JR Simplot Company Petition (13-022-01p) for Determination of Non-regulated Status of Low Acrylamide Potential and Reduced Black Spot Bruise Potato Events F10, F37, E12, E24, J3, J55, J78, G11, H37, and H50

OECD Unique Identifier: SPS-00F10-7, SPS-00F37-7, SPS-00E12-8, SPS-00E24-2, SPS-000J3-4, SPS-00J55-2, SPS-00J78-7, SPS-00G11-9, SPS-00H37-9, SPS-00H50-4

Plant Pest Risk Assessment

March 2014

Agency Contact
Cindy Eck
Biotechnology Regulatory Services
4700 River Road
USDA, APHIS
Riverdale, MD 20737
Fax: (301) 734-8669

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 10 genetically-engineered (GE) potato (*Solanum tuberosum* L.) events (F10, F37, E12, E24, J3, J55, J78, G11, H37 and H50) with low acrylamide potential and reduced black spot bruise are 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, 2013). This petition was assigned the number 13-022-01p, and is hereafter referred to as JR Simplot, 2013. Throughout this document, in cases where the ten potato events are discussed as a group, they will be collectively referred to as JRSLA 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 JRSLA potatoes are 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, or 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 10 JRSLA potato events were produced by *Agrobacterium*-mediated transformation and nine of the events 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*; Table 5, pp. 29-30, JR Simplot, 2013). Therefore, the JRSLA potatoes are considered regulated articles under APHIS regulations at 7 CFR part 340. JR Simplot has conducted introductions of JRSLA potatoes under APHIS-authorized notifications since 2009 (JR Simplot, 2013, Table 13, page 66) in part, to gather information to support that JRSLA 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 the 10 JRSLA potato events and their 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 10 JRSLA potato events are 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 nonregulated status. APHIS will assess information submitted by the applicant about the 10 JRSLA potato events 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 nontarget 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. The 10 JRSLA potato events are not engineered to express substances to protect the potatoes against plants pests, and are therefore not subject to EPA review.

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 have initiated the FDA consultation process (JR Simplot, 2013, page 15).

B. Development of the Ten JRSLA 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 lbs 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 varieties store well and are the source of most fresh market potatoes and French fries, while the variety Atlantic does not store well and is used primarily to produce potato chips (JR Simplot, 2013, pp. 25-26).

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.

JR Simplot has used a genetic engineering approach to introduce into the background of commercial potato cultivars two traits that are of interest to potato consumers, producers and processors: reduced acrylamide potential in certain processed or heated potato products and reduced black spot bruise. JR Simplot used the single construct pSIM1278 to transform 5 different commercial parent varieties and created the 10 events described in JR Simplot, 2013. The objective was to incorporate the same new phenotypes into each of these important varieties, while maintaining all of the desirable characteristics

originally selected by potato breeders. If a single variety were transformed, it would take decades to move these new traits into the other commercial varieties by conventional breeding, and even then, it would be difficult to reconstitute the desirable characteristics of the original variety.

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).

The intended purpose of the 10 JRSLA potato lines is to provide the potato processing industry with new varieties with low acrylamide potential and reduced black spot bruise. Both of these changes are intended to benefit potato consumers, producers, and processors. 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.

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 JRSLA 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 10 JRSLA potato events were produced by using *Agrobacterium*-mediated transformation of potato internode explants of 5 different varieties: Ranger Russet, Russet Burbank, Atlantic, variety G and variety H (JR Simplot, 2013, p. 26-29). The binary plasmid vector pSIM1278 (Figure 3 in JR Simplot, 2013), consisting of the vector backbone (JR Simplot, 2013, Table 4) and the DNA insert (JR Simplot, 2013, Table 5) was used to create all 10 events; only the DNA insert portion was 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 (JR Simplot, 2013, p. 25).

A marker-free selection system was used to eliminate plants in which vector backbone inserted into the genome due to inefficient cleavage at the Left Border site (Gelvin, 2003; Richael et al., 2008). 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, 2013, Figure 3 and Table 4). 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 the genes of interest, and thus do not contain the *ipt* gene.

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 an RNA interference (RNAi) mechanism to degrade transcripts for the genes. They are not designed to encode a full open reading frame to produce protein (for details, see pages 31 – 32 in JR Simplot, 2013). 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 JRSLA 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 ten JRSLA events was 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 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 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 5 in JR Simplot, 2013 provides additional detail for all of these genetic elements. Even though the same pSIM1278 was used to create the 10 JRSLA events, each insertion is unique with some being simple insertions, some double insertions and others more complex (Table 1). More detail for each event can be found in JR Simplot, 2013, Figure 5.

Table 1. The parent variety, number of inserts and a brief description of the nature of the inserts in the 10 JRSLA potato events.

Event	Parent Variety	Insert Copies	Comments
F10	Ranger Russet	Single	Complete insert
F37	Ranger Russet	Single	Complete insert
E12	Russet Burbank	Single	Complete insert
E24	Russet Burbank	Single	Nearly complete insert with small deletion at the left border
J3	Atlantic	Single	Nearly complete insert with deletion of the left border and a small portion of the *Agp* promoter of the first cassette, fused to an inverted copy of the first cassette
J55	Atlantic	Single	Complete insert fused to an inverted copy of the first cassette)

J78	Atlantic	Single	Partial insert comprising first cassette plus partial second cassette, truncated after the second *R1* fragment (so missing the second *PhL* fragment and *Gbss* promoter)
G11	G	Single	Partial insert comprising first cassette only (truncated in the first *PhL* fragment of the second cassette)
H37	H	Complex	One complete insert and three unlinked partial inserts, one of which includes a complete second cassette
H50	H	Double	One complete insert and one unlinked partial insert with the first cassette only (truncated in the first *PhL* fragment of the second cassette)

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 10 JRSLA events do not contain vector backbone sequences as determined through Southern blot and Polymerase Chain Reaction assays (JR Simplot, 2013, Appendix 2).
- The genomes of the 10 JRSLA potato events contain either one or two copies of full or partial inserts between the Left and Right borders in pSIM1278 (JR Simplot, 2013, Appendix 1; as summarized in Table 2 above). Events J78 and G11 only contain the first expression cassette and insufficient portions of the second expression cassette.
- The insertions in the 10 JRSLA potato events were stably inherited through at least three generations of vegetative propagation, as confirmed by DNA gel blot hybridization (all 10 JRSLA events) and a phenotypic assay for the suppression of PPO (all events except J3, J55, and J78) (JR Simplot, 2013, Appendix 3). All 10 of the JRSLA potato events contained the first expression cassette containing the genetic elements to silence *Asn1* and *Ppo5*. 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 JRSLA potatoes are likely to alter plant pest risk relative to the untransformed control. The assessment encompasses (1) a consideration of the specific effects on plant metabolism due to silencing *Asn1*, *Ppo5*, *PhL* and *R1*; and (2) evaluation of whether the nutrient and antinutrient levels in harvested tubers of the 10 JRSLA events are comparable to those in the respective parental varieties and other reference potato cultivars. 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 (reviewd 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 JRSLA potatoes against pests and diseases and to affect the suitability of JRSLA tubers as a food source for non-target organisms.

To assess the efficacy of the gene silencing constructs, JR Simplot examined *Asn1*, *Ppo5*, *PhL* and *R1* transcript levels in tubers, stolons, roots, stems, leaves, and flowers in the 10 JRSLA events. In addition, to assess the efficacy of gene silencing and determine whether there are any unintended compositional changes to JRSLA potatoes, JR Simplot compared the tuber composition of the 10 JRSLA events with the tuber composition of their respective parent varieties. The analyzed tubers were sampled from 23 different trials conducted in 2009, 2010 and 2011 in 8 different states; however, not all varieties were included in every trial. JR Simplot selected locations to represent the major potato production areas in the US; trials were conducted in Idaho, Washington, North Dakota, and Wisconsin, states that account for more than half of US potato production (USDA-NASS, 2013). The Ranger Russet, Russet Burbank and Atlantic events were sampled all three years and the data presented as combined analyses across locations and years. For the G and H varieties the compositional analyses are all based on 2010 samples (JR Simplot, 2013, Appendix 9).

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 below. 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, 2013 has further developed this concept in the design of JRSLA 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). JRSLA 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 10 JRSLA events and nontransgenic control varieties, *Ppo5* transcripts were down-regulated in tubers of all 10 events, in stems of nine events (all except G11), in roots of five events (F10, F37, E12, H37, and H50) and in stolons of three events (E24, G11, and H50) (JR Simplot, 2013, Table 6, p. 40, and Appendix 5). No transcripts hybridizing to the *Ppo5* probe were detected in leaves of nontransgenic control varieties or in leaves of any of the 10 JRSLA events. Similarly, transcripts hybridizing to the *Ppo5* probe were not detected in flowers of the eight JRSLA events assayed that produce flowers or of their nontransgenic control varieties. From the data provided, it is not possible to definitely determine whether the results reflect down-regulation of *Ppo5* transcripts alone, or whether the results also or instead reflect down-regulation of some of the other *Ppo* genes, particularly *POT41*, since its expression pattern has not been characterized nor its DNA sequence made available. However, it is unlikely that *NOR333* transcripts were down-regulated via RNAi since there is no homology between the *tPpo5* sequence introduced into the JRSLA potatoes and the *NOR333* gene.
- Using a catechol assay, there was a lack of functional PPO activity in tubers of seven of the 10 events (see Table 2; JR Simplot, 2013, Appendix 3). The three Atlantic events (J3, J55 and J78) were not tested with the catechol assay because the Atlantic parent variety is known to have some resistance to black spot bruising (JR Simplot, 2013, p.26) and did not react with catechol (JR Simplot, 2013, p. 47). In contrast, Russet Burbank and particularly, Ranger Russet, are more susceptible to black spot bruise (Potato Association of America, 2009).

