Okanagan Specialty Fruits

Petition for Determination of Nonregulated Status:
Arctic™ Apple (*Malus x domestica*)
Events GD743 and GS784

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR part 340.

Submitted by:

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Submitted February 21, 2012

CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

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Release of Information

Okanagan Specialty Fruits Inc. (OSF) is submitting the information in this petition for review by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) as part of the regulatory process to consider article deregulation under 7 Code of Federal Regulations (CFR) part 340.6. By submitting this information, OSF does not authorize its release to any third party. In the event the USDA should receive a Freedom of Information Act request, pursuant to 5 U.S. Code (USC) 552, and 7 CFR Part 1, covering all or some of this information, OSF expects that, in advance of release of the document(s), USDA will provide OSF with a copy of any and all material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g. responsiveness, confidentiality and/or competitive concerns. OSF understands that a copy of this information may be made available to the public, as part of a public comment period. Except in accordance with the foregoing, OSF does not authorize the release, publication or other distribution of this information (including online hosting) without OSF's prior notice and consent.

Executive Summary

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has the responsibility, under the Plant Protection Act (7 CFR USC sections 7701-7772), to prevent the introduction and/or dissemination of plant pests into the United States or interstate introduction and/or dissemination. APHIS regulations at 7 CFR 340.6 provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and therefore should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing the deregulated article to be introduced into the marketplace without restriction.

Okanagan Specialty Fruits Inc. (OSF) is submitting this request to APHIS for nonregulated status of Arctic™ Apple (*Malus x domestica*[1]) events GD743 and GS784 which are resistant to enzymatic browning. The "nonbrowning" phenotype of GD743 and GS784 was developed by inserting a polyphenol oxidase (PPO) suppression sequence derived from apple. When apples containing the inserted gene are subjected to mechanical damage, such as slicing or bruising, the apple flesh does not brown as an untransformed apple does, but rather remains its original color. This nonbrowning trait reduces the need for antibrowning agents on cut fruit, and minimizes shrinkage caused by harvest and postharvest damage.

Cultivated apple (*Malus x domestica*) is one of the major temperate fruit-tree crops. OSF is developing Arctic™ Apple cultivars that will function as direct replacements for current cultivars in situations where the Arctic™ Apple trait would provide an advantage and add value.

Arctic™ Apple events were produced using an *Agrobacterium*-based plant transformation system. The transformation vector, GEN-03, contains a chimeric PPO suppression transgene consisting of partial coding sequences of four members of the apple PPO gene family – consisting of PPO2, GPO3, APO5 and pSR7 – in the sense orientation under control of the cauliflower mosaic virus (CAMV35s) promoter and nopaline synthase (NOS) terminator. The transgene is designed to simultaneously reduce expression of PPO2, GPO3, APO5 and pSR7 to induce a nonbrowning phenotype in apple fruit. The transgene sequences were derived from the donor organism apple. Molecular characterization of Arctic™ Apple events showed that GD743 contains two copies of the transfer DNA (T-DNA), while GS784 arose from the insertion of multiple copies of the T-DNA, with no evidence for the inclusion of vector backbone in either case.

Phenotypic data and other information presented in this petition demonstrate that it is no more likely that the regulated articles will present a plant pest risk than conventional apple for the following reasons:

(i) no biologically meaningful agronomic or phenotypic differences in apple populations containing either event GD743 or GS784 were detected over multiple years and locations compared to control and conventional apple populations, indicating that

[1] See Nomenclature and Biology of Cultivated Apple (OSF, 2011) for synonyms of *Malus x domestica.*

there was no increased weediness or competitiveness in apple populations containing either of the Arctic™ Apple events;

(ii) apple populations containing either of the transformed events were no more susceptible to disease or insect pests than conventional apple populations;

(iii) the composition and quality of apples derived from the populations containing the Arctic™ Apple events were comparable to the composition and quality of apples from control and conventional apple cultivars; and

(iv) the likeliness of apple outcrosses due to pollen gene flow is low – as it is in conventional orchards – and even if pollen gene flow does occur, the environmental impact would be very low, limited to the transgene being expressed in a portion of seeds only.

The PPO suppression transgene does not confer plant pest characteristics to apple and as such, the transgenic GD743 and GS784 events containing the PPO suppression transgene do not represent a plant pest risk.

The use of Arctic™ Apple cultivars can provide benefit by (i) minimizing shrinkage caused by harvest and postharvest damage, (ii) reducing or eliminating the need for antibrowning agents on cut fruit, and (iii) promoting the inclusion of apple in the fresh-cut fruit market.

Data and information in this request demonstrate that events GD743 and GS784 do not represent a unique plant pest risk. Therefore, OSF requests a determination from APHIS that events GD743 and GS784, and progeny thereof, no longer be considered regulated articles under regulations in 7 CFR part 340.

Nomenclature

Throughout this petition, subject apples are referred to as “GD743” and “GS784”. The numbers “743” and “784” are the original designations of the selected clones. The letter codes were added here to identify the parent cultivars Golden Delicious (GD) and Granny Smith (GS).

In accordance with the Organization for Economic Cooperation and Development's (OECD) “Guidance for the Designation of a Unique Identifier for Transgenic Plants” (OECD, 2002) GD743 has been assigned the unique identifier OKA-NBØØ1-8 and GS784 has been assigned the unique identifier OKA-NBØØ2-9.

Certification

The undersigned certifies that, to his best knowledge and belief, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioners which may be unfavorable to the petition.

_____

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Key to Abbreviation and Acronyms

APHIS	Animal and Plant Health Inspection Service
BCA	bicinchoninic acid
bp	nucleotide base pairs
CAMV35s	Cauliflower Mosaic Virus 35s
cDNA	complementary DNA
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CSIRO	Commonwealth Scientific and Research Organization (Australia)
CTAB	cetyltrimethylammonium bromide
Cu	copper
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dsRNA	double stranded RNA
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EST	expressed sequence tag
FB	fireblight
FDA	Food and Drug Administration
g	gram
GAA	Green Apple Aphid
GAP	Good Agricultural Practices
GD	Golden Delicious
GD743	Arctic™ Apple event Golden Delicious 743
GS	Granny Smith
GS784	Arctic™ Apple event Granny Smith 784
GUS	beta-glucuronidase
HCl	hydrochloric acid
IPM	Integrated Pest Management
JB	Japanese beetle

Key to Abbreviations and Acronyms (cont.)

kD	kilodalton
LB	left border
M	mean
$MgCl_2$	magnesium chloride
mg	milligram
ml	milliliter
mM	millimolar
mRNA	messenger RNA
N	number of units in a population
n	number of units in a sample
NaCl	sodium chloride
NF	a non-patented strain of Fuji
ng	nanogram
nptII	neomycin phosphotransferase II gene
NptII	neomycin phosphotransferase II protein
NOS	nopaline synthase
ORAC	oxygen radical absorbance capacity
OSF	Okanagan Specialty Fruits Inc.
P	probability
PCR	polymerase chain reaction
PG	a patented strain of Gala
PM	Powdery Mildew
PPO	polyphenol oxidase
RB	right border
RNA	ribonucleic acids
S	standard deviation
SDS	sodium dodecyl sulfate
siRNA	small interfering RNA
SOFA	Statistics Open For All
SSII	Superscript II
T-DNA	transfer DNA

Key to Abbreviations and Acronyms (cont.)

TLM	tentiform leafminer
USA	United States of America
USC	United States Code
USDA	United States Department of Agriculture
UTR	untranslated region

1 RATIONALE FOR DEVELOPMENT OF ARCTIC™ APPLES

1.1 Basis for Request for Determination of Nonregulated Status under 7 CFR Part 340.6

The Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA) has the responsibility, under the Plant Protection Act (7 USC Sections 7701-7772) and the Plant Quarantine Act (7 USC Sections 151-167) to prevent the introduction and dissemination of plant pests into and within the United States. APHIS regulations at 7 CFR 340.6 provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not represent a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

1.2 Arctic™ Apple Events GD743 and GS784

Okanagan Specialty Fruits Inc. (OSF) has developed Arctic™ Apple events GD743 and GS784 that are resistant to enzymatic browning. These events were developed using *Agrobacterium*-mediated transformation to stably incorporate into the apple genome a chimeric polyphenol oxidase (PPO) suppression transgene derived from apple. The transgene is designed to simultaneously suppress expression of four members of the apple PPO gene family.

Browning discoloration of apple flesh due to damage – such as from cuts, bruises and cell death that cause disruption of cell membranes – is caused by an enzymatic reaction catalyzed by PPO. The brown pigment is a polymer formed from the nonenzymatic condensation of quinones, with lesser amounts of amino acids and proteins, into lignin-like compounds. The quinones are synthesized from diphenols in the reaction catalyzed by PPO (Whitaker and Lee, 1995b). Most PPOs also have monophenolase activity, and convert monophenols to diphenols (Mar - Sojo *et al.*, 1998).

Normally in apple, PPO is located in the plastid, physically separated from the phenolic substrates in the vacuole. When cells are damaged, loss of compartmentalization occurs and PPO comes into contact with its substrate. In events GD743 and GS784, little to no PPO enzyme is present, so cell disruption does not lead to browning.

Browning also reduces apple quality by causing detrimental flavor and nutritional changes (Eskin, 1990) that limit apple's fresh-market, fresh-cut and processing applications. Brown bruises are a significant cause of reduced grade for fresh-market apples for growers and of lost value for retailers. Enzymatic browning also limits the use of apple in fresh-cut produce products and is a significant problem limiting the widespread introduction and viability of prepared apple slices. Browning is also a major consideration in the manufacture of juice and dehydrated apple products.

Arctic™ Apple events will be used as direct replacements for their untransformed conventional counterparts in situations where the nonbrowning trait is considered desirable. They will also be used in conventional breeding efforts to produce new apple cultivars that are resistant to enzymatic browning.

A series of photographs in Appendix 1 help to document the development and characterization of GD743 and GS784.

