

Okanagan Specialty Fruits Inc.'s Petition (10-161-01p) for Determination of Non-regulated Status of Non- browning Arctic™ Apple Events GD743 and GS784

OECD Unique Identifier:

OKA-NB001-8

OKA-NB002-9

Plant Pest Risk Assessment

May 2014

Agency Contact

Cindy Eck

Biotechnology Regulatory Services

4700 River Road

USDA, APHIS

Riverdale, MD 20737

Fax: (301) 734-8669

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA'S TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.

Mention of companies or commercial products in this report does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned. USDA neither guarantees nor warrants the standard of any product mentioned. Product names are mentioned solely to report factually on available data and to provide specific information.

This publication reports research involving pesticides. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

TABLE OF CONTENTS

A.	Introduction.....	1
B.	Development of GD743 and GS784 Arctic™ Apple Events	2
C.	Expression of the Gene Product and Changes to Plant Metabolism.....	5
D.	Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities	8
E.	Potential Impacts on Nontarget Organisms (Including those Beneficial to Agriculture).....	11
F.	Potential for Enhanced Weediness of GD743 and GS784 Arctic™ Apple.....	11
G.	Potential Impacts on the Weediness of Any Other Plants with which GD743 and GS784 Arctic™ Apple Can Interbreed	12
H.	Potential Changes to Agriculture or Cultivation Practices	13
I.	Potential Impacts from Transfer of Genetic Information to Organisms with which GD743 and GS784 Arctic™ Apple Cannot Interbreed	14
J.	Conclusion	15
K.	References.....	15

Plant Pest Risk Assessment for Okanagan Specialty Fruits Inc.'s Non-browning Apple (*Malus x domestica*) Events GD743 and GS784

A. Introduction

Okanagan Specialty Fruits Inc. (OSF) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that the genetically engineered (GE) non-browning Arctic™ apple (*Malus x domestica*) events GD743 and GS784 are unlikely to pose a plant pest risk and therefore, are no longer regulated articles under the APHIS' 7 Code of Federal Regulations (CFR) part 340. This petition was assigned the number 10-161-01p, and is hereafter referenced as OSF, 2012. APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000¹. This plant pest risk assessment was conducted to determine if GD743 and GS784 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 Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and is also considered a plant pest. A GE organism is also regulated under 7 CFR part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk. Arctic™ apple events GD743 ('Golden Delicious') and GS784 ('Granny Smith') were produced by *Agrobacterium tumefaciens*-mediated transformation. Portions of the inserted genetic material are derived from plant pest sequences (i.e., 35S promoter from Cauliflower Mosaic Virus, and nopaline synthase terminator sequence from *A. tumefaciens*). Therefore, the genetically engineered apple events GD743 and GS784 are considered regulated articles under APHIS regulations at 7 CFR part 340. OSF has conducted field releases of GD743 and GS784 as regulated articles under APHIS-authorized permits or acknowledged notifications since 2003 (Tables 22 and 23, p. 61, OSF, 2012) in part, to gather information to support whether they are unlikely to pose a plant pest risk.

Potential impacts to be addressed in this risk assessment are those that pertain to the use of GD743 and GS784 and their progeny in the absence of confinement as compared to the unmodified apple cultivars from which GD743 and GS784 were derived. APHIS uses data and information submitted by the applicant, in addition to current literature, to assess if GD743 and GS784 are unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, then APHIS has no regulatory authority over that organism under 7CFR

¹ Section 403 (14) of the Plant Protection Act (7USC Sec 7702(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."

340. APHIS regulation 7 CFR 340.6(c) specifies the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities; indirect plant pest effects on other agricultural products; changes to agricultural or cultivation practices that may impact diseases and pests of plants; effects of the regulated article on nontarget organisms; weediness of the regulated article; any impacts on the weediness of any other plant with which it can interbreed; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with the APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies. The EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), regulates 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. The 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). GD743 and GS784 apple events are not engineered to express substances to protect against plant pests. 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 consultation process (57 FR 22984). OSF (2012) has indicated that they have submitted a food and feed safety and nutritional assessment for events GD743 and GS784 on May 30, 2011. FDA posts information about completed consultations for public review via the FDA Completed Consultations on Bioengineered Foods page at www.fda.gov/bioconinventory. Additionally, regulatory submission for GD743 and GS784 has been submitted to Health Canada and the Canadian Food Inspection Agency (CFIA) on December 7, 2011 (p.16, OSF 2012).

B. Development of GD743 and GS784 Arctic™ Apple Events

Apple has been cultivated in Europe and Asia from ancient times (Janick et al., 1996). Today, the United States is one of the top apple producers in the world and as of 2008, ranked second only to China (USDA, ERS, 2010a). Apples are consumed in many forms. During that same year, approximately two-thirds of U.S. apple production was for the fresh market, while the remaining was processed into various forms such as, fruit juice, cider, sauce, canned, fresh apple slices, and dried fruit products (USDA, ERS, 2010b). Apple cultivars suitable for the processing market should be large in size and depending on the processed product, high in soluble solids, specific sugar acid ratio, yellow flesh color, non-browning flesh, and should taste like an "apple" (Janick et al., 1996). Over the past 5 years there has been a substantial increase in retail sales of fresh apple slices, part of which can be contributed to the increased use of fresh apple slices in fast food establishments and single serve packaging (Boyd, 2011). The most significant challenge of

the fresh apple slice market is minimizing cut surface browning which is a major contributor to reduced shelf life of processed fruits (Chiabrando and Giacalone, 2012). Mechanical bruising during and after harvesting and processing is the largest contributor to apple fruit loss (Kader, 1983; Nicolas et al., 1994). The enzyme responsible for this browning is polyphenol oxidase (PPO). PPO can be found in bacteria, fungi, plants, arthropods, and mammals (Martinez and Whitaker, 1995). In general, PPOs convert o-diphenols to o-quinones utilizing oxygen as the second substrate (Figure 1). The reactive quinones polymerize to form brown pigments, melanins. The major phenolics identified in apple fruit are: quercetin glycosides, chlorogenic acid, epicatechin, phloretin glycosides, and procyanidin B₂ (Lee et al., 2003). Several phenolics serve as substrates for PPO in apple; especially chlorogenic acid (the principle one in mature apple fruit), epicatechin, and catechin, however, catechin plays a major role in browning because its oxidative product has a higher intensity of color (Murata et al. 1995).

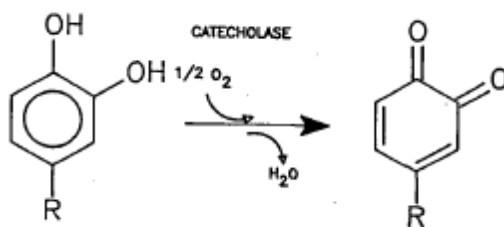


Figure 1. Reaction catalyzed by PPO (also known as catecholase). Dehydrogenation of o-diphenol to o-quinone (Nicolas et al., 1994).