JRSLA potatoes were designed to have reduced levels of free asparagine by silencing *Asn1* as well as reduced levels of reducing sugars by silencing *PhL* and *R1*. 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*, and *PhL* transcript levels in tubers from field and greenhouse grown plants and in stolons, roots, stems, leaves, and flowers of

greenhouse grown plants (JR Simplot, 2013, Appendix 5). These data are summarized below and in Table 6, page 40, of JR Simplot, 2013. Free glutamine, free asparagine, and reducing sugar levels in tubers were also examined (see Table 2), and tubers were processed into French fries and potato chips in order to analyze acrylamide content (JR Simplot, 2013, Appendix 9). JR Simplot provides evidence demonstrating that:

- *Asn1* transcripts were down-regulated in tubers of all 10 events, in stolons of nine events (all but H37), in flowers of all eight flowering events (all except H37 and H50), in roots of six events (F10, F37, E12, J3, J55, and H50), in stems of four events (F37, E24, J3, and H50), and in leaves of one event (F10).
- Seven of the 10 JRSLA events (F10, F37, J3, J55, G11, H37, and H50) displayed a statistically significant reduced content of free asparagine while three events (E12, E24 and J78) had only numerically reduced levels.
- Five of the 10 JRSLA events (F10, F37, G11, H37, and H50) displayed a statistically significant increased content of free glutamine while the other five events (E12, E24, J3, J55 and J78) had only numerically increased levels. Increased content of free glutamine is an expected consequence of reducing the content of free asparagine. This is because the enzyme asparagine synthetase also functions to deaminate glutamine to glutamate (JR Simplot, 2013, Figure 6).
- *R1* transcripts were down-regulated in greenhouse grown tubers of eight events (all except J78 and G11) and in field grown tubers of some of these events (JR Simplot, 2013, Appendix 5, Figure 4). *R1* transcripts were also down-regulated in stolons of the same eight events, roots of three events (F10, F37 and H50), stems of one event (G11) and leaves of two or three events (G11 and H50, and possibly H37) (JR Simplot, 2013, Table 6, and see Appendix 5, Figure 12 for *R1* in leaf tissue) . *R1* transcripts were not down-regulated in G11 tubers due to a truncation of the inserted DNA which removed the *pR1* fragments (JR Simplot, 2013, Figure 5). *R1* transcripts were also not down-regulated in greenhouse grown tubers of J78, but were down-regulated in field grown tubers of that line. *R1* transcripts were unexpectedly down-regulated in stems and leaves of event G11, which does not contain the *PhL/R1* silencing cassette. The reason for this apparent down-regulation, which was not observed in tubers, stolons, or roots, is unknown.
- *PhL* transcripts were down-regulated in tubers of six events (E12, E24, J3, J55, H37, and H50) and in stolons of seven events (F10, F37, E12, E24, J55, H37, and H50) (see also JR Simplot, 2013, Appendix 5, Figure 7). *PhL* transcripts appear to be slightly down-regulated in leaves of three events (F10, J55 and J78) (see also JR Simplot, 2013, Appendix 5, Figure 11), but were not down-regulated in roots or stems of any event. *PhL* transcripts were not down-regulated in J78 or G11 tubers due to a truncation of the inserted DNA which removed some or all of the *PhL/R1* silencing cassette (JR Simplot 2013, Figure 5).
- Six events (F10, F37, E12, E24, J3 and J55) had significant decrease in tuber reducing sugar (fructose + glucose) content after 1 month in storage (JR Simplot, 2013, Appendix 9). However, these differences were not detected in fresh tubers (except for J3 and J55) or after longer periods of storage. A decrease in reducing sugars was not tightly correlated with the presence of the complete silencing cassette for the *R1* or *PhL* genes. In particular, there is no significant decrease in

reducing sugars in fresh tubers in events F10, F37, E12, E24, H37 and H50, all of which contain this cassette. An unexpected decrease in reducing sugars was observed in fresh J78 tubers which do not contain the full silencing cassette for the *R1* or *PhL* genes.

- Cooked potato products from fresh tubers of all 10 events, as well from tubers after up to 2 or 3 months in storage, had statistically significant reductions in acrylamide content. For events F10, F37, E12 and E24, these reductions were statistically significant through 7 months of storage (JR Simplot, 2013, Appendix 9).

Cooked potato products of events J78 and G11, which lack the second cassette of pSIM1278, had levels of acrylamide similar to those in the other 8 JRSLA events. Consistent (although not always statistically significant) reductions in free asparagine and increases in free glutamine across all 10 JRSLA events suggest that silencing *Asn1* was sufficient to achieve the desired low acrylamide phenotype, and reductions in reducing sugar content may not be required. Considering the variable and often insignificant differences in reducing sugar content in the JRSLA tubers relative to their respective parent varieties (JR Simplot, 2013, Appendix 9), the efficacy of silencing *PhL* and *R1* conferred by pSIM1278 appears limited. pSIM1278 did not confer the desired phenotype of lower levels of reducing sugars in JRSLA tubers.

Table 2. Tuber composition phenotypes of JRSLA Potatoes

Event	**PPO activity**	**ASN/GLN level**	**Reducing sugars level**	**Acrylamide level**
F10	absent	↓/ ↑	↓at 1 month	↓, through 7 months
F37	absent	↓/ ↑	↓at 1 month	↓, through 7 months
E12	absent	*/*	↓at 1 month	↓, through 7 months
E24	absent	*/*	↓at 1 month	↓, through 7 months
J3	not tested	↓/*	↓at 1 month	↓, through 2 months
J55	not tested	↓/*	↓at 1 month	↓, through 2 months
J78	not tested	*/*	no change	↓, through 2 months
G11	absent	↓/ ↑	no change	↓, through 2 months
H37	absent	↓/ ↑	no change	↓, through 2 months
H50	absent	↓/ ↑	no change	↓, through 2 months

*Reduction in Asn and/or increase in Gln occurred but was not statistically significant

No functional enzymes or proteins are expressed by the inserted DNA. The *ipt* gene used for negative selection resides in the vector backbone and is therefore not expressed in the 10 JRSLA events, all of which are free of vector backbone (JR Simplot, 2013, Appendix 2).

JR Simplot also analyzed tubers of the 10 JRSLA events and their respective parents 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, sugars, and glycoalkaloids

(JR Simplot, 2013, Appendix 9). This compositional analysis of tubers confirmed the following:

- For key proximates, vitamins, and minerals, there were no statistically significant differences between any of the 10 events and their respective parent varieties for content of protein, fat, ash, crude fiber, carbohydrates, calories, copper, magnesium or potassium. However, events F10 and F37 had statistically significant increases in niacin (vitamin B3) and vitamin C content, and events J3 and J55 had statistically significant decreases in pyridoxine (vitamin B6) content (JR Simplot, 2013, Appendix 9).
- For amino acids, event F37 had statistically significant increases in free aspartate and free arginine content, event G11 had statistically significant increases in free lysine and free proline content while event H37 had a statistically significant decrease in free lysine content, and events J3 and J55 had statistically significant decreases in free valine content (JR Simplot, 2013, Appendix 9). Consistent with the decreased content of free asparagine and increased content of free glutamine in all events as mentioned above (see Table 2 above), as expected most of the events (F10, F37, E12, E24, G11, H37, and H50) also had corresponding significantly decreased levels of total aspartate and asparagine and increased levels of total glutamate and glutamine.
- Events E12 and E24 had statistically significant decreases, and event H37 had a statistically significant increase, in sucrose content.
- In all cases, these differences were within the 99% tolerance intervals generated from nine non-GE commercial varieties grown concurrently at the same field sites (JR Simplot, 2013, Appendix 9).
- There were no significant differences between any of the 10 events and their respective parents for mean glycoalkaloid toxin content (JR Simplot, 2013 Appendix 9). Although the upper end of the range of glycoalkaloid content was higher in event H50 and much higher in event G11 than the limit of 20 mg per 100 g of potato that is generally accepted as safe, the upper ends of the ranges for the control varieties were also high. The mean values for H50 and its control were below 20 mg per 100 g of potato, while the mean values for G11 and its control were very slightly above and below 20 mg per 100 g of potato, respectively, and did not differ statistically. A third event, E24, also exhibited elevated levels of glycoalkaloids at the upper end of the range while its control did not. However, the mean glycoalkaloid value for event E24 was well below 20 mg per 100 g potato and did not differ statistically from its control. JR Simplot notes that handling conditions can result in higher glycoalkaloid levels (JR Simplot, 2013, Appendix 9, p. 67).