1.3 Benefits of Arctic™ Apple Events GD743 and GS784

Arctic™ Apple events GD743 and GS784 will offer growers, packers, processors, wholesalers, retailers, foodservice and consumers nonbrowning variants of many of the popular apple cultivars they have become accustom to purchasing.

Key benefits of the Arctic™ Apple cultivars will include:

- reduced shrinkage caused by finger bruising and scuff marks,
- reduced need for antibrowning agents on fresh sliced and dehydrated apple products,
- new uses of apples in high-quality, prepared-produce items, and
- promotion of consumption of this healthy snack food.

1.4 Submissions to Other Regulatory Agencies

GD743 and GS784 are within the scope of the U.S. Food and Drug Administration's (FDA) policy statement concerning regulation of products derived from new plant cultivars, including those developed through biotechnology. That statement, which was published in the *Federal Register* on May 19, 1992 (57 FR 22984), clarified the FDA's interpretation of the Federal Food, Drug, and Cosmetic Act. In compliance with that policy, OSF submitted to the FDA a food and feed safety and nutritional assessment for events GD743 and GS784 on May 30, 2011. Furthermore, regulatory submissions for product approvals were made to Health Canada and the Canadian Food Inspection Agency (CFIA) on December 7, 2011.

2 THE APPLE FAMILY

A companion document entitled "Biology of Cultivated Apple" (OSF, 2011), submitted to the United States Department of Agriculture by Okanagan Specialty Fruits, provides an extensive review of the apple family. The following sections provide a summary of that content, including information on apple's origin, use, biology, taxonomy, genetics, related species and history.

2.1 Apple as a Crop

Apple cultivars are used for fresh fruit, fresh-cut products and processing into juice, pie filling, sauce, alcoholic cider, juice concentrate, fruit leather, dehydrated fruit bars and other products (Downing, 1989). Byproducts of manufacturing, such as pomace left over from juice production, may be fed to livestock, wild animals or used as a food ingredient in, for example, baked goods, for extraction of ester flavors, etc. Hobbyists in cold areas grow minor cultivars, selected for hardiness, many of which may have crab apple in their background (Ferree and Warrington, 2003). Several *Malus* crab apple species and crab apple hybrids are used as ornamental and amenity trees, and as specialized pollinator cultivars interplanted in commercial apple orchards (Mayerd *et al.*, 1989).

Fresh apples are the primary apple product consumed in the United States. Processed apples account for less than 50 percent of the U.S. crop on average, with some variation in production location and from year to year (Outlook, 2007). Apples compete with processed snack foods and confectionaries, as well as other fruits and fresh vegetables. The apple is notable for its nutritional qualities. It is a natural snack food with low fat content, sugar content of 11-16 percent, is a good source of potassium, soluble fiber including pectin and other complex carbohydrates, phenol and flavonoid antioxidants (Vinson *et al.*, 2001). Children and infants consume a disproportionate amount of fresh and processed products of apple (Dennison, 1996).

Cultivated apple is the world's most important temperate fruit crop. Countries with significant apple production are: China, Europe, USA, India, Turkey, Russia, Iran, Japan, Chile, New Zealand, Canada, Australia, South Africa, Argentina and Brazil (O'Rourke, 1994). Per capita consumption in the USA and Canada is in the order of 16 pounds of fresh apples and 29 pounds of processed apple products per year (O'Rourke, 2010). The farm-gate value of apples in the USA is about $1.6-2.0 billion (Perez and Pollack, 2008).

Cultivated apple trees are grown in orchards throughout temperate regions of the world (Westwood, 1993). Fruit cultivars with desirable quality and production characteristics are propagated by grafting a scion onto a rootstock, since apple does not root easily from cuttings (Rom and Carlson, 1987). Rootstock cultivars, also usually *Malus* x *domestica*, are propagated in layer beds and affect the scion cultivar by inducing traits such as dwarf growth habit, precocity, resistance to root diseases and cold temperatures, or have other characteristics useful for efficient apple production (Jackson, 2005). Seedlings of hardy cultivars such as cv. Wealthy and crab apples are also sometimes used as rootstock. Modern orchards are planted at a density of 200-2000 trees per acre, and trees are seldom higher than 15 feet tall at maturity. Production averages about 15 tons per acre, but can vary considerably depending on orchard age, management practices and weather.

Climatic areas with a hardiness zone rating of 5a (McKenney *et al.*, 2001) or warmer, but also cool enough to result in 1200 or more hours of chilling, are most suitable for commercial apple production. Production is centered in, but is not exclusive to, areas with low to moderate rainfall, in order to promote good fruit size and color as well as to avoid diseases such as apple scab and canker that are problematic in damper climates. Orchard trees are usually replaced after about 20 years as new and improved cultivars become available or land use changes. Specimen apple and pear trees growing in favorable locations can be very long lived, probably as long as 400 years old (Wikipedia(1), 2011).

Apple trees in commercial plantings reach maturity at an age of 4-5 years. Flowering in the early spring produces white or pink flowers about 1 inch in diameter that develop on fruiting spurs in groups of six flowers. Cultivated apple often develops a biennial bearing habit, tending to produce abundant flowers in one year but fewer in the next; this is a significant production hindrance that can be managed by thinning fruit soon after bloom. Fruits can be uniformly red, green or yellow, or bicolored such as striped or blushed red on yellow or green background (Wikipedia(2), 2011). The most desirable weight of commercial apples ranges from 6-10 ounces per piece. Apples are picked between mid-August and November in northern temperate zones, and some cultivars can be stored for up to a year. There are about 11 significant commercial apple cultivars in international trade, with many of these grown in all major production areas. Widely grown international cultivars are: Golden Delicious, Red Delicious, Granny Smith, Gala, Fuji and Jonagold. Minor international cultivars include: McIntosh, Cox's Orange, Braeburn, Mutsu and Pink Lady. Many additional apple cultivars are sold into regional or niche markets (O'Rourke, 1994).

Apple is a labor intensive, highly managed crop, requiring about 200 hours of labor per acre per year. The major crop management operations are: fruit harvesting, tree pruning, fruit thinning, irrigation, plant nutrition, growth regulation, and pest and disease control. While varying considerably, mature commercial orchards generate gross sales of $4,000 to $10,000 per acre per year (Gallardo *et al.*, 2010). Apple scab and powdery mildew are the major fungal diseases; Fireblight is the major bacterial disease; codling moth and leaf rollers the key insect pests in most production areas, while aphids, mites, and other pests are of concern in most areas. Virus diseases can also infect apple, with most viruses spread during propagation through use of contaminated grafting wood (WSU, 2010). However, virus diseases have become a limited concern to apple growers more recently because of the success of certified budwood schemes that distribute virus-free wood for propagation. Apple can also be infected by a number of quarantinable diseases, and certification standards are in place for import and export of fruit, propagating wood and trees with trading partners.

2.2 Taxonomy of Apple

The cultivated apple is a member of Rosaceae, the rose family, which is made up in general of herbs, shrubs or trees that are deciduous or evergreen and often thorny. Rosaceae consists of about 100 genera with more than 2,000 species distributed throughout the world; it is most common in temperate regions and is sometimes divided into tribes or subfamilies based on fruit characteristics (Rehder, 1958). Rosaceae species of agronomic importance include: apple, pear, quince, peach, cherry, apricot, plum, strawberry, raspberry and blackberry. Species of ornamental importance include: crab apple, pyrocantha, spirea, mountain ash and rose. The

genus *Malus*, to which cultivated apple and crab apple belong, includes about 25 species worldwide. The center of diversity of cultivated apple is central Asia, but native or naturalized *Malus* occur in central Europe and a number of *Malus* species are native to temperate Asia and western China (Way *et al.*, 1990).

The following *Malus* species have been identified as native or naturalized in the United States (Little, 1979):

- *Malus angustifolia* (southern crab apple) is found on the coastal plain from Maryland to northwest Florida and west to Louisiana and southern Illinois;

- *Malus coronaria* (sweet crab apple) is found from central New York to northern Georgia and eastern Kansas;

- *Malus fusca* (Oregon crab apple) is found along the Pacific coast from southern Alaska to northern California;

- *Malus ioensis* (prairie crab apple) is found from southeastern Minnesota to central Texas;

- *Malus platycarpa*, thought to be a hybrid between cultivated apple and native species of crab apple, has been recorded from southern Illinois east to Delaware and south to North Carolina (McVaugn, 1943);

- *Malus x domestica* (apple) escaped from cultivation and naturalized locally from southern Nova Scotia to Ontario, Maine, eastern Washington and northern Idaho;

- *Malus x soulardii* (Soulard crab), a hybrid of *ioensis x domestica* is found from southern Minnesota to eastern Texas.

Malus baccata (Siberian crab apple) and *Malus prunifolia* (pear leaf apple), both introduced from Asia, have escaped from cultivation in the northeastern United States but apparently are not naturalized (Little, 1979). *Malus* is described as "a taxonomically difficult genus with numerous intergrading variations and hybrids for which many scientific names have been given" (Little, 1979).

2.3 Genetics of Apple

Rudimentary breeding efforts, or selection and propagation of exceptional chance seedlings, in Canada and the USA have resulted in cultivars such as McIntosh, Delicious and Golden Delicious. The National Apple Register of the United Kingdom describes over 8,000 historical and modern named cultivars (Smith, 1971).

Government-sponsored breeding programs became well established in the 20th century, mostly as a component of government research programs, and resulted in the introduction of cultivars with improved fruit quality, storage and production characteristics, as well as improved rootstock cultivars (Janick and Moore, 1996). Many significant introductions of apple cultivars are clonal improvements originating from spontaneous mutants occurring in trees in commercial orchards. Over 180 clones of Delicious cv. originating in this way have been named and introduced

(Higgins, 2005) (Wikipedia(3), 2011). Clonal improvements of the cultivars McIntosh, Gala, Fuji, Pink Lady and Braeburn are also commercially important. Some important apple cultivars such as McIntosh, Red Delicious and Golden Delicious originated as chance seedlings a century or more ago; most have been improved through clonal selection of naturally occurring mutations.