Levels of PPO and concentrations of total polyphenols, proanthocyanidins (polymers of catechin and epicatechin), (+)-catechin, and phloridzin appear to have a strong positive correlation with the degree of browning in various apple cultivars (Song et al., 2007). A number of treatments have been used to prevent browning of fresh cut apples in effort to increase the shelf life, some of which include natural browning inhibitors, salt and chemical treatments, edible coating agents, and reduced oxygen atmospheres. One of the most common methods is the application of a reducing agent such as ascorbic acid (vitamin C) in combination with calcium chloride (firming agent) (Chiabrando and Giacalone, 2012). OSF has developed Arctic™ apple events GD743 and GS784 which exhibit a non-browning phenotype via the suppression of four apple PPO genes. These non-browning apples are intended to benefit the fresh cut apple and dehydrated apple markets by reducing browning associated with bruising and cutting, eliminating the need for chemical treatments to reduce browning of fresh cut apple slices and thereby promoting the inclusion of the apple in the fresh cut and prepared produce fruit market (pp. 15-16 in OSF 2012).

Description of the genetic modifications

Arctic™ Apple events GD743 and GS784 were produced by using *Agrobacterium*-mediated transformation of apple leaf tissue from the cultivars ‘Golden Delicious’ and ‘Granny Smith’ respectively (pp. 34-36 in OSF, 2012). The *Agrobacterium tumefaciens* strain used during transformation was disarmed of DNA sequences within the T-DNA (transfer-DNA), which upon integration into the plant genome are normally responsible for the formation of crown gall tumors in plants. The disarmed *Agrobacterium tumefaciens* harbors a binary plasmid vector GEN-03 (Figure 2, p. 35 in OSF 2012). GEN-03 was based on a derivative of the pBIN19 vector (Bevan, 1984), pBINPLUS (van Engelen et al., 1995) and contains the polyphenol oxidase

(PPO) silencing and the *nptII* expression cassettes flanked by *Agrobacterium tumefaciens* T-DNA borders (Table 5, p. 35 in OSF 2012). The suppression of PPO in GD743 and GS784 results in apples that have a non-browning phenotype.

The first gene cassette consists of three genetic elements:

- P_{NOS}, nopaline synthase promoter sequence from *A. tumefaciens*, which directs constitutive transcription of the *nptII* selection marker (Bevan et al., 1983a).
- *nptII*, neomycin phosphotransferase type II gene from *E. coli* Tn5 (Rothstein et al., 1981; Bevan, 1984). This gene provides resistance to the antibiotic kanamycin.
- T_{NOS}, nopaline synthase terminator sequence from *A. tumefaciens*. This sequence is involved with termination of transcription and polyadenylation. (Depicker et al., 1982; Bevan et al., 1983b).

The second gene cassette consists of three genetic elements:

- P_{CAMV35S}, the duplicated-enhancer 35S cauliflower mosaic virus promoter with untranslated translational leader sequence from alfalfa mosaic virus RNA4 designed for constitutive high level expression (Datla et al., 1993). This promoter directs transcription of the PGAS transgene.
- PGAS, a chimeric sense suppression transgene from *Malus × domestica*. PGAS consists of 394 to 457 bp regions of four apple PPO genes (PPO2, GPO3, APO5, and pSR7) in tandem that upon transcription is designed to suppress the expression of these four members of the apple PPO gene family (Armstrong and Lane, 2009; pp. 31-36 in OSF, 2012).
- T_{NOS}, nopaline synthase terminator sequence from *A. tumefaciens*. This sequence is involved with termination of transcription and polyadenylation. (Depicker et al., 1982; Bevan et al., 1983b).

The production of the neomycin phosphotransferase type II enzyme in the transformed tissue allows apple tissue containing the *nptII* and PGAS gene expression cassettes to be selected on medium containing the antibiotic kanamycin. Expression of *nptII* confers no other benefit to the regenerated transformed apple plant.

Due to the lack of sequence similarity between the four PPO genes targeted for suppression (with the exception of APO5 and GPO3) (Table 3 and 4, p. 34 in OSF, 2012) it was necessary to design the PGAS PPO suppression transgene with sequences from all four PPO genes. None of the four PPO genes alone shared 100% homology over regions of 22 bp with all four of the PPO genes. This level of homology has been suggested to be necessary for effective gene targeting and silencing using RNA interference (RNAi) because the longer double stranded RNA (dsRNA) produced from the suppression transcript is processed into small interfering RNAs (siRNAs) that are 21-23 nucleotides in length that in turn direct the cleavage of the target mRNA through sequence complementarity (Sharp, 2001). The suppression of PPO results in apples with a non-browning phenotype.

OSF provided evidence demonstrating:

- The *A. tumefaciens* strain EHA105 that was used to transform ‘Golden Delicious’ and ‘Granny Smith’ is a nonpathogenic strain of *Agrobacterium* that contains a disarmed Ti

plasmid devoid of functional sequences capable of inducing tumor formation in plants (Hood et al., 1993);

- The final product does not contain backbone sequences corresponding to the gene for *NptIII* outside of the T-DNA borders (OSF, 2012, Figure 4, p. 40) from the transformation vector, GEN03 (OSF, 2012, Figure 6, p.43);
- The genome of ‘Golden Delicious’ (GD743) contains two copies of GEN03 inserted at two separate loci, and the genome of ‘Granny Smith’ (GS784) contains 4 copies of GEN03 inserted at four separate loci (OSF, 2012, Figure 5, p.42, and analysis p. 41); and multiple insertions at a single site in the form of tandem or inverted repeats was ruled out for the GS784 event (OSF, 2012, pp. 44-47);
- Inheritance of the GEN03 silencing construct was confirmed by Polymerase Chain Reaction (PCR) analysis of the linked *nptII* gene and Enzyme-Linked Immunosorbent Assay (ELISA) analysis of the expressed NPTII protein demonstrating the Mendelian inheritance of *nptII*/NPTII from seed or plant material generated from seed (OSF, 2012, Table 11, p.48). The results were consistent with segregation ratios expected for two unlinked insertion sites in GD743 and four unlinked insertion sites in GS784.
- Clonal stability of the GEN03 silencing construct in the apple genome was confirmed by PCR and ELISA analysis of *nptII*/NPTII present in apple leaf tissue that was clonally propagated (OSF, 2012, Table 12 and 13, p.49 and 50). Unlike most annual crops, apple trees are vegetatively propagated by grafting a fruit-producing scion onto a rootstock; therefore each vegetatively propagated apple tree scion is a genetically identical clone, and little or no genetic variability is expected. Evaluation of the clonal stability of vegetatively propagated apple tree scions is important for determining gene stability from year to year and graft to graft (OSF, 2012; Janick et al., 1996).

C. Expression of the Gene Product and Changes to Plant Metabolism

APHIS assessed whether changes in plant metabolism or composition in GD743 and GS784 are likely to alter plant pest risk. The assessment encompasses a consideration of the expressed PGAS sense silencing RNA and its effect on plant metabolism, and an evaluation of whether GD743 and GS784 are nutritionally equivalent to the cultivars from which they were derived, GD and GS, as well as published nutritional data for apple (NDB09003) provided by the USDA (2009). The NDB09003 reference standard is based on analytical data for ‘Red Delicious’, ‘Golden Delicious’, ‘Gala’, ‘Granny Smith’, and ‘Fuji’ raw apples with the skin. Phenolics are the primary substrates of PPO enzymatic activity. The main apple phenolics (quercetin glycosides, chlorogenic acid, epicatechin, phloretin glycosides, and procyanidin B₂) exhibit antioxidant capacity (Lee et al., 2003); and therefore, silencing of PPO in apple may contribute to the retention or increase of these compounds. 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 (Awmack and Leather 2002). Similarly a vast array of secondary metabolites in plants is known to provide defense against microbes (Dixon, 2001). Thus APHIS assessed whether changes in host plant quality could have the potential to affect GD743 and GS784’s performance against pest and disease.

