.g. Dicer, Argonaute, RdRP, RNA and DNA helicases) (Lundgren and Duan, 2013).

Therefore, based on the compositional similarity of W8 potato tubers with the control and reference tubers, the observed interactions of W8 potatoes with insects and pathogens, and the unlikely impacts of nontarget effects due to RNAi silencing, APHIS concludes that exposure to and/or consumption of W8 tubers or other plant parts is unlikely to have adverse impacts on organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of W8 Potatoes

APHIS assessed whether W8 potatoes are likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the non-transgenic progenitor (Russet Burbank control) from which they were derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop and the situations in which crop volunteers or feral populations are considered weeds. The assessment also compares the GE crop event to the Russet Burbank control, when cultivated under field conditions characteristic for the regions of the US representing the major production areas for potatoes especially Russet Burbank. Multiple field trials with the W8 potato did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, delayed senescence or other key agronomic characteristics associated with weediness or survival outside of cultivation.

Potatoes are not known to be weedy or persistent; they are incapable of survival outside of cultivation (Holm et al, 1979; Muenscher, 1980; Love 1994; OECD, 1997). Potato tubers have a fairly low frost tolerance; shallow tubers and those exposed to the surface are often destroyed by frost, but in temperate climates up to 20% of tubers left in the soil show no dormancy and will sprout the next season (Andersson and de Vicente, 2010). Volunteer potatoes, growing from overwintered tubers, can be a weed problem in the subsequent potato crop but are easily controlled with cultivation and herbicides and do not persist as weeds for more than one or a few years (Andersson and de Vicente, 2010). Since tubers are a source of volunteers, tuber yield is directly related to volunteer potential.

Another potential source of volunteers is true potato seed (TPS). While many potato cultivars are partially or fully sterile, rarely producing fruits, some cultivars are capable of prolific fruit and seed production. Russet Burbank, the variety used to develop W8 potatoes is fully sterile. Regardless, plants produced from TPS are no weedier than volunteer plants produced from over-wintered tubers and are relatively easy to control in rotational crops.

JR Simplot evaluated phenotypic and agronomic characteristics of W8 potatoes relative to their parental and reference varieties at several field locations over 2 years at geographically distinct sites that represent most of the main production areas for potatoes in the USA (JR Simplot, 2014, Table 11-2. Field Trial Locations)

- Relative to the Russet Burbank control, there was no consistent trend among the W8 event for significant increases in final emergence, stems per plant, or vine desiccation.
- The W8 event was lower than the control for early emergence (39.5 vs 61.5%), W8 was less vigorous than the control for plant vigor (3.0 vs 3.7) and shorter than the control for plant height (40.4 vs 45.1 cm). All of these detected differences were within the conventional variety range (CVR).
- There were no statistically significant reductions in total yield, US#2 yield, tubers per plant, tubers <4oz, tubers 4-6oz, tubers 6-10oz, tubers >14oz. W8 had fewer tubers in the 10-14oz group (14.0 vs 19.0%) than the control which could be associated with lower yield, however total yield was not significantly different as mentioned above.
- W8 potatoes did not display any notable differences to the control in tolerance to abiotic stresses (JR Simplot 2014, Table 11-5) or biotic stresses.

The data presented by JR Simplot demonstrate that the W8 potatoes are for the most part phenotypically and agronomically similar to the respective Russet Burbank parent variety and do not exhibit meaningful changes in characteristics that would make them weedier or more persistent than the parent variety. Because of the reductions in early emergence and large tuber yield, the W8 potatoes appear to have a reduced potential for weediness. Furthermore, JR Simplot did not observe any differences during the completed post-harvesting volunteer monitoring of the W8 potato field test sites from 2 years of field testing that would lead them to believe that these potatoes have properties that would increase their survivability compared to conventional potatoes (JR Simplot, 2014, Section 12.1).

Based on the agronomic field data and literature survey concerning weediness potential of the crop, the W8 potatoes are unlikely to persist as a troublesome weed or to have an impact on current weed management practices. Furthermore, post-harvest monitoring of field test plots planted with the GE crop event under USDA-APHIS notifications and permits did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. All field sites were monitored for 2 years after harvest for volunteer activity as required by USDA-APHIS compliance. No differences were observed that would suggest that the W8 potatoes are more likely to become weeds than the conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants with which W8 Potatoes 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., 2013). 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 (Ellstand et al., Table 1, 1999). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from the GE crop event 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 the engineered plants.