Based on the compositional analyses presented in JR Simplot, 2013 (Appendix 9), APHIS concludes that although raw tubers of some of the JRSLA potato events had some statistically significant differences in nutritional components (vitamins C, B3 and B6; certain amino acids, sucrose and reducing sugars) compared to their respective parent varieties, they are nutritionally equivalent to other commercial potato varieties. No new proteins are expected to be produced based on the inserted genetic elements and the

genetic modification. For the few cases when significant differences were detected between the JRSLA events and their respective parents, the mean values for the JRSLA events all fell within the tolerance interval and in most cases, also fell within the combined literature range for potatoes. The primary *Ppo* gene expressed in roots, tubers, and stolons is suppressed in the JRSLA events, and reduction in PPO activity in JRSLA event tubers has been demonstrated. As discussed in the next section, the JRSLA 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.

APHIS therefore concludes that the JRSLA potatoes pose no more of a plant pest risk from new gene products, changes to plant metabolism or composition than their respective parent varieties 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, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in the JRSLA potatoes that are known 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 or whether the JRSLA 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) (USDA-APHIS 2013b). 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, 2013). *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 for the 10 JRSLA events from two distinct sources: (1) They took observational data on any insects or diseases present in the regulated field trials conducted in 2009, 2010 and 2011 (JR Simplot, 2013, Appendix 6); and (2) they conducted laboratory and field disease assays to generate response data to two important diseases of potato: late blight caused by *P. infestans* and bacterial soft rot caused by *Erwinia carotovora* (JR Simplot, 2013, Appendix 8).

In 18 field trials conducted in 2009, 2010 and 2011, JR Simplot collected data on insect, disease and abiotic stressors. Field trial sites were selected to represent the major production areas for each variety. The Ranger Russet and Russet Burbank types were primarily field tested in Idaho, while the bulk of the field trials for the G and H types were conducted in Wisconsin, Florida and Michigan. The Atlantic types were field tested in 8 states (JR Simplot, 2013, Appendix 6, Table 1). 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 or pressure, and abiotic stressor symptoms. A list of common potato disease and insect pests is presented (JR Simplot, 2013, Appendix 6, Table 26). Not all insects or diseases were evaluated or found at each site. Rather, data were collected on those insect and disease pests which were observed or were specifically looked for in each field trial. Stressors were rated on a 1-5 scale (1=high, 3=average, 5=low), and the ranges of the observed data for each JRSLA event were compared to those of its respective parent variety. If a stressor was specifically looked for but not found, then a rating of 0 was recorded. These data are summarized below. "Incidental" stressor data were also recorded for diseases at mid and late season and for insects at mid-season and observations were recorded as present or absent for the JRSLA event and its respective parent variety. However, these data were recorded simply as incidental insect, Verticillium, virus, and "other" and rarely was the presence of the stressor encountered, except for Verticillium.

For the insect stressors, JR Simplot collected 630 observations, of which nine were reported with minor differences (JR Simplot, 2013, Appendix 6) (see Table 3 below). In seven of these nine cases, the JRSLA events had more insect stress than the controls: event F37 had greater stress from seed corn maggots during one observation in Adams County, Washington in 2011; event G11 had greater stress from Colorado potato beetles and potato leafhopper during one of eight observations, and from grasshoppers during two of eight observations in Oneida County, Wisconsin in 2011; event H50 had greater stress from blister beetles and grasshoppers during one of eight observations each in Oneida County, Wisconsin in 2011 (JR Simplot, 2013, Appendix 6).

Table 3. Field Trial Insect and Disease Stressor Observations for Ranger Russet Events F10 and F37 in 2010-2011, Russet Burbank events E-12 and E-24 in 2009 and 2011, Atlantic Events J3, J55, and J78, G variety Event G11, and H variety Events H37 and H50 in 2011 (adapted from JR Simplot, 2013, Appendix 6).

Stressor	**Total # Observations for each Event (number of observations where the range of ratings for the event was outside the range of its parental control variety)**									
Insects	**F10**	**F37**	**E12**	**E24**	**J3**	**J55**	**J78**	**G11**	**H37**	**H50**
Aphids	18	12	18	18	21	21	21	8	8	8
Blister Beetle								8	8	8 (1)
Colorado Potato Beetle	18	12	18	18	65	65	65	8 (1)	8	8
Flea Beetle					8	8	8	8	8	8
Grasshopper								8 (2)	8	8 (1)
Looper	3	3	3	3	3	3	3			
Potato Leafhopper					6	6	6	8 (1)	8	8
Seed corn maggot	3	3 (1)	3 (1)	3 (1)	3	3	3			

Total observations	42	30	48	48	106	106	106	48	48	48
Diseases	**F10**	**F37**	**E12**	**E24**	**J3**	**J55**	**J78**	**G11**	**H37**	**H50**
Bacterial										
Black leg	3	3	3	3	3	3	3			
Fungal										
Botrytis	12	6 (1)	12	12	24	24	24			
Early Blight	15 (1)	9 (2)	15 (1)	15 (1)	51 (1)	51 (1)	51			
Late Blight					3	3	3			
Rhizoctonia	3	3	3	3	6	6	6			
Verticillium	3 (1)	3	3	3	3 (1)	3 (1)	3 (1)	4	4	4
White Mold	9	9	9	9 (1)	6	6	6			
Virus	3	3	3	3	3	3	3			
Total observations	48	36	48	48	99	99	99	4	4	4

For the disease stressors, JR Simplot collected 489 observations, of which 13 were reported with differences (see Table 3 above adapted from JR Simplot, 2013, Appendix 6). In two of these 13 cases, the JRSLA events had less disease stress than the controls, in four cases the range of disease stress observed was slightly broader than the control and skewed towards less disease, and in one case the range of disease stress observed was slightly broader than control in both directions. The JRSLA event had more disease stress than the control in the other six cases: F10 - early blight (*A. solani*), Grand Forks Co, ND in 2011; E24 - white mold or Sclerotinia stalk rot (*Sclerotinia sclerotiorum*), Bingham Co, ID in 2011; F10, J3, J55, and J78 - *Verticillium* spp., Bingham Co, ID in 2011). However, with the exception of early blight in event F10 at Grand Forks, ND in 2011, these observed differences were small, and 14 other observations demonstrated no difference between F10 and controls in stress due to early blight. Among important diseases affecting potato tubers, there were many observations collected for black leg (soft rot in tubers; *E. carotovora*) and Rhizoctonia (*R. solani*), but few observations for late blight (*P. infestans*), and no observations were systematically collected for Fusarium dry rot (*Fusarium* spp.), ring rot (*C. michiganensis* spp. *sepedonicus*), and water rot/Pythium leak (*P. ultimum*). However, there were no significant differences between events J3, J55, J78, H37, and H50 and their controls in the number of tubers discarded (pick-outs) at harvest due to quality issues, i.e. rot or mold, while the number of tubers discarded for event G11 was significantly lower than its control (JR Simplot, 2013, Appendix 6). Pick-outs for Events F10, F37, E12 and E24 were not recorded. During the cold storage period for trials in three locations in either 2009 or 2010, pink rot (*Phytophthora erythroseptica*) and soft rot and Pythium leak diseases were occasionally observed on both untransformed and unspecified JRSLA potato events, but no data were provided, and the disease incidence was attributed to environmental soil conditions that favored infection (JR Simplot, 2013, pg. 46). While a number of viruses also affect

tubers (e.g., potato leaf roll virus), these also have above ground symptoms and such symptoms were not observed during the field trials.

JR Simplot also recorded data on abiotic stressors (frost, hail, heat, herbicide, and wind) for 7 of the 10 JRSLA events (JR Simplot, 2013, Appendix 6). For events F10, F37, E12, E24, J3, J55 and J78, JR Simplot recorded 341 stressor observations and in no case did the JRSLA potatoes fall outside the range of their respective parental varieties. No abiotic stressor data was presented for the G11, H37 and H50 events.

JR Simplot evaluated resistance to late blight (*P. infestans*) in both laboratory and field experiments and to soft rot (*E. carotovora*) in laboratory experiments. Two separate laboratory assays were conducted in 2009 and one in 2011. All 10 JRSLA events were not included in every test for late blight and soft rot; Table 4 summarizes which events were included in each test and their disease response/level of susceptibility relative to their parental control. In 2009, the Ranger Russet and Russet Burbank types (E12, E24 and F10) were tested in one assay and the G and H types (G11, H37, H50) were tested in a second assay. The 2011 laboratory assay included the Ranger Russet, Russet Burbank and Atlantic types, but not the G and H types. The 2011 field experiment included the E12, E24, F10, J3, J5 and J78.

Table 4. Summary of disease ratings for late blight (*P. infestans*) and soft rot (*E. carotovora* subsp. *carotovora*) lab and field studies for JRSLA events compared to their parental controls conducted by JR Simplot (JR Simplot, 2013, Appendix 8). (< = less susceptible, > = more susceptible, nsd = no significant difference).