Systemic induction of PPO gene expression has been studied in response to injury, herbivory, fungal pathogens, and bacterial pathogens in one or more of the following species: tomato, poplar, and tobacco (Thipyapong and Steffens, 1997; Thipyapong et al., 2004; Thipyapong et al., 2007; Constabel and Ryan, 1998; Barbehenn et al., 2007; Wang and Constabel, 2004). More specifically, tomato plants that over expressed PPO were more resistant to *Pseudomonas syringae* and to insect pests of tomato, such as common cutworm (*Spodoptera litura*), cotton bollworm (*Helicoverpa armigera*) and beet army worm (*Spodoptera exigua*). However, in the case of antisense PPO-suppressed tomato, susceptibility to these pathogens and pests was increased as evidenced by increase bacterial growth and lesion numbers, higher larvae growth rates and foliage consumption (Thipyapong et al., 2004; Thipyapong et al., 2007). To date, steady state levels of PPO have not been correlated with scab (Kolodziejczyk et al., 2010) or fire blight resistance (Korba et al., 2008; Sobiczewski et al., 2006). Increased expression of certain members of the PPO gene family and increased PPO enzymatic activity have however been demonstrated in various apple tissues upon wounding (Boss et al., 1995; Kim et al., 2001).

GD743 AND GS784 are genetically engineered with a silencing construct designed to reduce the expression of four apple PPO genes: PPO2, GPO3, APO5, and pSR7 (OSF, 2012); therefore, the gene product is a chimeric, sense-silencing RNA rather than a functional protein or new enzyme.

PPO enzymatic activity studies and controlled bruising studies were conducted to determine the level of PPO enzymatic activity in GD743 and GS784 relative to their un-transformed counterparts ‘Golden Delicious’ (GD) and ‘Granny Smith’ (GS) (OSF, 2012, Tables 12-21, p 51-59). PPO enzymatic activity studies were conducted on unwounded leaf tissue (tissue culture, greenhouse, and field leaves) and fruit tissue (immature and mature fruit). The controlled bruising experiments were used to further verify a reduction in PPO activity. In these experiments, the level of fruit bruising was reported qualitatively by visual inspection and quantitatively with a Chromo Meter and reported as “change in lightness” or “total change in color” following manual bruising of mature fruit of GD743, GD, GS784, and GS at two different temperatures, either directly out of cold storage (2°C) or at 18°C (2 hrs following removal from cold storage).

OSF has provided evidence demonstrating that,

- Overall, PPO activity was reduced by 76% or more in GD743 and GS784 leaf tissue with the highest reduction in GD743 and GS784 greenhouse leaves (93%) (OSF, 2012, Tables 14, 15, 16, p.51-52);
- For immature and mature fruit of GD743, PPO enzymatic activity was reduced by more than 90%. PPO activity was reduced by 90% in GS784 mature fruit; however, PPO activity for GS784 immature fruit was not determined due to limited sample availability (OSF, 2012, Tables 17, 18, p.53-54).
- Visual bruising of GD743 and GS784 mature fruit was undetectable whereas, visual observation of bruising in GD and GS was detectable. Quantitative results for GD743 and GS784 indicated that there was a statistically significant difference in the change in lightness (delta L) or the change in color (delta E) in the mechanically bruised area as compared to the GD and GS non-transformed controls regardless of the fruit temperature at the time of the bruising (OSF, 2012, Tables 19, 20, 21, p.57-59).

- Photographic images of bruised, sliced, or juiced fruit of GD743 and GS784 and their respective controls (Figures 14, 15, and 16, respectively, pp. 115-117 in Appendix 1 of OSF 2012) also demonstrate reduced browning or changes in color for the transgenic lines and support the quantitative data.

The only new functional protein/enzyme produced by the inserted transgenic material is the selectable marker NPTII. The production of this enzyme is used solely for the purposes of selecting apple tissue containing the *nptII* and PGAS gene expression cassettes on medium containing the antibiotic kanamycin. NPTII protein was not found to accumulate in mature fruit of GD743 and GS784 (levels were within the same range found in nontransgenic controls) (Table 41, p. 92 of OSF2012).

Detailed compositional and nutritional comparisons of GD743 and GS784 and the conventional apple fruit controls GD and GS were conducted on a limited number of composite samples collected from New York and Washington States in 2009. GD and GD743 samples were harvested one month prior to the GS and GS784 samples and stored at 2°C as harvesting dates can vary depending on the cultivars and environmental conditions (Kupferman, 1992; Kupferman, 1994). The primary nutrients in apple are sugar, dietary fiber, potassium, phenolic antioxidants and, to a lesser extent, vitamin C. Apple events GD743 and GS784 and their respective controls (GD and GS) were analyzed for fat, protein, moisture, ash, carbohydrates, calories, sugar profile, dietary fiber, potassium, Vitamin C, total phenolics and water-soluble oxygen radical absorbance capacity (ORAC) (OSF, 2012, Tables 28 and 29 p.82-83).

The compositional analyses confirmed the following

- A statistically significant cultivar effect was observed for GD and GS. GS had higher moisture (OSF, 2012, Table 30, p.84), protein (OSF, 2012, Table 31, p.85), potassium (OSF, 2012, Table 36, p.87), and fiber (OSF, 2012, Table 34, p.86) content and lower carbohydrate (OSF, 2012, Table 32, p.85), calorie (OSF, 2012, Table 33, p.86), and sugar (OSF, 2012, Table 35, p.87) content than GD.
- A statistically significant field effect was observed for the New York and Washington Field Trials. The Washington field trials produced apples with higher moisture content (OSF, 2012, Table 30, p.84) and lower protein (OSF, 2012, Table 31, p.85), carbohydrate (OSF, 2012, Table 32, p.85), calories (OSF, 2012, Table 33, p.86), sugar (OSF, 2012, Table 35, p.87) and dietary fiber (OSF, 2012, Table 34, p.86) than apples produced at the New York field trial.
- No statistically significant effect was observed for Arctic™ apple events GD743 and GS784 for protein, moisture, carbohydrates, calories, sugar profile, dietary fiber, or potassium (as per the OSF 2012 Tables 30-36, p.84-87) or for fat (below the reporting limit) (OSF 2012 Tables 28 and 29, p.82-83).
- A statistically significant effect was observed for Arctic™ apple events GD743 and GS784 and for the New York and Washington field trials for Vitamin C (OSF, 2012, Table 39, p.90), ORAC (OSF, 2012, Table 37, p.89), and total phenolics (OSF, 2012, Table 38, p.89). GD743 and GS784 had higher Vitamin C, ORAC and total phenolics as compared to the GD and GS controls. However, the values for GD743 and GS784 fell within or close to the range for apple, raw with skin standard (NDB09003) provided by

the USDA (2009); whereas, the values for GD and GS were outside of this range. These observed effects appeared to be greater in the New York field trial.