Few private breeding programs have been established because of the long period from hybridization to commercial establishment of a new cultivar. In addition, a new apple cultivar faces the challenge of being recognized as a distinctive and individual consumer product at the retail level, where it must compete for shelf space with other recognizable apple cultivars – unlike, for example, sweet cherry or onions, for which one cultivar is not easily distinguished from another.

A few breeding programs experimented with induced mutations for clonal improvement, but this approach is now out of favor because of associated disadvantages such as reversion to the original type due to chimeras (Van Harten, 1998).

There is less scientific basis for the breeding of apples than for agronomic crops because it is difficult to describe, for example, heritability of traits due to the long reproductive cycle of apple – normally five years or so. Rather, the approach has been to create an inventory of a number of high-quality breeding selections with different characteristics and ripening times, that can then be drawn on as market opportunities arise and interest by growers is sufficient to support their testing and market introduction.

The primary quality characteristics noted by apple breeders are:

- Taste, consisting of: texture that must be crisp and juicy, sugar acid balance that must be pleasant and rich to give ‘body’ to the taste, and a distinctive aroma that is pleasant and in many ways defines the cultivar.

- Appearance must be bright and attractive, usually red stripes around the fruit on a yellow or light-colored background rather than a green one. Individual apples should be consistent in color to facilitate high pack-outs and uniform retail displays; shape should be smooth and uniform, not irregular; size should be consistently larger than 2-1/2 inches in diameter.

- Good storage characteristics that allow good quality fruit to be sold for most of the year, and pleasant taste after the apple begins to soften.

- Tree growth habit and derived production characteristics are also important. These components are: vigorous growth with favorable partitioning of that growth (carbon accumulation) into flowers/fruit to give high yield; meeting thresholds for resistance to pests and diseases; and ripening time matched to grower requirements. Since it is difficult to combine excellent examples of all of these traits into a single hybrid, breeders often look for excellence in several important traits, such as taste and growth habit, and set minimum acceptable threshold values for the remaining ones

Most commercially grown apple cultivars are diploid with a chromosome number of 34 (n =17); a few are triploid (Darlington and Wylie, 1955). Natural or induced mutations of many cultivars, especially red-skinned ones, have been selected by horticulturalists and are commercially important. The most common characteristics of these are enhanced fruit color, altered tree growth habit and earlier fruit maturity time. The genetic differences between the strain and its parent are usually small, and have not been described even with molecular genetic techniques. Darlington (Darlington and Wylie, 1955) list the chromosome number of cultivated native and naturalized *Malus* as follows: *baccata,* 34; *fusca,* 34 *ioensis,* 34; *prunifolia,* 34 and 51; *angustifolia,* 34 and 68; *coronaria,* 51 and 68.

2.4 Propagation and Pollination of Apple

Apple is propagated by grafting a scion bud to a rootstock, which consists of a rootball and a short stem. Most trees are produced in specialized fruit tree nurseries. Nursery companies have access to favorable growing locations, to specialized technology, to certified propagating wood of the most popular cultivars, and to information useful to growers to help with planting decisions. Some growers produce their own nursery trees. The sequence of operations in apple propagation is: rootstock liners are planted in a nursery in the spring; they are budded in August; the stock is cut back to the bud the following spring; the scion bud develops into a nursery tree in the following growing season. Nursery trees are dug up in the fall, graded and stored; then distributed to growers for planting the following spring. Flowering occurs in year 2 following planting, with full fruit production in years 4 or 5 (OVTFA, 1993).

Only 2-10 percent of flowers of cultivated apple develop to mature fruit; the others fail because of lack of pollination, competition between developing fruits, or cultural practices to remove fruit to promote size and quality of harvested fruit. Nearly all apple cultivars require cross-pollination for consistent cropping, with about 2-5 percent of the orchard area devoted to pollinizer cultivars, either another fruit cultivar or a specialized crab-apple pollinizer cultivar. Genes conferring incompatibility have been described and are sufficient in number that the diversity between cultivars results in nearly all cultivars being cross-compatible. Some cultivars are at least partially self-fertile due to an environmentally-induced breakdown of the incompatibility system when flowers age or temperatures are high (Warmund, 1996).

Insects carry out apple pollination, primarily domesticated or wild honeybees or other insect pollinators such as bumble bees, *osmia* bees and other species (Dupree *et al.*, 1987). The period of flowering during which viable pollen is produced by an apple plant varies depending on weather conditions from about 7-30 days. Anthers open sequentially, making fresh pollen available for a week or so; some flowers on the tree open before others. Wind pollination is inconsequential for apple; and emasculated flowers seldom, if ever, set fruit even though flowers on nearby branches produce abundant pollen. Rootstock cultivars seldom flower, since rootstock-derived shoots that may develop are removed to prevent competition with the scion.

Pollen fertility of most apple cultivars is close to 100 percent, but is reduced in some cultivars such as McIntosh by unknown factors and in others, such as Jonagold, by its triploidy. Apple flowers have up to 25 ovules but more commonly 10-15, depending on the cultivar, some of which fail to develop into seeds. Cultivars vary in average seed number per fruit from 3-12, with pollination conditions influencing this average. A small percentage of fruit may develop by

parthenocarpy (the natural or artificially induced production of fruit without fertilization of ovules) (Pauwels *et al.*, 1998). Seeds require conditioning by stratification at an optimum temperature of about 40° Fahrenheit for several months to germinate (Bradford and Nonogaki, 2007). Seedling trees have a juvenile growth phase of 5-10 years before producing flowers and characteristically have lanky shoot growth and thorny spurs, adaptations for growing in communal thickets.

2.5 Weediness of Apple

While cultivated apple is not regarded as a weedy species, it has been reported to be a successional species in abandoned pasture but not abandoned cultivated field (Stover and Mark, 1998). Animals, such as bears, can carry fruit containing seed away from cultivated areas, and occasionally escaped trees establish in previously undisturbed habitat. Such cultivated apple-tree seedlings can be persistent; the species has escaped cultivation and naturalized in southern Canada, in the eastern USA, and from British Columbia south to California (Little, 1979).

Four species of crab apples within the section *Chloromeles* are native to North America. One, Pacific Crab (*Malus fusca*), occurs on the west coast in British Columbia; three species that are similar to each other and closely related to *Malus fusca* occur in the east (Hosie, 1979). The introduced ornamental species *Malus baccata* and *Malus prunifolia*, originating in Asia, have escaped from cultivation in the northeastern USA but are not naturalized (Little, 1979). Ornamental crab apple is grown in gardens and as a street or specimen tree (Draper and Chatfield, 1996).

Research using molecular techniques found no introgression of cultivated apple genes to native *Malus* species of North America (Dickson *et al.*, 1991). Hybrids between cultivated apple and crab apple are expected to be easily recognized because fruit of the hybrid is intermediate between the parents, and fruit size of the domestic apple and crab is distinctly different; with fruit size of domestic apple large, crab apples small, and hybrids intermediate. Cultivated apple can be artificially cross-pollinated to produce hybrids with many if not all crab apple species, but the fertility and ecological fitness of such possible hybrids hasn't been well described. A large, mature cultivated apple tree can produce in the order of 2,000 fruits per year, potentially yielding 10,000 seeds; it may live for 50 years or longer, theoretically producing 500,000 seeds in its lifetime. For a naturalized apple tree population to be at equilibrium, each individual apple tree need reproduce just one other plant to replace itself. This suggests that the probability of an individual apple seed developing into a mature tree is small when compared to annual plants that produce fewer seeds per plant and require a replacement plant each year to maintain a stable population. Volunteer plants originating from seed in apple orchards are very rare due to the perennial nature of this tree crop and the associated orchard management practices, such as herbicide treatment of the tree row and mowing of the alley between rows.

2.6 Modes of Gene Escape in Apple

In considering the potential environmental impact of an unconfined release of genetically modified apple, it is important to understand the possible development of hybrids through interspecific and intergeneric crosses with the crop and related species. The development of hybrids could result in the introgression of the novel traits into these related species, resulting in:

- the related species becoming more weedy; and/or
- introduction of a novel trait with the potential for ecosystem disruption into the related species.

Interspecifically, for a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediaries, as well as survival and fertility of the resulting offspring, is necessary.

Several crab apple species are native to North America (see Section 2.5 above) and hybridization between cultivated apples and native and/or introduced crab apple species of the group *Chloromeles* is possible. Domesticated apple has been grown in North America for several centuries, allowing time for hybrids between cultivated apple and native or introduced *Malus* species to naturalize should they occur frequently and successfully compete. Hybrids involving cultivated apple have been reported and are expected to be recognizable by their intermediate fruit characteristics. Plants of these hybrids *malus x platycarpa* (*platycarpa* is sometimes united taxonomically with *coronaria*), *malus* x *soulardii* and *malus* x *ioensis* appear to be local and not very common (Little, 1979).

Native crab apples such as *Malus fusca* and others tend to grow in thickets, and it is unclear if local populations are maintained primarily through seed or by vegetative regeneration of shoots from roots in a manner similar to aspen. If vegetative regeneration from roots predominates then the importance of seed propagation is proportionately diminished.

Bee-mitigated cross-pollination is thought to be an important consideration for gene escape. Bee colonies are moved to orchards to facilitate pollination, by transferring pollen from cultivated apple or crab apple pollinizer trees to receptor trees with incidental pollen transfer in the opposite direction. Effective transfer of pollen from orchards to native crab apple flowers depends on synchronous flowering of the two species, and would require close proximity. Pollination efficiency decreases rapidly as the distance between the pollen source and the receptor tree increases (Wertheim, 1991); the frequency of transfer is also influenced by the size and proximity of competing sources of pollen. Commercial growers have traditionally planted about 2-5 percent of orchard trees as pollinizer cultivars, and arranged them so pollinating bees need travel only 50 feet or less to reach receptor trees (Winson, 1991). Thus, the vast majority of cross-pollination of apple in commercial orchards is between receptive flowers and immediately adjacent pollen sources. Further, when pollinating, individual bees are usually loyal to the location and species and/or cultivar from which they are collecting nectar or pollen. Loyalty to species can be monitored in the field by examination of pollen pellets from individual bees for uniform color and appearance. It is uncommon to observe hybrid pellets, indicating that mono-specific pollen pellets are most common.