Disease	Assay	Pathogen Strain	Year	F10	F37	E12	E24	J3	J55	J78	G11	H37	H50
Late Blight	Lab Tubers	US8	2009	<		>	nsd				<	<	>
Late Blight	Lab Tubers	US10	2009								nsd	nsd	nsd
Late Blight	Lab Tubers	US22	2009								<	>	>
Late Blight	Lab Tubers	US8	2011	nsd	nsd	<	nsd	>	nsd	nsd			
Late Blight	Field Foliar	US22	2011	<		nsd	nsd	nsd	nsd	nsd			
Soft Rot	Lab Tubers		2009		nsd	nsd	nsd				nsd	nsd	
Soft Rot	Lab Tubers		2011	nsd	nsd	nsd	nsd	<	<	nsd	nsd	nsd	nsd
Total assays				4	3	5	5	3	3	3	5	5	4

The data from all laboratory late blight assays was highly variable, and in some cases, large differences were not found to be significant (JR Simplot, 2013, Appendix 8, Tables 1, 2 and 3), and no trends were apparent (see Table 4 above). E12 had significantly more

disease than the controls in 2009, but had significantly less disease than the controls in 2011, using the same US8 strain of *P. infestans* in both years. In the 2009 assay that included the G and H types, three different late blight strains were used (US8, US10 and US22) and the results were inconsistent across strains and between the events G11, H37 and H50, except that all three events were similar in having no significant difference in their response to strain US10 relative to their respective controls.

The most robust data for late blight reactions comes from the 2011 field trial conducted in Michigan (JR Simplot, 2013, Appendix 8, Table 4). This trial received a foliar inoculation of *P. infestans* strain US22, a relatively new genotype that has been prevalent in the Midwestern US (Gevens et al., 2011), and percent foliar infection was recorded at several points in time. Event F10 has significantly less foliar infection, and there were no differences for the other five events tested (see Table 4 above). These data provide no indication that the six JRSLA events tested have increased susceptibility to late blight. Events F37, G11, H37 and H50 were not included in this test, however there appears to be no strong correlation with presence of one or both expression cassettes and an increase in susceptibility. In addition to the challenge experiments, there were no differences observed in late blight in the field in the absence of challenge inoculum for J3, J55 and J78 compared to the Atlantic control (see Table 3).

JR Simplot evaluated resistance to bacterial soft rot by percent weight loss of inoculated tubers. In the 2009 assay, only 5 of the 10 JRSLA events were tested. No significant differences were detected; however, this data was only minimally informative due to the large standard errors (JR Simplot, 2013, Appendix 8, Table 5). All 10 JRSLA events were included in the 2011 soft rot assays. The Atlantic events J3 and J55 had significantly less weight loss than their parental varieties, and six other events had numerically less weight loss (JR Simplot, 2013, Appendix 8, Table 6). These data (see Table 4 above) provide no indication that any of the 10 JRSLA events have increased susceptibility to bacterial soft rot.

The biological function of PPO in plants remains elusive (Mayer, 2006). The oxidation products of PPO appear to play a role in general plant defense mechanisms against pathogens and pests; Mayer 2006 has reviewed these interactions in potato. Much of this literature is focused on specific phenolic compounds, rather than on PPO activity *per se*. Phenolics can inhibit the growth of specific pathogens and can also inhibit enzymes involved in pathogenesis (Lyon, 1989). Additional factors that complicate the analysis of PPO relative to pest and pathogen resistance include: (1) both the substrates and the products of PPO have been implicated in resistance to plant pathogens; and (2) PPO can exist in latent forms such that no *Ppo* transcription is required to initiate the PPO activity (Partington et al., 1999).

Lyon 1989 reviewed the biochemical basis for resistance of potato to bacterial soft rot caused by *Erwinia* spp. Because it is the dominant monophenol, chlorogenic acid has been a focus in many of these studies. Chlorogenic acid did not inhibit the *in vitro* growth of *Erwinia* spp. or *P. infestans* (the causal agent of late blight), and there remains

no proof that phenols are important in the interaction between potato and *Erwinia* spp. (Lyon 1989).

Kroner *et al* 2012evaluated the role of specific phenolics in quantitative resistance to the elicitors of two pathogens, *P. infestans*, the causal agent of late blight, and *Pectobacterium atroseptiucm* (synonym: *E. carotovora* subsp. *atroseptica*), the causal agent of bacterial soft rot. Increasing concentrations of total phenolics tended toward a positive correlation with quantity of symptoms due to the late blight pathogen, but were negatively correlated with increased tuber rot severity due to the soft rot pathogen. Because chlorogenic acid accumulates in response to soft rot elicitors, these authors suggest that chlorogenic acid could be used as a marker for resistance to soft rot (Kroner et al., 2012). Since chlorogenic acid is a PPO substrate, silencing of PPO would not be expected to reduce the level of chlorogenic acid in potato tubers.

All of the JRSLA potato events contain the gene cassette for silencing the *Ppo5* gene in tubers and *Ppo5* transcripts were down-regulated in tubers of all 10 events, and reduced levels of PPO activity were confirmed for all but the Atlantic events J5, J55, and J78. Taking this into consideration, the weight of evidence from field observations, challenge experiments with the late blight pathogen and tuber soft rot, and the level of tuber discards (pick-outs) due to quality issues including rots, suggests that JRSLA potatoes are unlikely to be more susceptible than their control variety to either late blight or tuber rots due to silencing of the *Ppo5* gene in tubers.

The relationship between PPO and resistance to herbivores has also been studied. PPO activity increases when potato leaves are wounded and at higher rates in response to regurgitant from the pest Colorado potato beetle (Kruzmane et al., 2002). In other plants the increase of PPO activity is a direct induced defense against insect pests that decreases nutrient availability (Baldwin and Preston, 1999). In addition, PPO in glandular trichomes of wild potatoes (and other plants) is involved in resistance to insects. However, the trichomes of cultivated potatoes contain low amounts of PPO which is not thought to be involved resistance to pests (Friedman, 1997).

In the JRSLA potatoes, *Ppo5* has been silenced in tubers by use of a tuber-specific promoter. JR Simplot provided expression data showing that PPO transcripts are down-regulated in tubers of all 10 events, stems of 9 events, roots of 5 events and stolons of 3 events (JR Simplot, 2013, Table 6, page 43). Transcripts hybridizing to the *Ppo5* probe were not detected in greenhouse grown leaves or flowers of the JRSLA events or their controls. Another *Ppo* gene transcript (*NOR333*) has been shown to be more highly expressed in young leaves and flowers of field-grown potatoes. APHIS has determined that *NOR333* shares no homology with the *tPpo5* sequence used to silence the *Ppo5* gene in the JRSLA events and thus should not be down-regulated via RNAi in JRSLA potatoes. This suggests that the JRSLA potatoes are expected to be unchanged relative to PPO gene expression and PPO levels in leaves and therefore unchanged in any potential interactions between potato foliar PPO and foliar pathogens or pests. No consistent differences were observed in foliar pest and pathogens on JRSLA potatoes compared to their control varieties. Nematode damage of JRSLA potatoes was not assessed. The

results of Osman et al 2012, suggest that PPO might be involved in resistance to some plant parasitic nematodes, but do not provide data demonstrating such a role or address the species that affect potatoes (Osman et al., 2012). Conversely, lower PPO levels might increase the resistance of JRSLA potatoes to some nematodes. Other researchers found that tubers of potato cultivars resistant to *G. pallida* have lower levels of phenols and discolored less than tubers of susceptible cultivars (Mondy et al., 1985). They suggest that PPO-mediated tanning of nematode cysts enables eggs to remain viable in soil for a longer time. It is not possible to draw a conclusion regarding the susceptibility of JRSLA potatoes to *G. rostochiensis* or *G. pallida* based on PPO silencing.

Changes in amino acid composition have also been implicated in plant pest interactions or defenses. L-glutamic acid and its derivatives α and γ-aminobutyric acids and L-glycine are chemoattractants for *G. rostochiensis* and *G. pallida* (Riga et al., 1997; Rasmann et al., 2012). However, there are no significant changes in glutamic acid or glycine levels in tubers of the JRSLA events (JR Simplot, 2013, Appendix 9). In another instance, researchers demonstrated that changes in the amino acid profile in potato phloem sap as the plants mature may affect aphids (Karley et al., 2002). Aphids performed relatively poorly on more mature plants in the tuber-filling phase; these mature plants had reduced levels of glutamine and asparagine and increased levels of glutamate in leaf phloem. JRSLA potato tubers have reduced levels of asparagine; however they have increased levels of glutamine (JR Simplot, 2013, Appendix 9). No leaf compositional data was presented by JR Simplot, 2013. It is difficult to accurately predict from the tuber compositional data whether there would be changes in amino acid composition in JRSLA tissues that aphids feed upon that would enhance aphid performance. However, no increase in aphid infestation or damage was reported in the field observations for all 10 JRSLA potato events (JR Simplot, 2013, Appendix 6). Similarly, although aphids are vectors for viruses such as potato virus Y (USDA-APHIS, 2013b), no increase in viral disease was reported in field observations for seven of the events (JR Simplot, 2013, Appendix 6).