The sample preparation methodology for compositional testing of Arctic™ apple events GD743 and GS784 and respective controls deviated from the sample preparation methodology used for the NDB09003 USDA standard. Arctic™ apple events GD743 and GS784 fruit and their controls were pre-sliced, placed on ice and shipped to the laboratory for testing. A period of up to 24 hours passed prior to the apple slices being tested. While the Arctic™ apple events GD743 and GS784 fruit have reduced PPO expression the controls do not and therefore the controls had some PPO-driven browning prior to compositional testing. Phenols and Vitamin C are substrates for the PPO-driven browning reaction and were most likely partially consumed during the 24 hour period of time between slicing/shipping and testing.

In summary, GD743 and GS784 apple fruit are nutritionally equivalent to their respective controls GD and GS and fall within or close to the range for NDB09003 USDA standard (USDA, 2009). Differences in Vitamin C, ORAC and total phenolics were observed between GD743 and GS784 and their respective controls (GD and GS), and these compounds have had varying impacts on pests and diseases in other plant species (Awmack and Leathers, 2002; Goggin et al., 2010; Mukherjee et al., 2010). However levels of vitamin C, ORAC and total phenolics fall within or close to the range for the USDA standard NDB09003; therefore APHIS concludes that GD743 and GS784 Arctic™ apple fruit pose no more of a plant pest risk from gene silencing, new gene products, or changes to plant metabolism or composition than conventional apple fruit. Moreover, the resulting elevated Vitamin C content and increased total phenolics after slicing contributes to an increase in chemical compounds with antioxidant capacity for the GD743 and GS784 events as these compounds would be retained in fresh apple slices for a longer period of time than in GD and GS.

D. Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities

APHIS assessed whether GD743 and GS784 apples are likely to have significantly increased disease and pest susceptibility. This assessment encompasses a thorough consideration of introduced traits, their impact on agronomic traits and plant composition, and quantitative and/or observational data on pest and disease responses. Important changes are those which would (1) affect not only the new GE crop, but that would also result in significant introduction or spread of a damaging pest or disease to other plants; and/or (2) result in the introduction, spread, and/or creation of a new disease 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 should be evaluated with respect to the context of currently cultivated varieties, the intensity of the impact and the ability to manage the pest or disease.

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, or weed programs exist (USDA APHIS, 2013). Currently, the Light Brown Apple Moth (LBAM) and the Japanese Beetle are two insect management programs in place that have potential implications for apple. While Japanese Beetle is quite widespread, the LBAM in the United States is restricted to only a few counties in California where eradication efforts are ongoing (USDA APHIS, 2011).

The apple orchard is a highly managed environment which incorporates integrated pest management (IPM) strategies. IPM programs are tailored to specific areas of the country; however, nearly every IPM program specifically addresses the most common diseases of apple: apple scab, fire blight, and powdery mildew as well as the most common insect pests of apple which include codling moth, aphids, mites, and tentiform leaf miners (MacHardy, 2000; McCamant, 2007; Beckerman, 2006). While viral diseases can infect apple, primarily through the use of infected grafting wood, the use of certified budwood programs has had a significant impact on reducing the spread of viral disease of apple (WSU, 2010).

OSF used qualitative or quantitative techniques (OSF, 2012, Appendix 3, p.141-163) to measure field trials for damage due to diseases (apple scab, powdery mildew, fire blight, leaf spot, post-harvest fruit rot), insect pests (aphids, mites, Japanese beetle, Codling Moth, Tentiform Leaf Miner, Campylopus bug), and two other conditions that affect apple, Burr Knot and russet. Data were collected on pest and disease damage for field trials in New York and Washington State in one or more of the following years: 2003, 2004, 2005, 2008, 2009 and 2010.

OSF collected data for the following fungal diseases of apple with the most causal agent for each disease included in parentheses: apple scab (*Venturia inaequalis*), powdery mildew (*Podosphara leucotricha*), leaf spot (*Alternaria mali* or *Botryosphaeria obtusa*), and black (*Botryosphaeria obtusa*) and tan (*Colletotrichum spp.*) post-harvest rot.

No incidences of apple scab were observed at either the Washington or New York field trial. This was most likely due to the routine fungicide application used to control powdery mildew in susceptible cultivars such as GD and GS. No statistically significant differences in the incidence of powdery mildew, leaf spot, or post-harvest tan rot on GD743 and GS784 compared to their respective controls GD and GS were observed. A significant difference in the incidence of black rot on GD compared to GD743 (GD>GD743) fruit collected from the 2005 New York field trial in June of 2009 was observed. However, in December of 2010, no statistically significant difference was observed for the same field trial.

OSF collected data at both the Washington and New York field trials for fire blight, which is caused by the bacterial pathogen *Erwinia amylovora*. No incidences of fire blight were reported for the New York field trial. Between May 2005 and September 2007, thirteen incidences of fire blight were detected in the Washington 2003 and 2004 field sites. Of the thirteen affected trees, only two GD743 trees were affected, and the remaining affected trees were other GEN03 events in various backgrounds and the GD control. No incidences of fire blight were reported for GS or GS784.

OSF collected data for the following insect pests: aphids, Woolly Apple Aphid (*Eriosoma lanigerum*), Green Apple Aphid (*Aphis pomi*), mites (*Tetranychus McDanieli*, *Tetranychus urtica*, *Aculus schlechtendal*), Japanese beetle (*Popillia japonica*), Codling moth (*Lapeyresia pomonella*), Campyloomma bug (*Campyloomma verbasci*), and Tentiform Leaf Miner (*Phyllonorycter blancardella*). Both the Washington and New York field trials were monitored for mites. No incidences of mites were observed for the New York field trial, and only a single incidence of mites was observed for the Washington field trial in 2009. This observation did not include any events subject to this petition or their respective controls. Codling moth damage was not observed for the New York field trial in the 2010-2011 growing season. Although codling moth damage was reported for the Washington field trials, no significant difference in the incidence of codling moth on GD743 and GS784 and their respective controls were observed. A general observation of aphid incidence was recorded for the New York field trial. No difference was observed between GD743 and GD or GS784 and GS. For the Washington field trials, observations of Woolly Apple Aphid and Green Apple Aphid were recorded. No significant difference between GD743 and GD or GS784 and GS were observed. Japanese beetle incidence was observed for the New York field trial only, and no significant difference was observed between GD743 and GD or GS784 and GS. A significant difference in the incidence of Tentiform Leaf Miner (TLM) between GS784 and GS was observed in 2005 at the Washington field trial with a higher incidence of TLM being observed on GS784. Incidences of Campyloomma Bug were not detected at the New York field trial. Campyloomma Bug was detected in the Washington 2004 and 2008 field blocks in May of 2010; however, a significant difference in Campyloomma Bug incidence was observed in the 2004 block, but not in the 2008 block, with the incidence of Campyloomma Bug being higher in the GD control than in the GD743 event.

OSF collected data on two conditions affecting apple, Burr Knot and Russet. Burr Knot is a condition where knot like structures occur where adventitious shoots are trying to form. The exact cause of Burr Knot is unknown, but Burr Knot can predispose the apple tree to secondary infections or infestations (Roos, 2005). The incidence of Burr Knot was not influenced by the incorporation of the GEN03 transgene for the New York field trial, and Burr Knot was considered a normal condition for the Washington field trial. Apple russetting results in a brown, corky net-like condition on apple skin, and can be caused by a number of factors such as environmental conditions, pesticide use, and various microbes (du Toit et al., 2012; Brown, 1998). Russet appeared to be influenced by the incorporation of the GEN03 transgene with a higher incidence of Russet observed in GD versus GD743 in the 2004 block of the Washington State field trial in 2010 but not in the 2008 field block. No difference in the incidence of Russet at the New York State field trial was observed in 2010.