Intergeneric hybridization is unlikely; Hybrids with *Pyrus* or *Sorbus* have not been documented, although grafts of these intergeneric combinations may survive for a while.

Gene escape can also occur due to seed movement (Dennis *et al.*, 2007). Apples are eaten by animals and seed can be distributed by them. Examples of animals that could contribute to escape in this way are bears, mice and squirrels. Apples are often discarded by travelers on

roadways, or in compost piles. Seeds distributed in this way can result in seedling trees. Apples float and can be carried by water streams or currents, resulting in germination and escape. Extracted apple seeds sink in water. However, *M. domestica* typically occurs in commercial orchard plantings, and as fruit trees in gardens or pastures. It is not common to find wild seedling trees; therefore, gene escape is not thought to be a widespread problem.

3 DESCRIPTION OF THE TRANSFORMATION SYSTEM

Agrobacterium tumefaciens-mediated transformation was carried out using kanamycin selection (De Bondt *et al.*, 1994). The plant transformation vector GEN-03 used to generate GD743 and GS784 is a binary vector based on pBINPLUS (van Engelen *et al.*, 1995), a derivative of BIN19 (Bevan, 1984). The disarmed *Agrobacterium tumefaciens* strain EHA105 has been previously described (Hood *et al.*, 1993). The recipients for transformation were virus-free cultures of the apple cultivars Golden Delicious (GD) and Granny Smith (GS).

Leaves of three-week-old apple tissue culture plantlets were excised and cut into segments perpendicular to the mid-rib; they were then inoculated with *Agrobacterium tumefaciens* EHA105 carrying the GEN-03 vector at a density of 3×10^8 cells/ml for 5-10 minutes. Leaf segments were blotted on filter paper to remove excess bacterial cells, and then placed onto co-cultivation medium with the adaxial surfaces in contact with the medium for four days (all spent in the dark). Infected leaf segments were washed and placed onto regeneration medium containing 6 µg/ml kanamycin with the adaxial surfaces in contact for four weeks (2 weeks dark, 2 weeks light). Leaf segments were transferred to a regeneration medium containing 50 µg /ml kanamycin for four weeks. Green shoots, considered to be transformed, were transferred to proliferation medium with 50 µg /ml kanamycin for four weeks. Surviving shoots were transferred to fresh proliferation medium. Shoots regenerating on 50 µg /ml kanamycin were selected by polymerase chain reaction (PCR) using primers specific to the transgene or selection marker. Shoots identified to be transgenic by PCR were measured for total PPO activity, after 2-3 successive subcultures. Any shoot that was PCR-positive for the transgene and selection marker and significantly suppressed for PPO activity (defined as more than 80 percent suppressed) was micrografted to M9 rootstock in preparation for field testing (Lane *et al.*, 2003).

The steps involved in the development of GD743 and GS784 are shown (Figure 1).

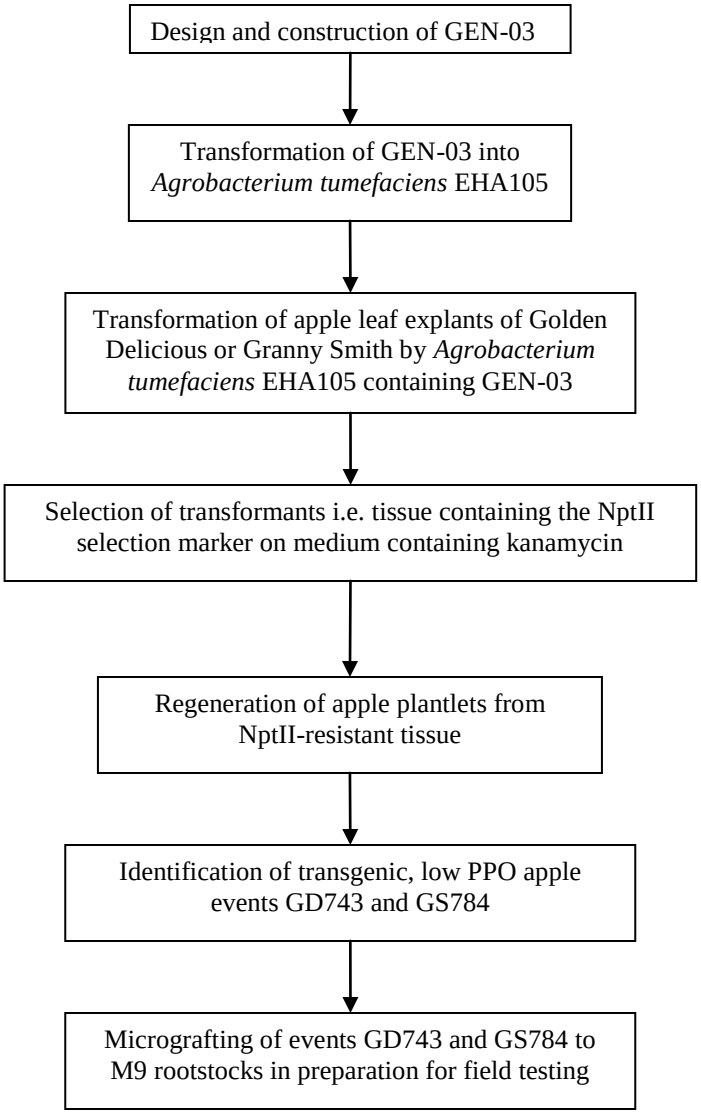


Figure 1: Steps in the Development of GD743 and GS784.

4 DONOR GENES AND REGULATORY SEQUENCES

4.1 Biology of Polyphenol Oxidase (PPO)

4.1.1 PPO Evolutionary History

PPO is found in higher plants, although some have only trace amounts; it is not present in conifers, Ginkgo, mosses or green algae (Mayer, 1987) (Sherman *et al.*, 1991). Enzymes similar in structure and function to plant PPOs are found in other phyla and kingdoms, notably tyrosinases that cause a strong browning reaction in mushrooms (Jolivet *et al.*, 1998), melanin-forming enzymes in animals, and haemocyanins in crabs, lobsters and their relatives. PPO is also present in some fungi and bacteria. True albino mutants do not have functional PPO enzyme. The similarity of PPO and related enzymes from 23 species was compared in a review considering these characteristics: gene structure; primary, secondary and tertiary protein structure; domain structure; copper-binding sites; and maturation mechanism and activation mechanism (van Gelder *et al.*, 1997).

PPO is held in inactive form in healthy plant cells and the browning reaction proceeds only when the phenolic substrate, concentrated in the cell vacuole, and the activated enzyme come together after cell membranes are ruptured. The lack of introns in PPO genes described to date suggests that PPO evolved as a chloroplast gene which subsequently migrated to the nucleus, becoming a nuclear gene. The protein is synthesized, usually in young cells of developing tissues, and then transported to the lumen membrane in the chloroplast where it can remain stabilized for several months.

4.1.2 Plant PPOs and Browning

The PPO protein

Plant PPO proteins are made up of about 510 amino acids and are monomers with a weight of about 40 kilodalton (kD), but the protein is translated as a larger protein of about 66-71 kD. The N-terminal 5' end has a transit protein of approximately 9-11 kD and the C- terminal 3' end, a weight of about 15 kD. The transit peptides of PPO isozymes differ within an individual species. There is a conserved region in the amino terminal part of PPO transit proteins of 22 amino acids in a hydrophobic domain, which is preceded by two arginine residues. These same amino acids are present in all plant PPOs examined, and this sequence is characteristic of lumen-targeted proteins. The total transit protein is rich in hydroxy amino acids characteristic of transit proteins targeted to the chloroplast. The C terminal portion is cleaved once the protein is within the chloroplast and is thought to be involved with activating the protein in some way, possibly by directing the binding of copper, and this prevents PPO activity in the cytoplasm during transit to the chloroplast. Sommer (Sommer *et al.*, 1994) reported the details of importation and processing of PPO. A typical example of PPO protein processing is grape PPO (Dry and Robinson, 1994), where a 1990 base pair cDNA was found to code for a 67kD protein consisting of a 10.6 kD chloroplast transit peptide, a 40.5 kD catalytic unit and a 16.2 kD C terminal extension.

An unusual feature of PPO proteins is their requirement for two copper molecules. Two copper-binding sites are highly conserved in PPO proteins from a diversity of species. The Cu A binding site is more conserved than the Cu B site. The two sites are separated by about 110 amino acids.

Both have histidine residues which are absolutely conserved in all PPOs and in related enzymes from many different species. The A site consists of 19 amino acids and the B site consists of usually around 50, but up to 64, amino acids.

Apple browning caused by PPO

Browning of apple flesh and juice reduces its quality, and reducing or eliminating browning in processed fruit has been studied extensively. Several phenolics serve as PPO substrate in apple tissue, with chlorogenic acid usually the principal one. Apple cultivars differ in their polyphenol content and in browning reaction (Murata *et al.*, 1995a) (Murata *et al.*, 1995b). Apple PPO has also been shown to be associated with plastids in young fruit, but the enzyme begins to degrade and is partially solubilized in mature fruit (Murata *et al.*, 1997). Other phenolics present in apple cortex are also substrates in the reaction, with 4-methyl catechol and (-) epicatechin being important contributors to browning because of their involvement in synergistically promoting the oxidation of other phenolics (Bajaj *et al.*, 1997). Chlorogenic and neochlorogenic acids, (+) catechin, (-) epicatechin and rutin are the major phenolic substrates of PPO in apricot (Radi *et al.*, 1997). In pear, similar substrates have been used with purified PPO to show that peroxidase can contribute to brown color with quinones (derived from the PPO reaction) when quinones are used as substrate (Richard-Forget and Gauillard, 1997).