There are no indications that JRSLA potatoes would have an adverse impact on the implementation of APHIS PPQ pest management programs for nematode pests of potato. These pests are of limited distribution and there are ongoing efforts to detect them and prevent their spread. Even if JRSLA potato varieties were to be more susceptible to nematodes than their parent varieties, this should not prevent the successful implementation of APHIS PPQ best management practices and control efforts, which include cultural practices, crop rotations including resistant varieties and non-crops, planting certified nematode free seed potatoes, and preventing movement of infected soil, equipment and plant material (USDA-APHIS, 2008a; 2009)

The introduced genes did not significantly alter the observed insect pest infestation and disease occurrence or resulting damage in JRSLA potatoes relative to the control line under the typical recommended pest management conditions. However, the only observations reported for insect pests that affect potato tubers or seed potatoes were for flea beetle and seed corn maggot (no observations were reported for nematodes), and the only field observations for pathogens recorded for events G11, H50 and H37 were for

Verticillium. Nonetheless, the tubers discarded due to tuber rot and inoculated disease assays did not provide any indication that the JRSLA potatoes have increased incidence of pathogens or disease symptoms (with the possible exception of increased susceptibility to late blight strain US8 in events H50 and J3 events or to strain US22 in events H50 and H37). As discussed earlier there were no significant changes in the composition of JRSLA potatoes that would render them more susceptible to pests and diseases over their control or reference potato varieties. As presented later in this document, the observed agronomic traits also did not reveal any significant changes that would indirectly indicate that JRSLA potatoes are or could be relatively more susceptible to pests and diseases than control or reference potato varieties. Thus JRSLA potatoes are unlikely to be more susceptible to plant pathogens and insect pests than existing commercial varieties of conventional potatoes and are not expected to adversely impact APHIS PPQ pest management programs for potato pests. For these reasons, JRSLA potatoes are unlikely to differ from conventional potatoes in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

JRSLA potatoes are not engineered for pest resistance, thus there are no "target" species and thus no "nontarget" species either. APHIS assessed whether exposure to or consumption of JRSLA 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 JRSLA potatoes compared to the non-GE counterpart (or reference varieties) for any substances (proteins, RNAi, nutrients, antinutrients, 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.

As discussed above, JRSLA potato tubers are nutritionally and compositionally similar to their respective parental varieties and/or other commercial potato varieties, with the exception of the intentional changes conferred by the introduced genes. These intentional changes fall into three broad categories: (1) reduction of PPO enzyme levels in tubers; (2) alteration of the levels of asparagine and glutamine in the pool of free amino acids in tubers; and (3) reduction in the levels of the reducing sugars glucose and fructose in tubers. The altered composition of JRSLA tubers is unlikely to alter the suitability of potato tubers as a foodstuff for beneficial organisms. Furthermore, any organism that feeds on potato tubers is likely to be considered a plant pest.

Although the composition of other plant parts was not assessed in JRSLA potatoes, as noted above, JR Simplot collected 630 observations of insect stressors during field trials of the JRSLA potatoes, of which only 9 were reported with minor differences from the parental varieties. In only two cases did the JRSLA potatoes exhibit less insect stress than controls, indicating that JRSLA potatoes do not cause harm to insect pests that feed on potato leaves. By extension, JRSLA 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 JRSLA flowers of the eight varieties that produce flowers (JR Simplot, 2013, Appendix 5), suggesting that asparagine levels might be reduced in the flowers of these events. 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 JRSLA 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 JRSLA potato responses to disease stressors in the 489 observations made, including bacterial and fungal pathogens, so by extension, JRSLA potatoes are unlikely to adversely impact soil or plant-associated beneficial fungi or bacteria.

RNAi mediated gene suppression 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 JRSLA events would contribute to silencing of 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 JRSLA 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 the RNA, 4) and they may not have the appropriate receptors to allow transmembrane movement of dsRNA or the appropriate enzyme to direct RNAi (e.g. Dicer, Argonaute, RdRP, RNA and DNA helicases) (Lundgren and Duan, 2013).

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

F. Potential for Enhanced Weediness of JRSLA Potatoes

APHIS assessed whether JRSLA 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 nontransgenic progenitors 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 events to the nontrangsenic progenitors, when cultivated under field conditions characteristic for the regions of the US where the GE crop is intended to be grown, for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For this crop, such characteristics include tuber sprouting (emergence), vigor, vine size, tuber set (as measured by yield), and tolerance to biotic and abiotic stress.

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 following 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. Lawson reports that TPS produced by the cv. Desiree can survive in the soil for up to 7 years in a potato rotation in Scotland (Lawson, 1983). Of the five parent varieties used to develop the 10 JRSLA events, Russet Burbank and "H" are fully sterile, precluding any possibility of TPS production. However, the JRSLA events derived from Ranger Russet, Atlantic and "G" are fertile and may produce TPS. 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 JRSLA potatoes relative to their parent varieties at several field locations over 2-3 years at geographically distinct sites that represent most of the main production areas for potatoes in the USA (JR Simplot, 2013, Appendix 6). The data presented indicated:

- Relative to their respective parent varieties, there was no consistent trend among the 10 events for significant increases in early emergence, final emergence, plant vigor or total yield (JR Simplot, 2013, Appendix 6).
    - Five events (F37, E12, E24, G11 and H37) had statistically significant increases in plant vigor, but the relative difference was small (on the 1 to 5 scale, the means differed by less than 1 from the control), and no statistically significant differences were detected for this characteristic for the other events.
    - Five events (F10, F37, E24, G11 and H37) had statistically significant reductions in total yield and no statistically significant differences were detected for this characteristic for the other events. There was no obvious correlation between decreased yield and increased disease or pest stress

except possibly for event G11, since greater insect stress was observed than in the parental control at four of 48 insect observations.
    - Event J55 had a statistically significant reduction in early emergence.
- Relative to their respective parent varieties, events G11, H37, and H50 showed no difference in vine size (JR Simplot, 2013, Appendix 6). Vine size was not assessed for the other seven events.
- Relative to their respective parent varieties, most JRSLA potatoes did not display any differences in tolerance to abiotic stresses (JR Simplot, 2013, Appendix 6). Abiotic stress data was not presented for events G11, H37 and H55.
- As discussed above (see Potential Impacts on Disease and Pest Susceptibilities), JRSLA potatoes did not display notable differences in tolerance to biotic stresses.

The data presented by JR Simplot demonstrate that the JRSLA potatoes are for the most part phenotypically and agronomically similar to the respective parent varieties and do not exhibit meaningful changes in characteristics that would make them weedier or more persistent than their respective parent varieties. Because of the reductions in emergence and yield, some of the JRSLA 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 JRSLA potato field test sites from 3 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, 2013, Section 9.3, and Appendix 10). Volunteers were rarely observed, were easily controlled, and are not engineered for resistance to herbicides.

Based on the agronomic field data and literature survey concerning weediness potential of the crop, the JRSLA 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 trial plots planted with the GE crop event under USDA-APHIS notifications or permits (JR Simplot, 2013, Table 13, page 67) did not reveal any differences in survivability or persistence relative to other varieties of the same crop currently being grown. These data suggest that the JRSLA potatoes are no more likely to become weeds than conventional varieties of the crop.

G. Potential Impacts on the Weediness of Any Other Plants with which JRSLA 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 (see Table 1 in (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, 2013a). These two species are found only in Texas, New Mexico, and Arizona, and *S. jamesii* is further found in Colorado and Utah. In some cases these species are found in counties with commercial potato production (pages 21-22 in JR Simplot Company, 2013; USDA-NRCS, 2013c; 2013b). The distribution map for *S. jamesii* in the USDA Plants Database includes Iowa ; however this is not correct (Bamberg, 2012) and this map should be updated in the near future.

As stated above, the five parent varieties of the 10 JRSLA events vary in fertility. Since Russet Burbank produces few flowers and is male sterile and events derived from variety "H" are sterile (do not produce flowers) (JR Simplot, 2013, pp. 17 and 40), pollen-mediated gene-flow from events derived from these varieties would not be possible. The events derived from Ranger Russet, Atlantic, and "G" are likely to produce fertile pollen. The new traits engineered into JRSLA events 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 these 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 and 24 pollinations 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 the 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 cultivated potato is not expected to cross easily with *S. stoloniferum* in the environment, hybridization between the two species is theoretically

[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).

possible if they are growing in close proximity to each other (Andersson and de Vicente, 2010).

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, 2013b; USGS, 2013). 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 in 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 JRSLA 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. low acrylamide potential and reduced black spot bruise) incorporated into the JRSLA 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 or to plant pests or insects. As also discussed above, *S. stoloniferum* is not listed on US or State weed lists (USDA-NRCS, 2013a). *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 JRSLA 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 JRSLA potatoes is not expected to increase the potential for gene flow, 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 JRSLA 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 JRSLA potatoes are unlikely to impact the weediness of this wild species since the JRSLA potatoes do not exhibit characteristics that cause them to be any weedier than other cultivated potatoes.

Therefore, the JRSLA 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; therefore, no impact on plant diseases or pests or their management is likely to occur.