OSF's pest and disease field data and post-harvest rot data (OSF 2012, Appendix 3, p. 141-163, Tables 53-65) indicate that in a highly managed orchard environment GD743 and GS784's non-browning phenotype did not increase the pest and disease incidences on GD743 and GS784, with the exception of the slight increase in incidence of Tentiform Leaf Miner in GS784 compared to GS; therefore, GD743 and GS784 are expected to be no more susceptible to the same plant pathogens and insect pests as their conventional apple cultivars GD and GS. It therefore follows that there should be no indirect plant pest effects on other agricultural products that are grown or stored in proximity to GD743 and GS784

E. Potential Impacts on Nontarget Organisms (Including those Beneficial to Agriculture)

GD743 and GS784 Arctic™ apple are not engineered for pest resistance, thus there are no ‘target’ species, and thus no ‘nontarget’ species either. APHIS assessed whether exposure or consumption of GD743 and GS784 Arctic™ apple would have an adverse effect on beneficial species or wildlife associated with apple.

As discussed earlier, GD743 and GS784 are similar in nutritional and compositional analysis to their untransformed counter parts GD and GS except for the changes in the total phenolics and vitamin C. GD743 and GS784 apples are engineered to silence PPO gene expression and therefore do not express a PPO protein. The four apple PPO genes targeted for suppression lack significant sequence similarity to each other (with the exception of APO5 and GPO3) to design a single RNA sense silencing transgene capable of silencing all four genes. The PGAS transgene contains sequences unique to each individual transgene indicating that sense silencing of apple PPO genes requires a specific level of sequence similarity. 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 PGAS transgene would contribute to PPO silencing in other non-target organisms such as pollinators or herbivores whose PPO sequences are expected to be even more divergent than those in apple. The only functional protein expressed as a result of the genetic engineering of GD743 and GS784 is the NPTII protein which confers kanamycin resistance. This antibiotic resistance allows for the selection of apple tissue containing the *nptII* and PGAS gene expression cassettes on medium containing the antibiotic kanamycin. Expression of *nptII* confers no other benefit to the regenerated transformed apple plant. NPTII is a common protein found in genetically engineered plants that have been widely planted across the U.S. and in other countries. No issues related to health or environmental safety have been noted to date (APHIS petitions 04-317-01p, 04-264-01p, 01-137-01p, 01-206-02p, 01-206-01p, 95-352-01p, 96-051-01p, 95-045-01p, 94-308-01p) (USDA-APHIS, 2012). Therefore, APHIS has determined the presence of the *nptII* gene will have no significant environmental impacts.

OSF submitted food and feed safety data and nutritional assessment of GD743 and GS784 apple fruit to FDA on May 30, 2011, as well as, a regulatory submission for GD743 and GS784 to Health Canada and the Canadian Food Inspection Agency (CFIA) on December 7, 2011 (p.16, OSF 2012). Based on the nutritional composition data and lack of toxicity and allergenicity of *nptII* gene products, APHIS concludes that consumption of GD743 and GS784 plant or plant products by mammals and other nontarget organisms is unlikely to cause any adverse impact on their survival and reproduction.

F. Potential for Enhanced Weediness of GD743 and GS784 Arctic™ Apple

Apple is a highly domesticated fruit tree species, and cultivated varieties of apple in the U.S. are not listed as weeds (Muenscher, 1980) or as Federal noxious weeds (7 CFR part 360; USDA NRCS, 2010). Baker (1974) described a set of ideal characteristics of weeds. Apple, although

not classified as a weed, does possess a few of the characteristics described by Baker (1974) and Hancock and Hokanson (2001) such as high seed output, seed production in many habitats, and short and distant seed dispersal. Reichard and Hamilton (1997) developed a model specifically directed toward addressing the invasiveness of trees. Many of the characteristics considered in this assessment overlap with those suggested by Baker (1974) and Hancock and Hokanson (2001). However, additional characteristics such as native range, invades elsewhere, polyploidy, fruiting season, length of fruiting period, seed size, and leaf longevity were considered in Reichard and Hamilton's tree model. Hancock et al. (2003) describe apple as having compatible wild relatives, an intermediate number of weediness traits and capable of escaping and persisting in the environment. In the context of the genetically engineered trait introduced, non-browning, GD743 and GS784 are not likely to become weedier than their non-GE counterparts GD and GS.

Phenotypic and agronomic characteristics of GD743 and GS784 were evaluated in a comparative manner to their respective controls (GD and GS) for both the New York (NY) and Washington (WA) State field trials over a period of two years (OSF, 2012, Tables 24 and 25, Figure 9, p.65-66). These assessments included tree height (NY only), trunk cross sectional area, flower clusters (NY only), and fruit number at harvest. Based upon the data collected for the New York and Washington State field trials over a period of two years, OSF (2012) has indicated that GD743 and GS784 are phenotypically and agronomically similar to GD and GS. Together with the disease and pest resistance data described in the '*Potential Impacts of Genetic Modifications on Disease and Pest Susceptibilities*' section of this document these findings support the conclusion that GD743 and GS784 are no more likely to be a weed compared to conventional apple.

G. Potential Impacts on the Weediness of Any Other Plants with which GD743 and GS784 Arctic™ Apple Can Interbreed

Gene flow from crops to wild relatives is thought to have the potential to enhance the weediness of wild relatives, as observed in rice, sorghum, sunflower and a few other crops (Ellstrand et al., 1999). *Malus sieversii* is considered to be the wild progenitor of the cultivated apple (Richards et al., 2009). Apple (*Malus x domestica*), as we know it today, is a predominantly outcrossing species and is likely the result of countless interspecific crosses. There are over 25 apple species and several *Malus* crab apple species in the U.S.; however, only crab apples are native to this country (USDA NRCS, 2012). Crab apples native to the U.S. include *Malus angustifolia* (southern crab apple), *Malus coronaria* (sweet crab apple), *Malus fusca* (Oregon crab apple), and *Malus ioensis* (prairie crab apple) (Little, 1979). Both native crab apple species and cultivated apples may vary in their chromosome numbers (polyploidy) (Luby, 2003).