As described previously, potatoes (*Solanum tuberosum*) belong to the genus *Solanum*, section *petota*, subsection *potatoe*, and series *tuberosa*, which consists of approximately 54 species, of which only *S. tuberosum* is widely cultivated for food production (OECD 1997). *S. tuberosum* is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is the cultivated potato widely in use as a crop plant in North America and Europe. The subspecies *andigena* is also cultivated, but cultivation is largely restricted to Central and South America (OECD 1997). The center of diversity for wild tuber-bearing potatoes (sect. *petota*) and the center of origin for domesticated potatoes is in Latin America (Andersson and de Vicente, 2010).The genus *Solanum* includes over 1000 species, however, cultivated potato is only sexually-compatible with some of the other tuber bearing species in the section *petota* and rarely with the non-tuber-bearing species in the section *etuberosum*; there are very strong barriers to hybridization to other Solanum species (Jackson and Hanneman Jr., 1999; Andersson and de Vicente, 2010). Numerous *Solanum* spp. occur in the US, including other cultivated species, weeds, and three Federal noxious weeds (7 CFR part 360, subpart 200) - *S. tampicense*, *S. torvum*, and *S. viarum*; however cultivated potato is not sexually compatible with these species. Among native *Solanum* spp. in the US, cultivated potato is potentially sexually-compatible only with the two tuber-bearing species, *S. jamesii* and *S. stoloniferum* (previously *S. fendleri* (Spooner et al., 2004)). Neither of these species is listed on US or State weed lists (USDA-NRCS, 2014a). These two species are found only in Texas, New Mexico, and Arizona, and *S. jamesii* is further found in Colorado and Utah. In few cases, these species may be found in counties with commercial potato production (JR Simplot Company, 2014; USDA-NRCS, 2014c; 2014b). The distribution map for *S. jamesii* in the USDA Plants Database includes Iowa ; however this is not correct (Bamberg, 2003) and this map should be updated in the near future (Bamberg, 2012).

Russet Burbank produces few flowers and is male sterile (do not produce flowers) (JR Simplot, 2014, pp. 16, 33, and 183). Pollen-mediated gene-flow from events derived from Russet Burbank would not be possible. The new traits engineered into the W8 event are not expected to expand the range of environments or locations in which cultivated potatoes are grown or affect their outcrossing capacity.

Numerous biological and geographic obstacles make gene flow from cultivated potato varieties to the two wild relatives a highly unlikely occurrence , and there have been no reports that such crosses have ever occurred naturally (Love, 1994; US-EPA, 2011).

Ploidy level and endosperm balance numbers (EBN) are genetic barriers which reduce the likelihood of gene flow from cultivated potato varieties into *S. jamesii* and *S. stoloniferum*. The concept of EBN was developed to explain endosperm level post-zygotic barriers that prevent seed development after fertilization in crosses among various *Solanum* spp. (Carputo et al., 1999). These EBNs are hypothetical genetic factors, independent of ploidy (Spooner et al., 2004). The EBN represents the effective ploidy, and must be in a 2:1 maternal to paternal ratio in the hybrid endosperm for normal endosperm development (Carputo et al., 1999). In general, crosses between species with the same EBN are successful, while crosses between species of differing EBN are not (Spooner et al., 2004).

Modern potato varieties are tetraploid with an EBN of 4. *S. jamesii* is diploid with an EBN of 1, while *S. stoloniferum* is tetraploid with an EBN of 2 (Andersson and de Vicente, 2010). Since the EBNs of the two wild species differ from cultivated potato, these crosses would be expected to fail. Jackson and Hanneman evaluated the crossability of 200 wild relatives with tetraploid potato (*S. tuberosum* ssp. *tuberosum*) cultivars (Jackson and Hanneman Jr., 1999). For crosses involving *S. jamesii*, no fruits formed after 109 pollinations (2012) and 24 pollinations (2013) with *S. jamesii* as the male and female parent, respectively. These data support the conclusion that crosses between cultivated potato and *S. jamesii* are expected to fail and thus that gene flow from cultivated potato to *S. jamesii* is unlikely to occur.