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

APHIS examined the potential for the new genetic material inserted into JRSLA 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., 2011). In addition, the inserted gene cassettes in JRSLA 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 JRSLA 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). JRSLA potatoes contain no introduced DNA sequences derived from plant viruses (JR Simplot, 2013, Table 5). Therefore, the likelihood of HGT from JRSLA 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 an 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.) (non-native) are parasitic for potato (Asigh and Marquez, 2010). If JRSLA 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 JRSLA 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 JRSLA 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 JRSLA 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 10 JRSLA potato events compared to the unmodified varieties from which they were derived. APHIS concludes that the 10 JRSLA potato events are 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 10 JRSLA potato events 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 JRSLA potatoes compared to their nontransgenic counterparts or other comparators.
- No increase in plant pest risk was identified in the 10 JRSLA potato events 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 10 JRSLA potato events compared to their nontransgenic counterparts or other comparators in field trials conducted in growing regions representative of where the JRSLA 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 JRSLA potatoes are unlikely to have any adverse impacts on organisms beneficial to agriculture based on the compositional similarity of JRSLA potato tubers to the parent varieties, the observed interactions of JRSLA potatoes with insects and pathogens, and the unlikely impacts of nontarget effects due to RNAi.
- The 10 JRSLA potato events are 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 JRSLA potatoes as weeds.
- The 10 JRSLA potato events are 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 JRSLA potatoes to other sexually compatible relatives with which they can interbreed is 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 10 JRSLA potatoes were not identified.
- Horizontal gene transfer of the new genetic material inserted into the 10 JRSLA 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.

K. References

51 FR 23302 (1986) "Coordinated Framework for Regulation of Biotechnology."

57 FR 22984 (1992) "Statement of Policy: Foods Derived from New Plant Varieties."

Acuna, R; Padilla, BE; Florez-Ramos, CP; Rubio, JD; Herrera, JC; Benavides, P; Lee, SJ; Yeats, TH; Egan, AN; Doyle, JJ; and Rose, JK (2012) "Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee." *Proc Natl Acad Sci USA.* 109 (11): p 4197-202. http://www.pnas.org/content/early/2012/02/17/1121190109.abstract.

Andersson, MS and de Vicente, MC (2010) *Gene flow between crops and theri wild relatives*. Baltimore: Johns Hopkins University Press.

Arif, M; Azhar, U; Arshad, M; Zafar, Y; Mansoor, S; and Asad, S (2012) "Engineering broad-spectrum resistance against RNA viruses in potato." *Transgenic research.* 21 (2): p 303-11. http://www.ncbi.nlm.nih.gov/pubmed/21701953.

Asigh, J and Marquez, EE. "Dodder (*Cuscuta* spp.) biology and management." Ed. Service, New Mexico State University Cooperative Extension: New Mexico State University, 2010. http://aces.nmsu.edu/pubs/_a/A615/.

Awmack, CS and Leather, SR (2002) "Host plant quality and fecundity in herbivorous insects." *Annual Review of Entomology.* 47 p 817-44. http://www.annualreviews.org/doi/pdf/10.1146/annurev.ento.47.091201.145300.

Bachem, CWB; Speckmann, GJ; Van der Linde, PCG; Verheggen, FTM; Hunt, MD; Steffens, JC; and Zabeau, M (1994) "Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers." *Bio/Technology.* 12 (11): p 1101-05. http://www.nature.com/nbt/journal/v12/n11/pdf/nbt1194-1101.pdf.

Baldwin, IT and Preston, CA (1999) "The eco-physiological complexity of plant responses to insect herbivores." *Planta.* 208 (2): p 137-45. http://dx.doi.org/10.1007/s004250050543.

Bamberg, J to: William Doley, USDA-APHIS. (2012).

Bamberg, J; Rio, A; Huaman, Z; Vega, S; Martin, M; Salas, A; Pavek, J; Kiru, S; Fernandez, C; and Spooner, D (2003) "A decade of collecting and research on wild potatoes of the Southwest USA." *American Journal of Potato Research.* 80 (3): p 159-72. http://dx.doi.org/10.1007/BF02855688.

Barr, CM; Neiman, M; and Taylor, DR (2005) "Inheritance and recombination of mitochondrial genomes in plants, fungi and animals." *New Phytologist.* 168 (1): p 39-50. http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2005.01492.x/abstract.

Boudko, DY (2012) "Molecular basis of essential amino acid transport from studies of insect nutrient amino acid transporters of the SLC6 family (NAT-SLC6)." *Journal of Insect Physiology.* 58 (4): p 433-49. http://www.sciencedirect.com/science/article/pii/S0022191011003623.

Brown, JR (2003) "Ancient horizontal gene transfer." *Nature reviews. Genetics.* 4 (2): p 121-32. http://www.ncbi.nlm.nih.gov/pubmed/12560809.

Carputo, D; Monti, L; Werner, JE; and Frusciante, L (1999) "Uses and usefulness of endosperm balance number." *Theoretical and Applied Genetics.* 98 (3-4): p 478-84. http://link.springer.com/article/10.1007%2Fs001220051095#.

CEPA-OEHHA (2005) "No Significant Risk Level (NSRL) of the Proposition 65 Carcinogen Acrylamide." Agency, California Environmental Protection. Last Accessed: April 30, 2013 http://oehha.ca.gov/prop65/law/pdf_zip/Acrylamide_NSRL.pdf.

Chawla, R; Shakya, R; and Rommens, CM (2012) "Tuber-specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield." *Plant biotechnology journal.* 10 (8): p 913-24. http://www.ncbi.nlm.nih.gov/pubmed/22726556.

Cook, SM; Awmack, CS; Murray, DA; and Williams, IH (2003) "Are honey bees' foraging preferences affected by pollen amino acid composition?" *Ecological Entomology.* 28 (5): p 622-27. http://dx.doi.org/10.1046/j.1365-2311.2003.00548.x.

Corsini, D; Stark, J; and Thornton, M (1999) "Factors Contributing to the Blackspot Bruise Potential of Idaho Potato Fields." *American Journal of Potato Research.* 76 (4): p 221-26. http://download.springer.com/static/pdf/390/art%253A10.1007%252FBF02854225.pdf?auth66=1382815106_2d592b0403bf1fbfcfb882680534e19d&ext=.pdf.

Dixon, RA (2001) "Natural products and plant disease resistance." *Nature.* 411 (6839): p 843-47. http://www.scopus.com/inward/record.url?eid=2-s2.0-0035859039&partnerID=40&md5=228cf646470450a8fd6b4e954fce7f60.

Dröge, M; Pühler, A; and Selbitschka, W (1998) "Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern." *Journal of Biotechnology.* 64 (1): p 75–90. http://www.sciencedirect.com/science/article/pii/S0168165698001059.

EFSA (2009) "Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants." *EFSA Journal.* 1108. http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm.

Ellstrand, NC; Prentice, HC; and Hancock, JF (1999) "Gene flow and introgression from domesticated plants into their wild relatives." *Annual Review of Ecology and Systematics.* 30 p 539-63. http://www.annualreviews.org/doi/abs/10.1146%2Fannurev.ecolsys.30.1.539.

EPA-FIFRA-SAP (2006) "Plant Incorporated Protectants Based on Virus Coat Protein Genes: Science Issues Asosciated with the Proposed Rule." http://www.epa.gov/scipoly/sap/meetings/2005/december/minutes1205.pdf.

FAO (2013) "Top production, potatoes, 2011." FAOSTAT, Statistics Division, Food and Agriculture Organization of the United Nations. Last Accessed: June 17, 2013 http://faostat.fao.org/site/339/default.aspx.

FDA (2006) "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use." Last Accessed: 21 August 2013 http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096156.htm.

Friedman, M (1997) "Chemistry, Biochemistry, and Dietary Role of Potato Polyphenols. A Review." *Journal of Agricultural and Food Chemistry.* 45 (5): p 1523-40. http://pubs.acs.org/doi/pdf/10.1021/jf960900s.

Frischmuth, T and Stanley, J (1998) "Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus." *Journal of General Virology.* 79 (5): p 1265-71. http://vir.sgmjournals.org/content/79/5/1265.abstract.

Fuchs, M and Gonsalves, D (2007) "Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies." *Annual Review of Phytopathology.* 45 p 173-202. http://www.annualreviews.org/doi/full/10.1146/annurev.phyto.45.062806.094434?url_ver=Z39.88-2003.

Gelvin, SB (2003) "Agrobacterium-Mediated Plant Transformation: the Biology behind the “Gene-Jockeying” Tool." *Microbiology and Molecular Biology Reviews.* 67 (1): p 16-37. http://mmbr.asm.org/content/67/1/16.abstract.

Gevens, AJ; Seidl, AC; and Clark, R (2011) "Potato and tomoato late blight in Wisconsin: a new type of late blight in a new decade." *2011 Wisconsin Crop Management Conference:* p 123-26.

Grant, V (1981) *Plant Speciation*. Columbia University Press.

Hegde, SG; Nason, JD; Clegg, JM; and Ellstrand, NC (2006) "The Evolution of California's Wild Radish Has Resulted in the Extinction of Its Progenitors." *Evolution.* 60 (6): p 1187. http://www.bioone.org/doi/abs/10.1554/05-634.1#.Uhelsxtwq-x.

Holm, LG; Pancho, JV; Herberger, JP; and Plucknett, DL (1979) *A geographical atlas of world weeds*. Krieger Publishing Company.

Hoopes, RW and Plaisted, RL (1987) "Potato." *Principles of Cultivar Development*. Ed. Fehr, W.R. Macmillan. New York.