Apple trees undergo a juvenile phase; a phase in which the trees do not produce flowers. The juvenile phase of apple can range from three to ten years and is dependent on the cultivar and cultural practices (Janick et al., 1996). Mature apple trees produce an abundance of flowers of which only approximately seven percent of the flowers are necessary for commercial apple production. In order to produce quality fruit of sufficient size, apple growers typically thin apple blossoms through chemical or mechanical means (Hehnen et al., 2012). In general, apples produce ten seeds per fruit; however, some cultivars are capable of producing more (Janick et al.,

1996). Apple seeds can be produced through hybridization or asexual seed reproduction (apomixis) (Kron and Husband, 2009). Additionally, apple seeds require stratification (cold storage for a minimum length of time, usually 6-14 weeks) for germination to occur (Janick et al., 1996). Apple is a predominantly out-crossing species and is dependent upon insects for pollination. Apples are primarily pollinated by honeybees, but other insect pollinators including bumblebees and *Osmia* bees, as well as some other wild bee species, contribute to apple pollination (Park et al. 2012). There is potential for GD743 and GS784 to naturally outcross or hybridize with other cultivated apple varieties or native crab apples or crab apple cultivars with which they have overlapping flowering times (Fitzgerald, 2005; Wilson, 2009). Interspecific hybrids of *M. domestica* x *M. coronaria* (*M. x platycarpa*) have been documented in Eastern North America (Luby, 2003; Kron and Husband, 2009). However, GE pollen from GD743 or GS784 that pollinates other apple varieties will only move the transgene into the seed of the non-GE apple variety as the apple fruit is comprised of maternal tissue only and therefore would not contain the transgene. Furthermore only a portion of the seeds produced by an apple pollinated with GE pollen would carry the transgene. Apples are not true to seed and are typically produced through the vegetative propagation of shoots and grafting onto rootstock. Gene flow and introgression between GD743 and GS784 and apples in a natural environment is possible albeit dependent upon complex genetic and ecological interactions (Kron and Husband, 2009).

As noted in the previous section, '**Potential for Enhanced Weediness of ArcticTM GD743 and GS784 Apple**', the GD743 and GS784 do not exhibit characteristics that would cause them to be more weedy than GD or GS. GD 743 and GS784 were also observed to be no more resistant to pest and diseases of apple compared to GD and GS under highly managed orchard conditions (with the possible exception of the relatively minor pest, Tentiform Leaf Miner, for GD784). Therefore, the introduced gene cassettes (PGAS and *nptII*) are not likely to increase weediness or fitness in wild relatives of apple. Therefore, APHIS has determined that any adverse consequences of gene flow from GD743 and GS784 to wild or weedy species of apple in the U.S. are unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered whether there are likely to be significant changes to agricultural practices associated with cultivation of GD743 and GS784 apple, and if so are they likely to significantly exacerbate plant diseases or pests, especially those for which APHIS has a control program. Non-browning apple is the first GE crop that employs RNA silencing of PPO. No changes in cultivation or management practices such as propagation, planting times, irrigation, or pesticide use are anticipated with the introduction of non-browning GD743 and GS784 apple, which is comparable to other currently available 'Golden Delicious' and 'Granny Smith' apple varieties in agronomic, ecological, and most compositional characteristics as mentioned earlier in this document. The slight differences in pest susceptibility (OSF 2012, Table 27, p. 80) would not be sufficient enough to warrant a change in pest management practices. According to OSF, GD743 and GS784 apples are initially intended for the fresh cut apple slice market and eventually the fresh apple market, providing an alternative to the currently available 'Golden Delicious' and 'Granny Smith' apple varieties. Because GD743 and GS784 apples have similar agronomic characteristics to GD and GS, no change in general cultivation practices are anticipated.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which GD743 and GS784 Arctic™ Apple Cannot Interbreed

Since 1940, horizontal gene transfer (HGT) between unrelated organisms has been one of the most intensively studied fields, and has gained extra attention with the environmental release of transgenic plants (Dröge et al., 1998). HGT has been implicated as a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria and the emergence of increased virulence in bacteria, eukaryotes, and viruses; and has contributed to major transitions in evolution. Gene exchange has been documented between unrelated organisms for nearly all types of genes (Gogarten et al. 2002). For example, Yoshida et al. (2010), through a comparative genomics analysis, implicated HGT for the presence of a similar genetic sequence between the parasitic plant purple witchweed (*Striga hermonthica*) and sorghum (*Sorghum bicolor*).

APHIS examined the potential for the new genetic material inserted into GD743 and GS784 to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants. The GD743 and GS784 contain two noncoding sequences and one coding sequence from bacteria. Horizontal gene transfer and expression of DNA from a plant species to other bacterial species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi, bacteria, and parasitic plants (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), so far there are no reports of significant horizontal gene transfer between sexually incompatible or evolutionarily distant organisms (Keese, 2008). Accumulated evidence show that there are universal gene-transfer barriers, regardless of whether transfer occurs among closely or distantly related organisms (Koonin et al., 2001; Wood et al., 2001; Kaneko et al., 2002; Brown, 2003; Sorek et al., 2007). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2002; Wood et al., 2001). There is no evidence that these organisms contain genes derived from plants. In cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Koonin et al., 2001; Brown, 2003), which is also the case with the recent report about of HGT between purple witchweed and sorghum. According to Yoshida et al. (2010), the incorporation of a specific genetic sequence occurred between purple witchweed and sorghum prior to the speciation of purple witchweed (*S. hermonthica*), a parasitic plant of monocots, and cowpea witchweed (*S. gesnerioides*), a parasitic plant of dicots. In other words, HGT is an extremely rare event, with the majority of these events occurring over millions of years.

FDA has evaluated HGT related to the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA, 1998).

APHIS also considered whether horizontal transfer of genetic material inserted into GD743 and GS784 to plant viruses was likely to occur and would lead to the creation or selection of a more

virulent plant pathogen through recombination with other plant viruses. This issue has been considered before by other science review panels and government regulatory bodies (Keese 2008). The only virus sequences contained within GD748 and GS784 are the duplicated-enhancer 35S cauliflower mosaic virus promoter with the untranslated translational leader sequence from alfalfa mosaic virus RNA4. Regulatory elements such as promoters and terminators have not been implicated in viral recombination.

Therefore APHIS concludes that HGT is unlikely to occur from GD743 and GS784 to microorganisms, viruses or parasitic plants, and thus no significant plant pest risk is expected from HGT.

J. Conclusion

APHIS has reviewed the information submitted by the petitioner and conducted a plant pest risk assessment on GD743 and GS784 ArcticTM apples compared to the unmodified apple varieties from which they were derived. Due to the lack of plant pest risk from the transformation process and inserted genetic material; the lack of plant pest risk from the expression of the NPTII protein and suppression of endogenous PPOs, and the resulting effects on metabolites and composition; the lack of observed atypical responses to disease or plant pests in the field; the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem; the lack of increased weediness characteristics of GD743 and GS784 ArcticTM apples; the lack of potential of GD743 and GS784 to impact the weediness of plants with which they can interbreed; the lack of changes in agricultural practices that could impact plant pests and diseases; and the unlikely potential for horizontal gene transfer to occur and result in a plant pest risk; APHIS concludes that GD743 and GS784 ArcticTM apples are unlikely to pose a plant pest risk.

K. References

- Armstrong JD, Lane WD. 2009. Genetically modified reduced-browning fruit-producing plant and produced fruit thereof, and method of obtaining such. US Patent Application Number 12/919,735.
- Awmack, C. S., and Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* 47:817-844.
- Baker, H.G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5: 1–23.
- Barbehenn, R.V., Jones, C.P., Yip, L., Tran, L., and Constabel, C.P. 2007. Limited impact of elevated levels of polyphenol oxidase on tree-feeding caterpillars: assessing individual plant defenses with transgenic poplar. *Oecologia* 154:129-140.
- Beckerman, J. 2006. Fruit Diseases: Disease Susceptibility of Common Apple Cultivars. Purdue University, Purdue Extension. BP132: 1-4.