Reactions other than those catalysed by PPO can also cause browning. The Maillard reaction is perhaps the best known, but its products can also inhibit PPO (Tan and Harris, 1995). Nonspecific oxidation of phenolics results in browning, while formation of other brown polymers and heating that can produce caramelized sugars can also contribute. Enhanced browning is desirable in products such as tea, olives, pepper, cocoa and coffee, and the use of molecular biology technology to enhance PPO activity and promote brown colour development has been suggested as an approach for improving these crops. Breeders have produced cultivars and breeding lines with reduced PPO in crops such as potato and wheat amongst others, and mutations have resulted in clones with reduced browning in, for example, grape (Rathjen and Robinson, 1992).

Inhibiting PPO and reducing browning

A large body of literature reporting on browning caused by PPO, and its inhibition and control by conventional nongenetic means, has accumulated from many years of work on this subject. PPO enzyme activity and the amount of browning may be limited by low levels of phenolic substrate in several fruits including apple (Coseteng and Lee, 1987), but the effect depends on the cultivar with the amount of browning correlated with PPO activity in some cultivars and with phenolic levels in others. Metalothionein, proteins which can specifically bind metals, can in some cases regulate enzymes by influencing metal concentration (Robinson *et al.*, 1993). Cu deficiency induced by adding Cu-binding metalothionein protein to the reaction mixture inhibits PPO (Goetghebeur and Kermasha, 1996), since PPO is inactive without two bound Cu ions. The level of Cu in soils can determine the PPO activity of plants growing in it (Wang and Chen, 1997). Thiols derived from cysteine (Friedman and Bautista, 1995) and some phenolics such as cinamic acid, 2,4-dihydrobenzoic acid, p-coumaric acid (Bajaj *et al.*, 1997) (Janovitz-Klapp *et al.*, 1990) are a few of the compounds known to inhibit PPO to a greater or lesser degree. Low oxygen atmospheres and low temperature can reduce the amount or rate of the browning reaction

in lettuce (Heimdal *et al.*, 1995) and other produce and fruit since the PPO catalyses the oxidation of phenols. Some of the treatments reported to control or reduce browning include: ascorbate, glutathione and citrate (Jiang and Fu, 1998); and sulfites, suboptimal pH and high-pressure carbon dioxide (Chen *et al.*, 1992); natural products (Buta *et al.*, 1999); and 10 percent solution of honey (Oszmianski and Lee, 1990). For broader perspective on inhibition of browning, see: (Friedman and Bautista, 1991) (Iyengar and McEvily, 1992) (Whitaker and Lee, 1995a) (McEvily *et al.*, 1992) (Weemaes *et al.*, 1998) (Martinez and Whitaker, 1995).

4.1.3 PPO Biochemistry and Physiological Function

Analysis of PPO activity

PPO protein has been isolated, the protein characterized, the reaction conditions optimized and the kinetics described from numerous species including apple (Ridgway and Tucker, 1999) (Murata *et al.*, 1992) and stone fruit (*Prunus*) species (Fraignier *et al.*, 1995). A characteristic assay of PPO activity, as used in OSF's lab, begins with grinding samples in 0.1 M phosphate buffer, pH 6.1, containing 2 percent polyvinylpyrrolidone (PVP) to bind cell phenolics and 2 percent Triton X 100 to solubilize the enzyme, followed by centrifugation at 14,000 rpm. The activity is then assayed using added phenols such as catechol or 4-methyl catechol in 50 mM phosphate buffer as substrate. Many but not all PPOs are activated by sodium dodecyl sulfate (SDS). pH, SDS, substrate type and concentration and enzyme concentration should be optimized for each species studied. We found that added Cu (2 mM) increased PPO activity; perhaps a reflection of the Cu status of the source plant. The browning reaction of wheat grains has been examined using bioassays, with substrate in buffer added to the plant sample (Kruger *et al.*, 1994). These quick tests were found to correlate well with measured activities of extracted enzyme and the browning reaction of wheat noodle samples. The technique was useful for screening large numbers of breeding selections.

PPO synthesis and its stability in plant tissues

PPO is very stable, resisting proteolytic activity (Mari *et al.*, 1998). PPO is synthesized in young developing tissue with little if any PPO mRNA detected in intact mature tissues, yet mature tissues have high PPO activity (Murata *et al.*, 1997) (Kadioglu and Yavru, 1998). At different stages of development, apple fruits differ in the intensity of the browning reaction and have different levels of enzyme activity, with PPO uniformly distributed in immature fruit but localized near the core in mature apples and becoming partially solubilized and denatured as apples ripen (Murata *et al.*, 1997). Wound-induced synthesis of PPO mRNA has been described in apple (Boss *et al.*, 1995); this protein may be contributing to the browning of cut fruit.

Northern blots, a technique used to identify mRNAs in tissue samples, have been used to follow the time course of PPO synthesis in apricot (Chevalier *et al.*, 1999) and tomato (Thipyapong *et al.*, 1997). Little or no PPO mRNA was detected in mature apricot and tomato fruit, with most of the synthesis occurring in young fruitlets collected from flowering time to 10 weeks after flowering. In apricot (Chevalier *et al.*, 1999), the gene was highly expressed in young green fruit but was turned off early in the ripening process. This indicates that the turnover of PPO is very low and that the enzyme is unusually stable, since activity remains in apples and other fruits at

the end of the growing season even though little if any synthesis of significant new enzyme occurs in the mature fruit.

Wound-induced PPO

An exception to the common pattern of PPO synthesis in young developing tissue is wound-induced gene expression and activity reported after wounding in apple (Boss *et al.*, 1995), tomato (Thipyapong and Steffens, 1997) and litchi (Jiang and Fu, 1998). Synthesis likely occurs in metabolically competent cells close to the site of injury. The PPO isozymes induced by wounding of apple by cutting the flesh into cubes were also found in younger, undamaged fruit flesh.

PPO in defense response

PPO is not an essential enzyme since it is absent from some plant species, and potato clones with very low PPO activity have been produced using gene-silencing techniques without causing apparent harm to the plants (Bachem *et al.*, 1994). The functional role of PPO in plants is not completely understood, but a role in plant defense is indicated in some species. Systemic induction of PPO gene expression by injury was reported in tomato, where PPO synthesis occurred in young leaves after mature leaves were injured; it also occurred after infection by fungal and bacterial pathogens (Thipyapong and Steffens, 1997). In the same study, agents such as jasmonates, salicylic acid and ethylene – known to be involved in plant defense reactions – were shown to induce a gene promoter. Jasmonic acid methyl ester treatment also stimulated PPO activity in rice (Wu and Pan, 1997). The location of induction was different depending on the inducing agent.

In a study to investigate the possible role of PPO in insect herbivore defense, a survey of 18 plant species in five genera showed that PPO was induced by jasmonates in only three species (tomato, tobacco and poplar) (Constabel and Ryan, 1998). Tomato was unique among the plant species surveyed and had both high steady state PPO activity in leaf tissue and was strongly induced by both wounding and methyl jasmonates. Most species had low constitutive PPO levels and the enzyme was not induced by methyl jasmonates. Although it is possible that other signals might be involved in the induction of PPO in these latter species, this suggests that PPO may not play a major role in herbivore defense in these species. No Rosaceous species were tested in this study.

Tomato plants with modified PPO expression (suppressed PPO or over expressed PPO) were used to directly examine the role of PPO in defense response (Thipyapong *et al.*, 2004; Thipyapong *et al.*, 2007). Tomato plants that over expressed PPO were more resistant to *Pseudomonas syringae* and to several insects pests of tomato, including common cutworm (*Spodoptera litura*), cotton bollworm (*Helicoverpa armigera*) and beet army worm (*Spodoptera exigua*), while antisense PPO-suppressed tomato showed greater susceptibility. In similar studies in transgenic poplar, over expression of PPO has produced mixed results. Over expression of PPO caused decreased growth rates in caterpillars in some instances, but the effect was both species and growth stage specific (Barbehenn *et al.*, 2007) (Wang and Constabel, 2004). Transgenic apple, modified in PPO expression, has not been assessed for changes in resistance or susceptibility to pests and diseases outside of the data provided in this petition.

In a survey of scab-resistant apple cultivars it was shown that steady state levels of both PPO and phenolics antioxidants varied widely amongst the cultivars tested (Kolodziejczyk *et al.*, 2010). The steady state level of PPO is not correlated with scab resistance indicating that other factors are the primary determinant of resistance. Similar lack of correlation is found for apple fireblight when steady state PPO levels (Kolodziejczyk *et al.*, 2010) are compared to mean blight score (Korba *et al.*, 2008) or lesion length (Sobiczewski *et al.*, 2006). Browning potential was not correlated with resistance to apple fruit decay caused by *Penicillium expansum* (Valentines et al., 2005). Rather, less fruit decay is found in fruit with higher lignin content. These results suggest that peroxidase, through its involvement in lignification, was a factor in resistance of apple fruit to *P. expansum*.

PPO involvement with insect feeding

High PPO activity combined with phenolics in the trichomes of some *Solanaceous* plants results in entrapment of insects, by the polymers resulting from the PPO reaction when the insects damage the trichomes with their stylettes during feeding. PPO and phenolics added to reared insects' diets were shown to be antinutritional due to the reduced availability of amino acids, as well as other nutrients that became unavailable because they became trapped in the lignin-like polymer (Duffey and Felton, 1991); a dose gradient of PPO levels in potato genotypes has been shown to correlate with suitability for Colorado potato beetle growth and development (Castanera *et al.*, 1996). On the other hand, Urbanska (Urbanska *et al.*, 1998) reported that some insects have PPO in their saliva. The proposed beneficial function of this insect PPO was to convert detrimental phenolic compounds to more benign forms, thus enhancing nutritional value to the insect.

4.1.4 PPO Molecular Biology

PPO gene families

In many plant species, PPO is a multi-gene family with characteristic isozymes predominant in different organs or tissues. Tomato has a highly conserved seven-member gene family, with PPO expression largely confined to early stages of tissue development and expression patterns of isozymes related to the tissue type (Thipyapong and Steffens, 1997). Potato PPOs also occur in a multi-gene family, with gene expression and enzyme activity levels varying with tissue location and time of development (Thygesen *et al.*, 1995). Grape was found to have a single gene which was expressed in young developing leaves, root and berries, but with little gene expression in mature tissues (Dry and Robinson, 1994). Tobacco has 10 PPO genes and bean has five (Cary *et al.*, 1992). Four genes or gene fragments have been cloned from apple, although more may remain undiscovered (Boss *et al.*, 1995). A full-length cDNA clone was isolated from sugarcane (Bucheli *et al.*, 1996).