Hunt, MD; Eannetta, NT; Yu, H; Newman, SM; and Steffens, JC (1993) "cDNA cloning and expression of potato polyphenol oxidase." *Plant Molecular Biology.* 21 (1): p 59-68. http://link.springer.com/article/10.1007%2FBF00039618#.

Jackson, SA and Hanneman Jr., RE (1999) "Crossability between cultivated and wild tuber-and non-tuber-bearing Solanums." *Euphytica.* 109 (1): p 51-67. http://link.springer.com/article/10.1023%2FA%3A1003710817938#.

Johnson, DA (2007) *Potato Health Management*. St. Paul: American Phytophathological Society.

JR Simplot Company (2013) "Petition for determination of nonregulated status for Innate™ potatoes with low acrylamide potential and reduced black spot bruise: events E12 and E24 (Russet Burbank); F10 and F37 (Ranger Russet); J3, J55, and J78 (Atlantic); G11 (G); H37 and H50 (H)." Submitted by Registration Manager. JR Simplot Company. Boise ID.

Kaneko, T; Nakamura, Y; Sato, S; Minamisawa, K; Uchiumi, T; Sasamoto, S; Watanabe, A; Idesawa, K; Iriguchi, M; Kawashima, K; Kohara, M; Matsumoto, M; Shimpo, S; Tsuruoka, H; Wada, T; Yamada, M; and Tabata, S (2002) "Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium Bradyrhizobium japonicum USDA110." *DNA Research.* 9 (6): p 189-97. http://dnaresearch.oxfordjournals.org/content/9/6/189.abstract.

Karley, AJ; Douglas, AE; and Parker, WE (2002) "Amino acid composition and nutritional quality of potato leaf phloem sap for aphids." *Journal of Experimental Biology.* 205 (19): p 3009-18. http://jeb.biologists.org/content/205/19/3009.full.pdf+html.

Keeling, PJ and Palmer, JD (2008) "Horizontal gene transfer in eukaryotic evolution." *Nature Reviews Genetics.* 9 (8): p 605-18. http://www.nature.com/nrg/journal/v9/n8/full/nrg2386.html.

Keese, P (2008) "Risks from GMOs due to horizontal gene transfer." *Environmental biosafety research.* 7 (3): p 123-49. http://www.ncbi.nlm.nih.gov/pubmed/18801324.

Khoury, CK; Greene, S; Wiersema, J; Maxted, N; Jarvis, A; and Struik, PC (2013) "An Inventory of Crop Wild Relatives of the United States." *Crop Science.* 53 (4): p 1496. https://www.crops.org/publications/cs/abstracts/53/4/1496.

Koonin, EV; Makarova, KS; and Aravind, L (2011) "Horizonal Gene Transfer in Prokaryotes: Quantification and Classification." *Annual Review of Microbiology.* 55 p 709-42. http://www.annualreviews.org/doi/full/10.1146/annurev.micro.55.1.709?url_ver=Z39.88-2003.

Kroner, A; Marnet, N; Andrivon, D; and Val, F (2012) "Nicotiflorin, rutin and chlorogenic acid: phenylpropanoids involved differently in quantitative resistance of potato tubers to biotrophic and necrotrophic pathogens." *Plant physiology and biochemistry : PPB / Societe francaise de physiologie vegetale.* 57 p 23-31. http://www.ncbi.nlm.nih.gov/pubmed/22677447

Kruzmane, D; L., J; and levinsh, G (2002) "Effect of regurgitant from Leptinotarsa decemlineata on wound responses in Solanum tuberosum and Phaseolus vulgaris." *Physiologia Plantarum.* 115 (4): p 577-84. http://onlinelibrary.wiley.com/doi/10.1034/j.1399-3054.2002.1150412.x/pdf.

Lacey, LA; Horton, DR; Unruh, TR; Pike, K; and Marquez, M (2001) "Biological control of insect pests of potato in North America. Excerpts." *2001 Washington State Potato Conference and Trade Show*. Last Accessed: August 5, 2013 http://aenews.wsu.edu/May01AENews/May01AENews.htm.

Lawson, HM (1983) "True potato seeds as arable weeds." *Potato Research.* 26 (3): p 237-46. http://link.springer.com/article/10.1007%2FBF02357120#.

Lazo, GR; Stein, PA; and Ludwig, RA (1991) "A DNA transformation-competent Arabidopsis genomic library in Agrobacterium." *Bio/Technology.* 9 (10): p 963-67. http://www.nature.com/nbt/journal/v9/n10/pdf/nbt1091-963.pdf.

Love, SL (1994) "Ecological risk of growing transgenic potatoes in the United States and Canada." *American Potato Journal.* 71 (10): p 647-58. http://link.springer.com/article/10.1007%2FBF02851433#.

Lundgren, JG and Duan, JJ (2013) "RNAi-Based Insecticidal Crops: Potential Effects on Nontarget Species." *BioScience.* 63 (8): p 657-65. Last Accessed: 2013/09/12 http://www.bioone.org/doi/abs/10.1525/bio.2013.63.8.8.

Lyon, GD (1989) "The biochemical basis of resistance of potatoes to soft rot Erwinia spp.—a review." *Plant Pathology.* 38 (3): p 313-39. http://dx.doi.org/10.1111/j.1365-3059.1989.tb02152.x.

Martins, SIFS; Jongen, WMF; and van Boekel, MAJS (2000) "A review of Maillard reaction in food and implications to kinetic modelling." *Trends in Food Science & Technology.* 11 (9–10): p 364-73. http://www.sciencedirect.com/science/article/pii/S092422440100022X.

Mayer, AM (2006) "Polyphenol oxidases in plants and fungi: Going places? A review." *Phytochemistry.* 67 (21): p 2318-31. http://www.sciencedirect.com/science/article/pii/S0031942206004560.

Mondy, N; Chandra, S; and Evans, W (1985) "Enzymatic discoloration and phenolic content of potato tubers from cultivars resistant and susceptible to the golden nematode." *American Potato Journal.* 62 (5): p 207-13. http://dx.doi.org/10.1007/BF02852799.

Mottram, DS; Wedzicha, BL; and Dodson, AT (2002) "Acrylamide is formed in the Maillard reaction." *Nature.* 419 (6906): p 448-49. http://www.nature.com/nature/journal/v419/n6906/pdf/419448a.pdf.

Muenscher, WC (1980) *Weeds. Second Edition.*: Cornell University Press.

Nauerby, B; Billing, K; and Wyndaele, R (1997) "Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentrations suitable for elimination of Agrobacterium tumefaciens." *Plant Science.* 123 (1–2): p 169-77. http://www.sciencedirect.com/science/article/pii/S0168945296045694.

NTP (2011) "Report on Carcinogens." U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. Last Accessed: July 8, 2013 http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf.

OECD (1997) "Consensus document on the biology of *Solanum tuberosum* subsp. *tuberosum* (potato)." Directorate, Environment. http://www.oecd.org/science/biotrack/46815598.pdf.

Osman, H; Youssef, M; El-Gindi, A-E-M; Ameen, H; and Abd-Elbary, N, Lashein, A. (2012) "Effect of reniform nematode, *Rotylenchulus reniformis*, as biotic inducer of resistance against root-knot nematode, *Meloidogyne incognita* in potato." *Journal of Plant Protection Research.* 52 (3): p 333-36.

Partington, JC; Smith, C; and G. P. Bolwell, GP (1999) "Changes in the location of polyphenol oxidase in potato (Solanum tuberosum L.) tuber during cell death in response to impact injury: Comparison with wound tissue." *Planta.* 207 (3): p 449-60. http://www.ncbi.nlm.nih.gov/pubmed/9951737.

Peterson, CD; Pearman, DA; and Dines, TD (2002) *New Atlas of the British flora*. Oxford University Press.

Potato Association of America. "Varieties - Ranger Russet (*solanum tuberosum*)." 2009. http://www.potatoassociation.org/Industry%20Outreach/varieties/Russets/ranger_russet.html.

Rasmann, S; Ali, J; Helder, J; and Putten, W (2012) "Ecology and Evolution of Soil Nematode Chemotaxis." *Journal of Chemical Ecology.* 38 (6): p 615-28. http://dx.doi.org/10.1007/s10886-012-0118-6.

Richael, CM; Kalyaeva, M; Chretien, RC; Yan, H; Adimulam, S; Stivison, A; Weeks, JT; and Rommens, CM (2008) "Cytokinin vectors mediate marker-free and backbone-free plant transformation." *Transgenic research.* 17 (5): p 905-17. http://www.ncbi.nlm.nih.gov/pubmed/18320338.

Richards, TA; Soanes, DM; Foster, PG; Leonard, G; Thornton, CR; and Talbot, NJ (2009) "Phylogenomic analysis demonstrates a pattern of rare and ancient horizontal gene transfer between plants and fungi." *Plant Cell.* 21 (7): p 1897-911. http://www.plantcell.org/content/21/7/1897.long.

Richardson, AO and Palmer, JD (2007) "Horizontal gene transfer in plants." *Journal of experimental botany.* 58 (1): p 1-9. http://www.ncbi.nlm.nih.gov/pubmed/17030541.