- Bevan, M.W., Flavell, R.B., and Chilton, M.D. 1983a. A chimeric antibiotic resistance gene as a selectable marker for plant cell transformation. *Biotechnology* 24:4.
- Bevan, M., Barnes, W.M., and Chilton, M.D. 1983b. Structure and transcription of the nopaline synthase gene region of T-DNA. *Nucleic Acids Research* 11:369-385.
- Bevan, M. 1984. Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research* 12:8711-8721.
- Boss, P.K., Gardner, R.C., Janssen, B.J., and Ross, G.S. 1995. An apple polyphenol oxidase cDNA is up-regulated in wounded tissues. *Plant Molecular Biology* 27:429-433.
- Boyd, V. 2011. Sliced apple demand and sales continue to grow. *The Grower*.
<http://www.thegrower.com/news/Sliced-apple-demand-and-sales-continue-to-grow-128210203.html>, accessed 3/07/2012.
- Brown, D. 1998. Yard and Garden Brief: Apple Russeting. University of Minnesota Extension Service, H200A.
<http://www.extension.umn.edu/yardandgarden/ygbriefs/h200appleruss.html>. Accessed 5/15/2012.
- Brown, J.R. 2003. Ancient horizontal gene transfer. *Nature Review Genetics* 4:121-132.
- Chiabrando, V. and Giacalone, G. 2012. Effect of Antibrowning Agents on Color and Related Enzymes in Fresh-Cut Apples During Cold Storage. *Journal of Food Processing and preservation* 36(2): 133-140.
- Constabel, C.P. and Ryan, C.A. 1998. A survey of wound- and methyl jasmonate-induced leaf polyphenol oxidase in crop plants. *Phytochemistry* 47:507-511.
- Datla, R.S.S., Bekkaoui, F., Hammerlindl, J.K., Pilate, G., Dunstan, D.I., and Crosby, W.L. 1993. Improved high-level constitutive foreign gene expression in plants using an AMV RNA4 untranslated leader sequence. *Plant Science* 94:139-149.
- Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., and Goodman, H. M. 1982. Nopaline synthase:transcript mapping and DNA sequence. *Journal of Molecular and Applied Genetics* 1:561-573.
- Dixon, R.A. 2001. Natural products and plant disease resistance. *Nature* **411**:843-847.
- Dröge, M., Puhler, A., and Selbitschka, W. 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. *Journal of Biotechnology* 64:75-90.
- du Toit, L., Hamm, P.B., Ocamb, C.M., Parke, J., Pscheidt, J.W., Serdani, M., Skoglund, L.G., Ward, K.F., Weiland, J., Zasada, I. Pacific North West Plant Disease Management

- Handbook: Apple (*Malus* sp.)-Fruit Russeting.
<http://pnwhandbooks.org/plantdisease/host-disease-descriptions/apple-malus-sp-fruit-russeting>. Accessed 05/15/2012.
- Ellstrand, N. C., Prentice, H. C., and Hancock, J. F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30:539-563.
- FDA (U.S. Food and Drug Administration). 1998. Draft Guidance: Use of antibiotic resistance marker genes in plants.
<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Biotechnology/ucm096135.htm> (Accessed on 4/10/2012).
- Fitzgerald, T. 2005. Washington State University, Spokane County Extension: Pollination of Fruit Trees, C105.
- Gogarten, J. P., Doolittle, W. F., and Lawrence, J. G. 2002. Prokaryotic evolution in light of gene transfer. *Molecular Biology and Evolution* 19:2226-2238.
- Goggin, F.L., Avila, C.A., and Lorence, A. 2010. Vitamin C content in plants is modified by insects and influences susceptibility to herbivory. *BioEssays* 32:777-790.
- Hancock, J.F. and Hokanson, K.E. 2001. Proceedings of the First International Symposium on Ecological and Societal Aspects of Transgenic Plantations. S.H. Strauss and H.D. Bradshaw, eds. College of Forestry, Oregon State University. pp.187-102.
- Hancock, J.F. 2003. A Framework for Assessing the Risk of Transgenic Crops. *BioScience* 53(5): 512-519.
- Hehnen, D., Hanrahan, I., Lewis, K., McFerson, J., and Blanke, M. 2012. Mechanical flower thinning improves fruit quality of apples and promotes consistent bearing. *Scientia Horticulturae* 134: 241-244.
- Hood, E.E., Gelvin, S.B., Melchers, L.S., and Hoekema, A. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. *Transgenic Research* 2:208-218.
- Janick, J., Cummins, J., Brown, S., and Hemmat, M. 1996. Apples. In *Fruit Breed, Volume I: Tree and Tropical Fruits*, edited by Jules Janick and James N. Moore. John Wiley & Sons, Inc., pp. 1-77.
- Kader, A.A., 1983. Influence of Harvesting Methods on Quality of Deciduous Tree Fruits. *HortScience* Vol 18(4): 409-411.
- Kaneko, T., Nakamura, Y., Sato, S., and Minamisawa, K. 2002. Complete Genomic Sequence of Nitrogen-fixing Symbiotic Bacterium *Bradyrhizobium japonicum* USDA110. *DNA Research* 9: 189–197.

- Keese, P. 2008. Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* 7:123-149.
- Kim, J.Y., Seo, Y.S., Kim, J.E., Sung, S-K., Song, K.J., An, G., and Kim, W.T. 2001. Two polyphenol oxidases are differentially expressed during vegetative and reproductive development and in response to wounding in the Fuji apple. *Plant Science* 161:1145-1152.
- Kolodziejczyk, K., Milala, J., Sokjka, M., Kosmala, M., and Markowski, J. 2010. Polyphenol oxidase activity in selected apple cultivars. *Journal of Fruit and Ornamental Plant Research* 18:51 - 61.
- Korba, J., Sillerova, J., and Kudela, V. 2008. Resistance of apple varieties and selection to *Erwinia amylovora* in the Czech Republic. *Plant Protection Science* 44:91 - 96.
- Koonin, E. V., Makarova, K. S., and Aravind, L. 2001. Horizontal gene transfer in prokaryotes: quantification and classification. *Annual Review of Microbiology* 55:709-742.
- Kron, P. and Husband, B.C. 2009. Hybridization and the reproductive pathways mediating gene flow between native *Malus coronaria* and domestic apple *M. domestica*. *Botany* 87: 864-874.
- Kupferman, E. 1992. Maturity and Storage of Apple Varieties New to Washington State. *Tree Fruit Postharvest Journal* 3(1): 9-16.
- Kupferman, E. 1994. Packing Golden Delicious Apples in Washington State. *Tree Fruit Postharvest Journal* 5(2): 12-18.
- Lee, K.W., Kim, Y.J., Kim, D.O., Lee, H.J., and Lee, C.Y. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem.* 51: 6516-6520.
- Little, E.L.J. 1979. *Malus*. In: Check List of United States Trees (Native and Naturalized). Washington, DC: Forest Service, USDA. p 167-170.
- Luby, J. 2003. Taxonomic classification and brief history. p.1-14. *Apples, Botany, Production and Use*. In: Ferree, D.C. and Warrington, I.J. (eds.) CABI Publishing, Cambridge, MA.
- MacHardy, W.E. 2000. Current status of IPM in apple orchards. *Crop Protection* 19: 801-806
- Martinez, M.V. and Whitaker, J.R. 1995. The biochemistry and control of enzymatic browning. *Trends in Food Science & Technology* 6:195-200.
- McCammant, T. 2007. Integrated Pest Management Manual for Minnesota Apple Orchards, 2nd Edition. Minnesota Department of Agriculture. p. 20-40.