Unreduced gametes, which are not uncommon in *Solanum* spp., provide an exception to the general rule that crosses between species with differing EBN are not successful. Unreduced gametes of *S. stoloniferum* have an EBN of 4 and thus could potentially lead to successful hybridization with tetraploid potato cultivars. In the experiments of Jackson and Hanneman, when *S. stoloniferum* (including plants categorized as *S. fendleri* and *S. papita* (Spooner et al., 2004)) was used as the male parent, 14 fruits containing 180 seeds formed after 852 pollinations, for a crossing efficiency of 0.015[3]. When *S. stoloniferum* (including plants categorized as *S. fendleri* and *S. papita*) was used as the female, 18 fruits containing 43 seeds formed after 205 pollinations, for a crossing efficiency of 0.012. Seed germination rates in these latter crosses were approximately 50%. In comparison, when *S. tuberosum* ssp. *tuberosum* females were crossed to *S. tuberosum* ssp. *tuberosum* males, 14 fruits containing 252 seeds formed after 110 pollinations, for an overall crossing efficiency of 0.16 (Jackson and Hanneman Jr., 1999). These results demonstrate that gene flow from cultivated potato to wild *S. stoloniferum* is scientifically plausible, but that hybridization occurs at roughly 10% of the efficiency with which it occurs in crosses when both parents are cultivated potatoes. Andersson and de Vicente conclude that although the cultivated potato is not expected to cross easily with *S. stoloniferum* in the environment, hybridization between the two species is theoretically possible if they are growing in close proximity to each other (Andersson and de Vicente, 2010).

[3] Crossing efficiency = # seeds per fruit/# pollinations. Crossing efficiency "takes out some of the bias introduced by getting a large number of seeds from a few fruit, but requiring a great number of pollinations to get one or more fruit." (Jackson and Hanneman, 1999).

As noted above, *S. stoloniferum* (syn. *S. fendleri*) is found in several counties in Arizona (Apache, Cochise, Coconino, Gila, Graham, Greenlee, Pima, Pinal, Santa Cruz), New Mexico (Catron, Dona Ana, Grant, Lincoln, Otera, Sierra, Socorro) , and Texas (Brewster, Jeff Davis, Presidio) (Bamberg et al., 2003; USDA-NRCS, 2014b; USGS, 2014). Of these, the 2007 Census of Agriculture found that potatoes were harvested on a total of 59 acres distributed among 82 farms in Apache, Chochise, Coconino, Gila, Dona Ana, and Socorro Counties, plus an unknown number of acres distributed among 7 farms in Brewster, Grant, Pinal, and Sierra Counties (USDA-NASS, 2009a; 2009b; 2009c).

Since cultivated potatoes and *S. stoloniferum* co-occur in several counties in Arizona and New Mexico, and one in Texas, and hybridization between the two species is scientifically plausible, this risk analysis further considers the potential impact on the weediness of *S. stoloniferum* if gene introgression from W8 potatoes were to occur. To be clear, gene introgression *per se* is not a risk, rather it is an exposure pathway. The risk to be evaluated is the nature of the altered phenotype should transgenes become stably introgressed into populations of the wild potatoes.

As discussed above, the genetic material and resulting novel phenotypes (i.e. late blight resistance, low acrylamide potential, reduced black spot bruise and lowered reducing sugars) incorporated into the W8 potatoes did not impart any significant and consistently observed changes to the agronomic properties or responses to biotic or abiotic stresses of cultivated potatoes under recommended management practices that would cause them to be more weedy, and they are not engineered for resistance to herbicides, plant pests or insects. As also discussed above, *S. stoloniferum* is not listed on US or State weed lists (USDA-NRCS, 2014a). *Solanum stoloniferum* exhibits a fairly wide range of genotypic and phenotypic variability in the environment (Bamberg et al., 2003; Spooner et al., 2004), suggesting that introgression of genetic material from cultivated W8 potatoes may not substantially change its ability to act as a weed.

Based on the information presented in the petition and in relevant literature, APHIS has reached the following conclusions: The genetic modification in the W8 potatoes is not expected to increase the potential for gene flow or increase the potential for hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown.