Rieseberg, LH and Wendel, JF (1993) "Introgression and its consequences in plants." *Hybrid Zones and the Evolutionary Process*. Ed. Harrison, R.G. Oxford University Press.

Riga, E; Perry, R; Barrett, J; and Johnston, ML (1997) "Electrophysiological Responses of Male Potato Cyst NematodesGlobodera rostochiensis and G. pallidato Some Chemicals." *Journal of Chemical Ecology.* 23 (2): p 417-28. http://dx.doi.org/10.1023/B%3AJOEC.0000006368.52520.07.

Sharp, PA (2001) "RNA interference—2001." *Genes & Development.* 15 (5): p 485-90. http://genesdev.cshlp.org/content/15/5/485.short.

Soltis, DE; Soltis, PS; and Rieseberg, LH (1993) "Molecular Data and the Dynamic Nature of Polyploidy." *Critical Reviews in Plant Sciences.* 12 (3): p 243-73. http://www.tandfonline.com/doi/abs/10.1080/07352689309701903#.UhekuRtwq-w.

Spooner, DM; van den Berg, RG; Rodriguez, A; Bamberg, J; Hijmans, RJ; and Cabrera, SIL (2004) *Wild potatoes (Solanum section Petota: Solanacea) of North and Central America*. American Society of Plant Taxonomists. Last Accessed: July 16, 2010 http://www.jstor.org/stable/25027915.

Stace, CA (1987) "Hybridization and the plant species." *Differentiation Patterns in Higher Plants*. Ed. Urbanska, K.M. Academic Press. p 115–27.

Stadler, RH; Blank, I; Varga, N; Robert, F; Hau, J; Guy, PA; Robert, M-C; and Riediker, S (2002) "Acrylamide from Maillard reaction products." *Nature.* 419 (6906): p 449-50. http://www.imreblank.ch/Nature_2002_419_449.pdf.

Tareke, E; Rydberg, P; Karlsson, P; Eriksson, S; and Törnqvist, M (2002) "Analysis of acrylamide, a carcinogen formed in heated foodstuffs." *Journal of Agricultural and Food Chemistry.* 50 (17): p 4998-5006. http://pubs.acs.org/doi/pdf/10.1021/jf020302f.

Thompson, JR and Tepfer, M (2010) "Assessment of the Benefits and Risks for Engineered Virus Resistance." *Advances in Virus Research.* 76 p 33-56. http://www.sciencedirect.com/science/article/pii/S0065352710760024.

Thygesen, PW; Dry, IB; and Robinson, SP (1995) "Polyphenol oxidase in potato. A multigene family that exhibits differential expression patterns." *Plant Physiology.* 109 (2): p 525-31. http://www.plantphysiol.org/content/109/2/525.full.pdf.

US-EPA (2011) "Introduction to biotechnology regulation for pesticides." United States Environmental Protection Agency. Last Accessed: October 24, 2013 http://www.epa.gov/oppbppd1/biopesticides/regtools/biotech-reg-prod.htm.

US-FSAP (2013) "Select Agents and Toxins List." United States Federal Select Agent Program. Last Accessed: July 8, 2013 http://www.selectagents.gov/select%20agents%20and%20toxins%20list.html.

USDA-APHIS (2008a) "Golden nematode." United States Department of Agriculture, Animal and Plant Health Inspection Service. Last Accessed: August 28, 2013 http://www.aphis.usda.gov/publications/plant_health/content/printable_version/fs_golden_nematode_08.pdf.

USDA-APHIS (2008b) "New pest response guidelines: *Ralstonia solanacearum* race 3 biovar 2." United States Department of Agriculture, Animal and Plant Health Inspection Service. Last Accessed: July 8, 2013 http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg-ralstonia.pdf.

USDA-APHIS (2009) "Best management practices for preventing spread of potato cyst nematodes." United States Department of Agriculture, Animal and Plant Health Inspection Service. Last Accessed: August 28, 2013 http://www.aphis.usda.gov/plant_health/plant_pest_info/nematode/downloads/bestpractices.pdf.

USDA-APHIS (2013a) "Plant Pest Program Information." United States Department of Agriculture, Animal and Plant Health Inspection Service. Last Accessed: 21 August 2013 http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml.

USDA-APHIS (2013b) "Potato virus Y strains." United States Department of Agriculture, Animal and Plant Health Inspection Service. Last Accessed: September 12, 2013 http://www.aphis.usda.gov/plant_health/plant_pest_info/potato/pvy_main.shtml.

USDA-ERS (2010) "Vegetables and melons outlook." United States Department of Agriculture, Economic Research Service. Last Accessed: September 12, 2013 http://usda01.library.cornell.edu/usda/ers/VGS/2010s/2010/VGS-10-28-2010.pdf.

USDA-NASS (2009a) "2007 Census of Agriculture: Arizona State and County data." United States Department of Agriculture, National Agricultural Statistics Service. Last Accessed: September 12, 2013 http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_State_Level/Arizona/.

USDA-NASS (2009b) "2007 Census of Agriculture: New Mexico State and County data." United States Department of Agriculture, National Agricultural Statistics Service. Last Accessed: September 12, 2013 http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_State_Level/New_Mexico/.

USDA-NASS (2009c) "2007 Census of Agriculture: Texas State and County data." United States Department of Agriculture, National Agricultural Statistics Service. Last Accessed: September 12, 2013 http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_State_Level/Texas/.

USDA-NASS (2013) "Crop production: 2012 summary." United States Department of Agriculture, National Agricultural Statistics Service. Last Accessed: March 1, 2013 http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_State_Level/Texas/.

USDA-NRCS "Introduced, invase, and noxious plants." United States Department of Agriculture, Natural Resources Conservation Service. Last Accessed: September 12, 2013 http://plants.usda.gov/java/noxiousDriver.

USDA-NRCS "PLANTS Database: Plants profile for *Solanum fendleri* A. Gray ex Torr. (Fendler's horsenettle)." United States Department of Agriculture, Natural Resources Conservation Service. Last Accessed: September 12, 2013 http://plants.usda.gov/core/profile?symbol=SOFE.

USDA-NRCS "PLANTS Database: Plants profile for *Solanum jamesii* Torr." United States Department of Agriculture, Natural Resources Conservation Service. Last Accessed: September 12, 2013 http://plants.usda.gov/core/profile?symbol=SOJA.

USDA (2007) "Recovery plan for potato wart disease." United States Department of Agriculture. Last Accessed: July 8, 2013 http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/PotatoWart70109.pdf.

USGS "Biodiversity occurrence data accessed through Biodiversity Information Serving Our Nation (BISON)." United States Department of Interior, United States Geological Survey. Last Accessed: September 12, 2013 http://bison.usgs.ornl.gov/.

WHO-IARC (1994) *IARC monographs on the evaluation of carcinogenic risks to humans: some industrial chemicals*. Ed. International Agency for Research on Cancer. Lyon: World Health Organisation.

Wood, DW; Setubal, JC; Kaul, R; Monks, DE; Kitajima, JP; Okura, VK; Zhou, Y; Chen, L; Wood, GE; Almeida, NF, Jr.; Woo, L; Chen, Y; Paulsen, IT; Eisen, JA; Karp, PD; Bovee, D, Sr.; Chapman, P; Clendenning, J; Deatherage, G; Gillet, W; Grant, C; Kutyavin, T; Levy, R; Li, MJ; McClelland, E; Palmieri, A; Raymond, C; Rouse, G; Saenphimmachak, C; Wu, Z; Romero, P; Gordon, D; Zhang, S; Yoo, H; Tao, Y; Biddle, P; Jung, M; Krespan, W; Perry, M; Gordon-Kamm, B; Liao, L; Kim, S; Hendrick, C; Zhao, ZY; Dolan, M; Chumley, F; Tingey, SV; Tomb, JF; Gordon, MP; Olson, MV; and Nester, EW (2001) "The genome of the natural genetic engineer Agrobacterium tumefaciens C58." *Science*. 294 (5550): p 2317-23. http://www.sciencemag.org/content/294/5550/2317.long.

Xi, Z; Bradley, RK; Wurdack, KJ; Wong, K; Sugumaran, M; Bomblies, K; Rest, JS; and Davis, CC (2012) "Horizontal transfer of expressed genes in a parasitic flowering plant." *BMC Genomics.* 13 p 227. http://www.biomedcentral.com/1471-2164/13/227.

Xi, Z; Wang, Y; Bradley, RK; Sugumaran, M; Marx, CJ; Rest, JS; and Davis, CC (2013) "Massive mitochondrial gene transfer in a parasitic flowering plant clade." *PLoS Genetics.* 9 (2). http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003265 .

Yoshida, S; Maruyama, S; Nozaki, H; and Shirasu, K (2010) "Horizontal Gene Transfer by the Parasitic Plant Striga hermonthica." *Science.* 328 (5982): p 1128. http://www.sciencemag.org/content/328/5982/1128.full .

Zhu, B; Lou, MM; Xie, GL; Zhang, GQ; Zhou, XP; Li, B; and Jin, GL (2011) "Horizontal gene transfer in silkworm, Bombyx mori." *BMC genomics.* 12 p 248. http://www.biomedcentral.com/1471-2164/12/248 .