- Muenscher, W. C. 1980. Weeds. 2nd Edition. Cornell University Press, New York. 586 pp.
- Mukherjee, M., Larrimor, K.E., Ahmed, N., Bedick, T.S., Barghouthi, N.T., Traw, M.B., and Barth, C. 2010. Ascorbic acid deficiency in *Arabidopsis* induces constitutive priming that is dependent on hydrogen peroxide, salicylic acid, and the *NPR1* gene. *Molecular Plant Microbe Interactions* 23(3): 340-351.
- Murata, M., Tsurutani, M., Tomita, M., Homma, S., and Kaneko, K. 1995. Relationship between apple ripening and browning: Changes in polyphenol content and polyphenol oxidase. *Journal of Agricultural and Food Chemistry*, 43(5): 1115-1121.
- Nicolas, J.J., Richard-Forget, F.C., Goupy, P.M., Amiot, M.J., and Aubert, S.Y. 1994. Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition*, 34(2): 109-157.
- Okanagan Specialty Fruits, Inc. 2012. Petition for the Determination of Nonregulated Status for GD743 and GS784. Submitted by N. Carter. Okanagan Specialty Fruits, Inc. St. Louis, MO http://www.aphis.usda.gov/biotechnology/not_reg.html.
- Park, M., Danforth, B., Losey, J., Biddinger, D., Vaughan, M., Dollar, J., Rajotte, E., and Agnello, A. 2012. Wild Pollinators of Eastern Apple Orchards and How to Conserve Them. Cornell University, Penn State University, and The Xerces Society. <http://www.northeastipm.org/park2012>.
- Reichard, S.H. and Hamilton, C.W. 1997. Predicting invasions of woody plants introduced into North America. *Conservation Biology* 11(1): 193-203.
- Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Lockwood, D.R., Reeves, P.A., and Forsline, P.L. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor of domesticated apple. *Tree Genetics and Genomes* 5: 339-347.
- Roos, D. 2005. Growing Small Farms: Burr Knot in Apple. North Carolina Cooperative Extension. <http://www.ces.ncsu.edu/chatham/ag/SustAg/burrknot.html>. Accessed 05/15/2012.
- Rothstein, S.J., Jorgensen, R.A., Yin, J.C., Yong-di, Z., Johnson, R.C., and Reznikoff, W.S. 1981. Genetic organization of Tn5. *Cold Spring Harb Symp Quant Biol* 45: 99-105.
- Sobiczewski, P., Zurawicz, E., Berczynski, S., and Lewandowski, M. 2006. Fire blight susceptibility of new apple cultivars and clones from Poland. *Acta Horticulturae (ISHS)* 704:551-553.
- Sharp, P., 2001. RNA interference – 2001. *Genes and Development* 15:485-490.

- Song, Y., Yao, Y., Zhai, H., Du, Y., Chen, F., and Wei, S. 2007. Polyphenolic compound and the degree of browning in processing apple varieties. *Agricultural Sciences in China* 6(5): 607-612.
- Sorek, R., Zhu, Y., Creevey, C. J., Francino, M. P., Bork, P., and Rubin, E. M. 2007. Genome-wide experimental determination of barriers to horizontal gene transfer. *Science* 318:1449-1452.
- Thipyapong, P., and Steffens, J.C. 1997. Tomato polyphenol oxidase (Differential response of the polyphenol oxidase F promoter to injuries and wound signals). *Plant Physiology* 115:409-418.
- Thipyapong, P., Hunt, M.D., and Steffens, J.C. 2004. Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. *Planta* 220:105-117.
- Thipyapong, P., Stout, M.J., and Attajarusit, J. 2007. Functional analysis of polyphenol oxidases by antisense/sense technology. *Molecules* 12:1569-1595.
- USDA. 2009. Composition of Foods Raw, Processed, Prepared. USDA National Nutrient Database for Standard Reference : NDB09003. Release 22.
<http://www.ars.usda.gov/nutrientdata>.
- USDA APHIS. 2011. Situation Report: California Light Brown Apple Moth Cooperative Eradication Program.
http://www.aphis.usda.gov/plant_health/plant_pest_info/lba_moth/updates-2011.shtml, accessed 07/18/2013.
- USDA APHIS. 2012. Petitions for Nonregulated Status Granted or Pending by APHIS as of April 11, 2012. http://www.aphis.usda.gov/biotechnology/not_reg.html, accessed 04/11/2012.
- USDA APHIS. 2013. Plant Health: Plant Pests Program Information.
http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml, accessed 07/18/2013.
- USDA, ERS. 2010a. US Apple Statistics Table 40. World apple production, including top-producing countries, 1980-2008. Available from: Economics, Statistics and Market Information System, Albert R. Mann Library, Cornell University. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1825>, accessed 03/07/2012.
- USDA, ERS. 2010b. US Apple Statistics Table 2. Apples: Production, utilization, and season-average grower price, United States, 1980 to date. Available from: Economics, Statistics and Market Information System, Albert R. Mann Library, Cornell University. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1825>, accessed 05/15/2012.

- USDA NRCS. 2010. NRCS Invasive Species Policy, Invasive Species Executive Order 13112. <http://plants.usda.gov/java/noxious?rptType=Federal>, accessed 04/10/2012)
- USDA NRCS. 2012. Plants Profile *Malus* Mill. Apple. Curated and maintained by USDA NRCS National Plant Data Team. <http://plants.usda.gov/java/profile?symbol=MALUS>, accessed 04/10/2012.
- van Engelen, F.A., Molthoff, J.W., Conner, A.J., Nap, J.P., Pereira, A., and Stiekema, W.J. 1995. pBINPLUS: an improved plant transformation vector based on pBIN19. *Transgenic Research* 4:288-290.
- Wang, J. and Constabel, C.P. 2004. Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta* 220:87-96.
- Wilson, K. 2009. Crabapple Pollenizers for Apples. Ontario Ministry of Agriculture, Food, and Rural Affairs . Factsheet: ISN 1198-712X. Order # 00-011.
- Wood, D. W., Setubal, J. C., Kaul, K., Monks, D. E., Kitajima, J. P., Okura, V. K., Zhou, Y., Chen, L., Wood, G. E., Almeida Jr., N. F., Woo, L., Chen, Y., Paulsen, I. T., J. Eisen, J. A., Karp, P. D., Bovee Sr., D., Chapman, P., Clendenning, J., Deatherage, G., Gillet, W., Grant, C., Kutayavin, T., Levy, R., Li, M.-J., 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, Z-Y., Dolan, M., Chumley, F., Tingey, S. V., Tomb, J-F., Gordon, M. P., Olson, M. V., and Nester, E. W. 2001. The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* 294:2317-2323.
- WSU. 2010. 2010 Crop Protection Guide for Tree Fruits in Washington (EB0419). In. Pullman, WA: Extension Publishing and Printing.
- Yoshida, S., Maruyama, S., Nozaki, H., and Shirasu, K. 2010. Horizontal gene transfer by the parasitic plant *Striga hermonthica*. *Science* 328:1128.