Within a species, small groups or pairs of similar PPO isozymes can occur. These are found together as the dominant isozyme in tissues such as leaves, tubers or flowers. The grouping of similar PPOs may be due to the polyploid nature of many of the plant species studied. Homology (biological similarity) of PPO genes at the nucleotide level within a species is usually 70 percent or more. Homology between genes of different species is usually less, about 50 percent, but can

be high between related species such as tomato and potato where homologies are 80 to 95 percent. Broad bean and apple, exceptionally, are related at 71.3 percent similarity.

Apple PPO genes

Various research groups, including OSF, have made an effort to clone and sequence members of the apple PPO gene family. The PPO sequences known to OSF are summarized (Table 1). The apple PPO gene sequences were sorted into four groups (PPO2, GPO3, APO5, pSR7) and the groups were named using the name first assigned to each sequence (Table 2). The members within a given PPO group are either the same gene, or are closely related enough at the nucleotide sequence level that they are expected to be equivalent from a gene silencing point of view. Complete or nearly complete sequences for PPO2, GPO3, APO5 and pSR7 are available. The sequences and sources of the four PPO gene sequences are also described in the Patent application "Genetically modified reduced-browning fruit-producing plants and produced fruit thereof, and method of obtaining such" (Armstrong and Lane, 2009).

Table 1: List of Known Apple PPO Genes

PPO Gene Sequence[1]	Source	Genbank Accession	Sequence
pSR7	Robinson (WO9302195)	A27661	Partial
pSR8	Robinson (WO9302195)	A27663	Partial
APO3 (5' APO3 sequence.)	Boss (personal communication)		Partial
APO9 (5' APO9 sequence.)	Boss (personal communication)		Partial
APO3 (3' APO3 sequence.)	Boss (personal communication)		Partial
APO9 (3' APO3 sequence.)	Boss (personal communication)		Partial
GPO3	Boss (personal communication)		Partial
AP14	OSF		Partial
APO5	(Boss *et al.*, 1995)	L29450	Complete
PPO3	(Haruta *et al.*, 1998)	D87669	Complete
PPO7	(Depicker *et al.*, 1982)	D87670	Complete
PPO2	(Kim *et al.*, 2001)	AF380300	Complete
PPOJ	OSF		Partial
GPO3	HortResearch		Complete
pSR7	HortResearch, OSF	A27661	Complete

[1] Many PPO sequences, partial and complete, have been identified. To develop a more complete picture of the PPO gene family, OSF used PCR with degenerate PPO primers to screen apple for novel PPO gene sequences identifying: GPO3, APO5 and AP14 in genomic DNA; GPO3, APO5, PPO2 and PPOJ in apple fruit and apple leaf cDNA; and GPO3 and pSR7 immature apple fruit cDNA library (Eugentech). HortResearch (now Plant & Food Research) found PPO2, GPO3, APO5 and pSR7 in their apple EST library (personal communication).

Table 2: Overview of the Apple PPO Gene Family

Group	Members[1]
PPO2	PPO2, PPOJ, pSR8
GPO3	GPO3, AP14[2], APO9, APO3
APO5	APO5, PPO3, PPO7
pSR7	pSR7

[1] Apple PPO gene sequences were sorted into four groups and the groups were named for the PPO sequence type. The members of a group are either the same gene, or are expected to be equivalent from an antisense point of view.
[2] AP14 is a pseudogene that is highly related to GPO3.

Suppression of the apple PPO gene family

A pivotal review (Sharp, 2001) suggested that for a transgene to induce silencing of a related but not identical target gene, the two segments must share regions of "identical and uninterrupted sequences of significant length" in the order of 30-35 base pair at a minimum. Since double stranded RNA (dsRNA) is processed to 21-23 nucleotide segments, Sharp suggests that a single basepair mismatch between the small interfering RNA (siRNA) and target RNA dramatically reduces gene targeting and silencing.

Pairwise alignment of PPO2, GPO3, APO5, and pSR7 with ClustalW (Thompson *et al.*, 1994) showed and overall homology between these sequences of 61 to 75 percent (Table 3) and is generally lower (<70 percent) than might be expected for PPO sequences within a species. The low overall homology between the apple PPO sequences prompted a closer examination of the relatedness of the PPO genes. The four apple PPO sequences were compared pairwise, within a sliding 22 base-pair window for regions of 100 percent homology (Table 4). This pairwise analysis demonstrated that GPO3 and APO5, having an overall sequence similarity of 75 percent, have several regions of identical and uninterrupted length. Other gene pairs share virtually no 22-base pair (bp) regions of 100 percent homology required for gene suppression. In short, other than APO5 and GPO3, the nucleic acid sequences of the apple PPO genes are sufficiently different that it was determined that that all four would need to be suppressed to induce a PPO-suppressed nonbrowning phenotype.

Table 3: Homology of PPO2, GPO3, APO5 and pSR7

	Percent Identity[1]			
	PPO2	**GPO3**	**APO5**	**pSR7**
PPO2	100	61	63	66
GPO3		100	75	62
APO5			100	65
pSR7				100
[1]PPO genes PPO2, GPO3, APO5 and pSR7 were aligned using ClustalW (Thompson *et al.*, 1994). The pairwise similarly score (percent identity) is reported.				

Table 4: Micro-Homology of PPO2, GPO3, APO5 and pSR7

	Number of regions of 22 bp homology[1]			
	PPO2	**GPO3**	**APO5**	**pSR7**
PPO2	100	0	0	0
GPO3		100	25	1
APO5			100	0
pSR7				100
[1] The four PPO apple sequences were compared pair-wise, within a sliding (conservative) 22 base-pair window for regions of 100 % homology.				

4.2 GEN-03 Vector

Events GD743 and GS784 were developed through *Agrobacterium*-mediated transformation of apple leaf tissue using the binary vector GEN-03 (Figure 2). GEN-03 is based on the binary vector pBINPLUS (van Engelen *et al.*, 1995). Vector pBINPLUS is based on the widely used binary vector BIN19 (Bevan, 1984). The complete sequence of BIN19 (U09365) is available at Genbank (Benson *et al.*, 2005). The GEN-03 vector contains a region of DNA (T-DNA) which consists of the PPO suppression transgene and NptII selection marker flanked by *Agrobacterium tumefaciens* T-DNA borders. This region (6287 bp) was transferred into the apple genome by *Agrobacterium tumefaciens* during the transformation process. The portion of the plasmid transferred to the plant genome begins near the right border (RB), extends through the PGAS transgene and NptII selection marker, and ends near the left border (LB).

The components of the T-DNA used to develop GD743 and GS784 are provided (Table 5). The sequences of the components of the T-DNA used to develop GD743 and GS784 are available.

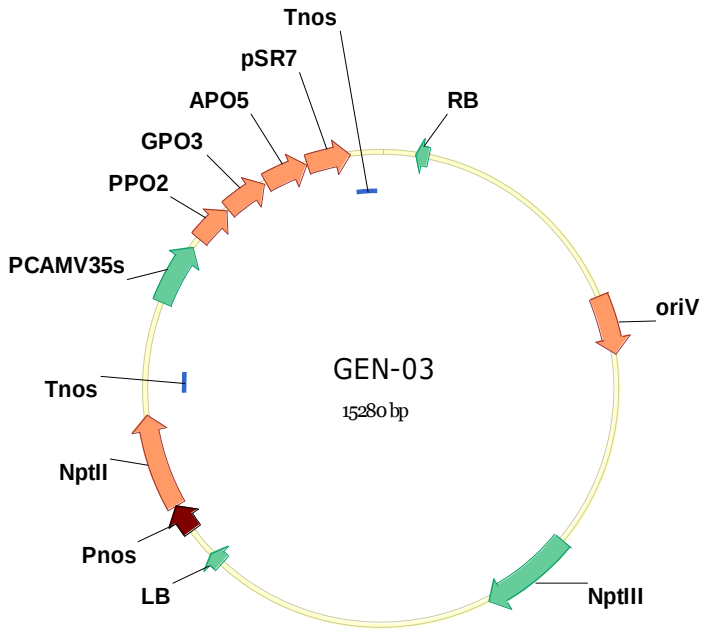


Figure 2: Map of the GEN-03 Vector

Table 5: Components of the T-DNA Used to Develop GD743 and GS784

Genetic Element	Size (Kb)	Function, Source, Reference
LB	0.15	A left border sequence derived from *Agrobacterium tumefaciens* pTiT37 (Depicker *et al.*, 1982)
P_{NOS}	0.31	A nopaline synthase promoter from *Agrobacterium tumefaciens* that directs transcription of the NptII selection marker (Bevan *et al.*, 1983b)
nptII	0.98	Neomycin phosphotransferase type II from Tn5 (Rothstein *et al.*, 1981) providing resistance to kanamycin
T_{NOS}	0.26	A 3' UTR from the nopaline synthase gene involved in transcription termination and polyadenylation (Depicker *et al.*, 1982) (Bevan *et al.*, 1983a)
$P_{CAMV35s}$	0.65	The duplicated-enhancer cauliflower mosaic virus promoter with untranslated leader sequence from alfalfa mosaic virus RNA4 (Datla *et al.*, 1993) that directs transcription of the PGAS transgene
PGAS	1.81	A sense suppression transgene designed to suppress four members of the apple PPO gene family
T_{NOS}	0.26	A 3' UTR from the nopaline synthase gene involved in transcription termination and polyadenylation (Depicker *et al.*, 1982) (Bevan *et al.*, 1983a)
RB	0.14	A right border sequence derived from *Agrobacterium tumefaciens* pTIT37 (Depicker *et al.*, 1982)

4.3 PPO Suppression Transgene

The PPO suppression transgene (PGAS) consists of 394, 457, 457 and 453 bp regions of apple PPO genes (PPO2, GPO3, APO5, pSR7, respectively), placed in the sense orientation under control of the cauliflower mosaic virus 35s promoter ($P_{CAMV35s}$) and nopaline synthase terminator (T_{NOS}). The use of a constitutive promoter such as the $P_{CAMV35s}$ is indicated here, as PPO is expressed both in early fruit development and in response to wounding. The transgene is designed to reduce overall expression of the entire apple PPO gene family, and to induce a reduced browning or nonbrowning phenotype in apple. The transgene was cloned into pBINPLUS to create the plant transformation vector GEN-03. The transgene was constructed using standard recombinant molecular biology techniques (Maniatis *et al.*, 1982) and was confirmed by DNA sequencing.