Gene flow, hybridization and/or introgression of the introduced genetic material from W8 potatoes to the wild relatives *S. stoloniferum* and *S. jamesii* are unlikely to occur. In the case of *S. stoloniferum*, where there is a remote possibility of gene introgression, APHIS concludes that even if such introgression were to occur, this species is not considered a weed, and the gene silencing cassettes originating from W8 potatoes are unlikely to impact the weediness of this wild species since the W8 potatoes do not exhibit characteristics that cause them to be any weedier than other cultivated potatoes. Therefore, the W8 potatoes are not expected to increase the weed risk potential of other species with which they can interbreed in the U.S. and its territories.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS could not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of the GE crop. W8 potatoes are resistant to some strains of a single disease, late blight, but it is not clear whether this will lead to a reduction in fungicide use. The W8 potatoes have no new properties to enhance their weediness, invasiveness, or pest resistance. Therefore, no impact on other plant diseases or plant pests or their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which W8 potatoes Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into W8 potatoes 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 genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and 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 the long run, to major transitions in evolution.

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

Horizontal gene transfer 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 GE plant is unlikely to occur. 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). 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 (Brown, 2003; EFSA, 2009; Koonin et al., 2001). In addition, the inserted gene cassettes in W8 potatoes are derived only from DNA from *Solanum* species, and are designed to be expressed and silence specific genes in potato. The 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. APHIS therefore concludes that the likelihood of HGT from W8 potatoes to bacteria or fungi is extremely low, and would not be expected to lead to an increased plant pest risk.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from the GE 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. 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). W8 potatoes contain no introduced DNA sequences derived from plant viruses (JR Simplot, 2014, Table 4-1, 4-2 & 4-3, p. 46-49). Therefore, the likelihood of HGT from W8 potatoes to plant viruses is expected to be very low and of no consequence.

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, Yoshida and colleagues (2010), through 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. 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. 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.

Some non-native species of dodder (*Cuscuta* spp.) are parasitic for potato (Asigh and Marquez, 2010). If W8 potatoes were infected by a parasitic plant or were naturally grafted to another plant in a potato field, there is a very low probability that HGT could result in the other plant acquiring DNA from W8 potatoes. 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. Dodder reproduces sexually (Asigh and Marquez, 2010). APHIS therefore concludes that the likelihood of HGT from W8 potatoes to another plant including parasitic plants is extremely low.

Overall conclusion for impacts from HGT: Based on the above analysis APHIS therefore concludes that HGT of the new genetic material inserted into the W8 potatoes 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 the Federal Register notice concerning this petition,] and other relevant information to assess the plant pest risk of the W8 potato event compared to the Russet Burbank control from which it was derived. APHIS concludes that the W8 potato event is unlikely to pose a plant pest risk based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in the W8 potato event because the *A. tumefaciens* vector was eliminated using antibiotics, no plant pest sequences were used other than *Agrobacterium* border sequences which do not confer plant pest risk, and no significant difference in disease and pest incidence were observed in the W8 potatoes compared to the nontransgenic control or other comparators.
- No increase in plant pest risk was identified in the W8 potato event from expression of the inserted genetic material or changes in metabolism or composition because disease and pest incidence and/or damage were not observed to be significantly increased or atypical in the W8 potato event compared to the nontransgenic control or other comparators in field trials conducted in growing regions representative of where

the W8 potatoes are expected to be grown and in laboratory, greenhouse studies. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that the GE crop event 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 W8 potatoes are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the compositional similarity of W8 potato tubers to the Russet Burbank parental control, the observed interactions of W8 potatoes with insects and pathogens, and the unlikely impacts of nontarget effects due to RNAi silencing.
- The W8 potato event is no more likely to become weeds than conventional varieties of the crop based on their observed agronomic characteristics, weediness potential of the crop and current management practices available to control W8 potatoes as weeds.
- The W8 potato event is not expected to increase the weed risk potential of other species with which they can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes, from W8 potatoes to other sexually compatible relatives with which they can interbreed are not likely to occur. These compatible relatives are not considered weedy or invasive. The new phenotype(s) conferred by genetic engineering are not likely to increase the weediness of these 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 the W8 potatoes were not identified.
- Horizontal gene transfer of the new genetic material inserted into the W8 potato events 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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