4.4 NptII Selection Marker

In addition to the PPO suppression transgene, the *nptII* gene from the *E. coli* Transposon Tn5 has been introduced into apple to be used as a selectable marker, under control of the nopaline synthase promoter (P_{NOS}) and terminator (T_{NOS}). This gene encodes for the enzyme neomycin phosphotransferase (NptII), which confers resistance to kanamycin in plants (Fraley *et al.*, 1986). NptII is an enzyme that inactivates the antibiotic kanamycin thereby allowing cells containing this gene to grow on medium containing kanamycin. The *nptII* gene is devoid of inherent plant pest characteristics (Fuchs *et al.*, 1993). Previously, APHIS has determined that the presence of the *nptII* gene will have no significant environmental impacts (APHIS 05-294-02r). As part of a study of clonal stability of the Arctic™ Apple transgene, it was shown that the *nptII* gene, as expressed by P_{NOS}, did not result in detectable amounts of the NptII protein accumulating in mature fruit of GD743 and GS784 (See Section 6.5, Table 41).

4.5 Conclusions

The following conclusions can be made concerning the selection of donor genes and regulatory elements combined in the GEN-03 vector used in the development of GD743 and GS784 events:

(a) PPO, responsible for apple browning, is encoded by a diverse, multi-gene family whose members are expressed both in early fruit development and in response to wounding;

(b) suppression of PPO, which is expected to lead to a nonbrowning phenotype, requires the use of a chimeric suppression transgene containing chosen sequences from the PPO gene family, all under control of a constitutive promoter element;

(c) the native function of PPO may be related to disease or insect defense, but the relationship in apple is weak and unconfirmed; and

(d) suppression of PPO is not expected to affect apple under cultivated conditions.

5 GENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION

5.1 Molecular Characterization

The intent of OSF's genetic modification was to insert the T-DNA from the vector GEN-03, including the PPO suppression transgene and NptII selection marker into the genome of apple. Polymerase chain reaction (PCR) was used to do the initial screening of kanamycin-resistant shoots arising from GEN-03 transformation. Southern analysis was used to confirm that a complete copy of the PPO suppression transgene was inserted in the apple genome, as well as, estimate copy number and show the absence of vector backbone. Reverse primer PCR (rpPCR) was used to show that multiple insertions detected in GS784 did not arise as the result of tandem or inverted repeat structures.

5.1.1 Polymerase Chain Reaction (PCR)

Initial PCR screening of kanamycin-resistant shoots arising from transformation of apple cultivars GD and GS with the GEN-03 vector was completed using primers specific for selected regions along the T-DNA insert (Table 6, Figure 3). One primer pair was specific for the NptII selection marker. Amplification of a 483 bp fragment from apple genomic DNA of both GD743 and GS784 with *nptII*-specific primers is evidence that n*ptII* is present and the tissue is transgenic (data not shown). A second set of primers was specific for the 3' end of the PPO suppression transgene. The forward primer was within the pSR7 segment of the PPO suppression transgene and the reverse primer was within NOS terminator. These primers amplify across the synthetic pSR7:NOS junction that is unique to the GEN-03 insert. Amplification of a 556 bp fragment from apple genomic DNA of both GD743 and GS784 using these primers is evidence that 3' end of the transgene is present and the tissue is transgenic (data not shown).

Method for PCR Screening GEN-03 Transformants

Genomic DNA was extracted from tissue culture leaf material using a modified CTAB extraction method (Lodhi et al., 1994). PCR was done in a 25 µl reaction containing 1 x PCR buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.5 µM forward primer, 0.5 µM reverse primer, 0.625 U Taq DNA polymerase, and 10 ng DNA. PCR reactions were overlayed with mineral oil and cycled: 30 x 94^oC for 1 min, 55^oC for 1 min, 72^oC for 1 min, followed by 1 x 72^oC for 10 min. DNA loading buffer (5 µl) was added to each reaction and 10 µl of each reaction was run on 1% TAE-agarose for 1.5 hours at 80V. Gels were stained with ethidium bromide, rinsed and photographed. Events that carry either the selection marker or the PPO suppression transgene were considered to be transgenic.

Table 6: Primers for PCR Screening GEN-03 Transformed Tissue

Target	Primer Name	Primer Sequence	Product (bp)
nptII	NptII-F	GAACAAGATGGATTGCACGCAG	483
	NptII-R	CTGATGCTCTTCGTCCAGATCA	
3' Transgene	pSR7-F (A81)	AAGCTTTTCCTTTCCACCGCATGT	556
	NOSTERM	TATGATAATCATCGCAAGAC	

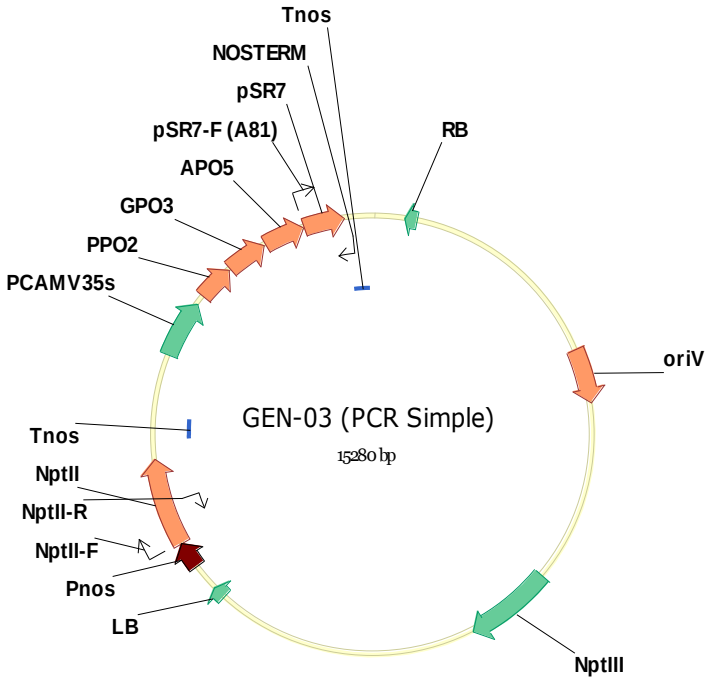


Figure 3: Location of the PCR Primers on the GEN-03 Vector

5.1.2 Southern Blot Analysis

Southern blot analysis is described in detail below, including the preparation of transgene and vector backbone-specific hybridization probes, relative location of the restriction sites and hybridization probes and hybridization blot results. Southern blot analysis was completed by LofStrand Laboratories (Gaithersburg, MD) from samples supplied by OSF.

Genomic DNA Extraction

Genomic DNA used for the molecular characterization of events GD743 and GS784 was extracted from apple leaf tissue using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol.

Southern Blot Analysis (Transgene Probe)

Ten micrograms of GS control and GD control genomic DNA were cut for 48 hours with 15 units of XbaI per microgram of DNA. Also 10 µg of GD743 and GS784 were cut for 48 hours with 15 units of XbaI per µg of DNA.

In a separate reaction 10 µg of GD743 and GS784 genomic DNA were cut for 48 hours with 4.8 units of AvrII per µg of DNA.

In a third set of digests, 10 µg of GD743 and GS784 genomic DNA were cut for 48 hours in a double digest using five units per µg of AscII and PacI.

In separate 48 hour reactions, 0.2 ng of each of GEN-03 plasmid DNA were cut with XbaI, or AvrII, or AscI and PacI.

All digested DNAs were run on a 300 ml, 0.65% TBE agarose gel along with Invitrogen 1Kb+ marker at 50 V for 18 hours.

The gel was stained with ethidium bromide and photographed. The gel was then treated with 0.25 M HCl for 20 minutes. The gel was treated with 0.5 M NaOH + 1.5 M NaCl two times for thirty minutes each followed by treatment with 0.5 M tris, pH 8.0 + 1.5 M NaCl two times thirty minutes each.

Transfer to a nylon membrane Nytran Supercharge (Whatman) was conducted per protocol using a TurboBlotter and 10X SSC, overnight. The membrane was UV linked and air dried.

The filter was prehybridized using 6X SSC, 5X Denhardt's solution, and 0.5% SDS at 68°C for 4 hours. The filter was hybridized using a random primed 300 base pair PCR amplified and gel purified Transgene Probe. A specific activity of greater than 1 x 10e9 dpm/µg for the probe was achieved. The probe concentration was 3.5x10e6 dpm/ml in the HYBE buffer (same as the prehybridization buffer). The hybridization was carried out at 68°C for 24 hours.

The filter was washed in 2X SSC + 0.1% SDS at 68°C with five buffer changes over a period of two hours. The filter was autoradiographed for 48 hours and 24 hours with an intensifier screen at -80°C.

Southern Blot Analysis (Backbone Probe)

The filter (which was previously probed with the Transgene Probe) was stripped using 0.1% SDS at 95°C for 20 minutes followed by an overnight wash in 0.05X SSC + 0.1% SDS at 70°C. The filter was checked and found to be at background levels using a survey meter. The filter was pre-hybridized using 6X SSC, 5X Denhardt's solution, and 0.5% SDS at 68°C for 4 hours. The filter was hybridized using a random primed 228 base pair PCR amplified and gel purified Backbone Probe. A specific activity of greater than 1 x 10e9 dpm/µg in the HYBE buffer (same as the pre-hybridization buffer) was achieved. The hybridization was carried out at 68°C for 48 hours.

The filter was washed in 2X SSC + 0.1% SDS at 68°C with five buffer changes over a period of 90 minutes. The filter was autoradiographed for two days with intensifier screen at -80°C.

Hybridization Probes

Probes were prepared by PCR using transgene and vector backbone specific primers (Table 7). Transgene and backbone probe sequences were used to search the Genbank nucleotide collection (Malus x domestica). No similarities were found, suggesting that the hybridization probes had no known native targets in apple. The relative location of the restriction enzyme and probes used are shown (Figure 4).

Table 7: Primers Used to Prepare the Hybridization Probes

Probe Target	Probe Size (bp)	Primer Name	Primer Sequence (5' to 3')
Transgene	300	Transgene F	TTGATGTGATGGTCCGATCT
		Transgene R	GTGCGTCATCCCTTACGTC
Backbone	228	Backbone F	CGGCTCCGTCGATACTATGT
		Backbone R	GCAGCGGTATTTTTCGATCA

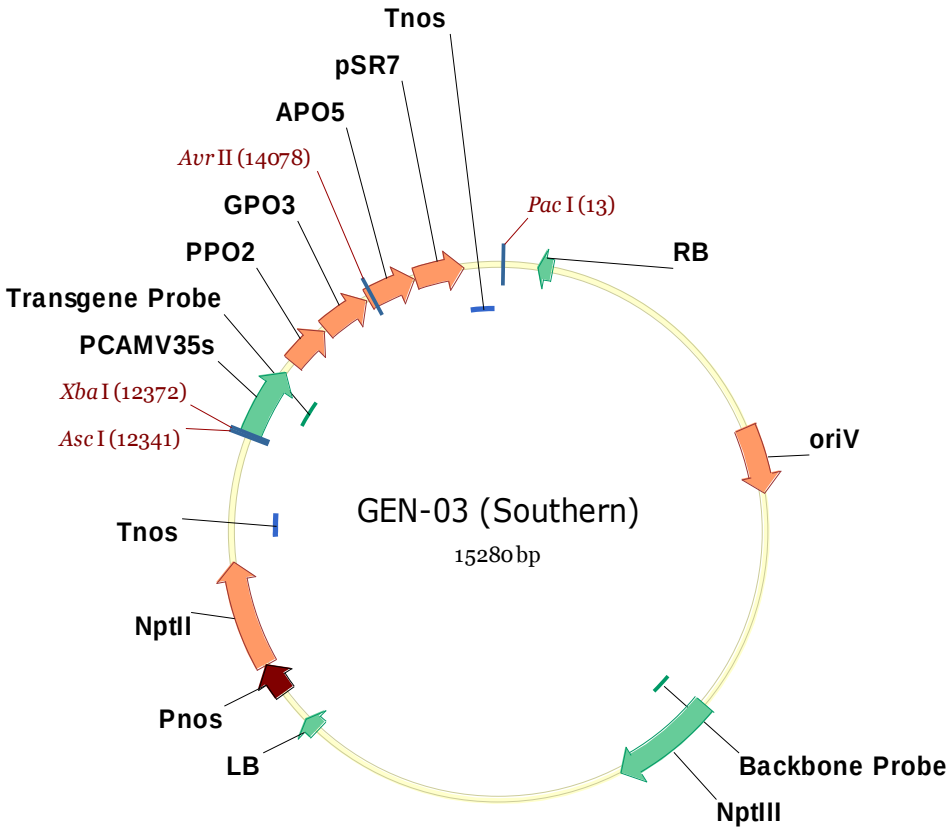


Figure 4: Relative Location of Probes and Restriction Enzymes on the GEN-03 Vector

Insert Integrity and Copy Number

Genomic DNA from GD743 and GS784 was digested with *Asc*I and *Pac*I to generate a 2.95 kb fragment that would hybridize with the Transgene Probe. A known amount of GEN-03 (0.2 ng) was also digested with *Asc*I and *Pac*I. Both the GD743 and GS784 had the predicted 2.95 kb fragment consistent with insertion of a complete copy of the PPO suppression portion of the insert. The intensity of the 2.95 kb band for GD743 represented 1 or 2 copies, while the intensity of the 2.95 kb band in the GS784 is several fold higher consistent with multiple copies of the insert in GS784 (see Figure 5: Southern Blot - Transgene Probe).

Genomic DNA of GD743 and GS784 was also digested with either *Avr*II or *Xba*I to generate one fragment that would contain part of the GEN-03 transgene (approximately 4.56 kb for *Avr*II and 3.24 kb for *Xba*I) and a fragment of the plant genome the size of which would depend upon where the T-DNA had integrated. The DNA from each of the transformed plants exhibited at least one unique fragment. No signal was detected in the control GD or GS DNA (*Xba*I).

GD743 revealed two bands (*Avr*II) or one band (*Xba*I) of the correct size. These results are consistent with one or two copies of the GEN-03 T-DNA in GD743.

GS784 revealed one band (*Avr*II) or three to five bands (*Xba*I) of the correct size. Of the three bands present in the *Xba*I digest, three are of similar intensity (4000, 4300, 6500) and two are approximately half that intensity (5300, 10000); these latter two possibly represent a partial digest. The sum intensity of these five bands would approximately equal that of the GS784 x *Asc*I/*Pac*I digest. Taken together with the results of the *Asc*I/*Pac*I digest, this indicates a copy number of about four in GS784.

To explain why the copy number in GS784 is under-represented in the *Avr*II digest, we examined the frequency of restriction sites for *Avr*II and *Xba*I in a number of apple BAC clones (GU295057 and FN832235). It was found that the frequency of *Avr*II sites was lower than *Xba*I in both cases. Thus, the genomic *Avr*II sites associated with the additional copies of the T-DNA in GS784 may be sufficiently distance to the insertion site that the associated restriction fragment would not be resolved.

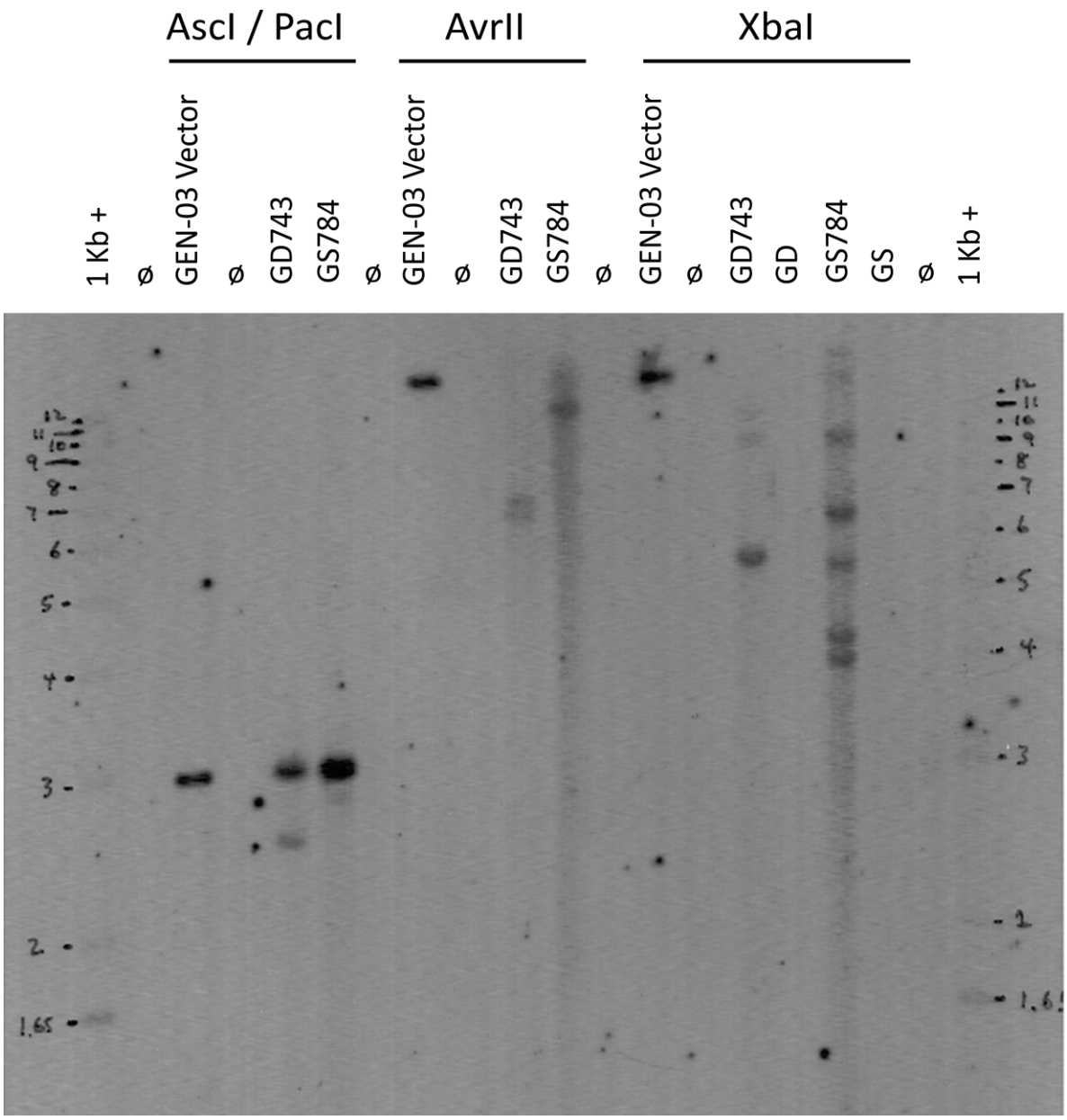


Figure 5: Southern Blot - Transgene Probe
Southern blot analysis of GD743 and GS784 and respective control cultivars GD and GS. A known amount of the plant transformation vector GEN-03 was also resolved on the same gel. The 2.95 kb *Asc*I-*Pac*I fragment detected in the GEN-03 vector and in the GD743 and GS784 represents the internal *Asc*I-*Pac*I fragment of the PPO suppression transgene. The >13,000 bp *Avr*II and *XbaI* fragments detected in the GEN-03 represents the entire plant transformation vector.

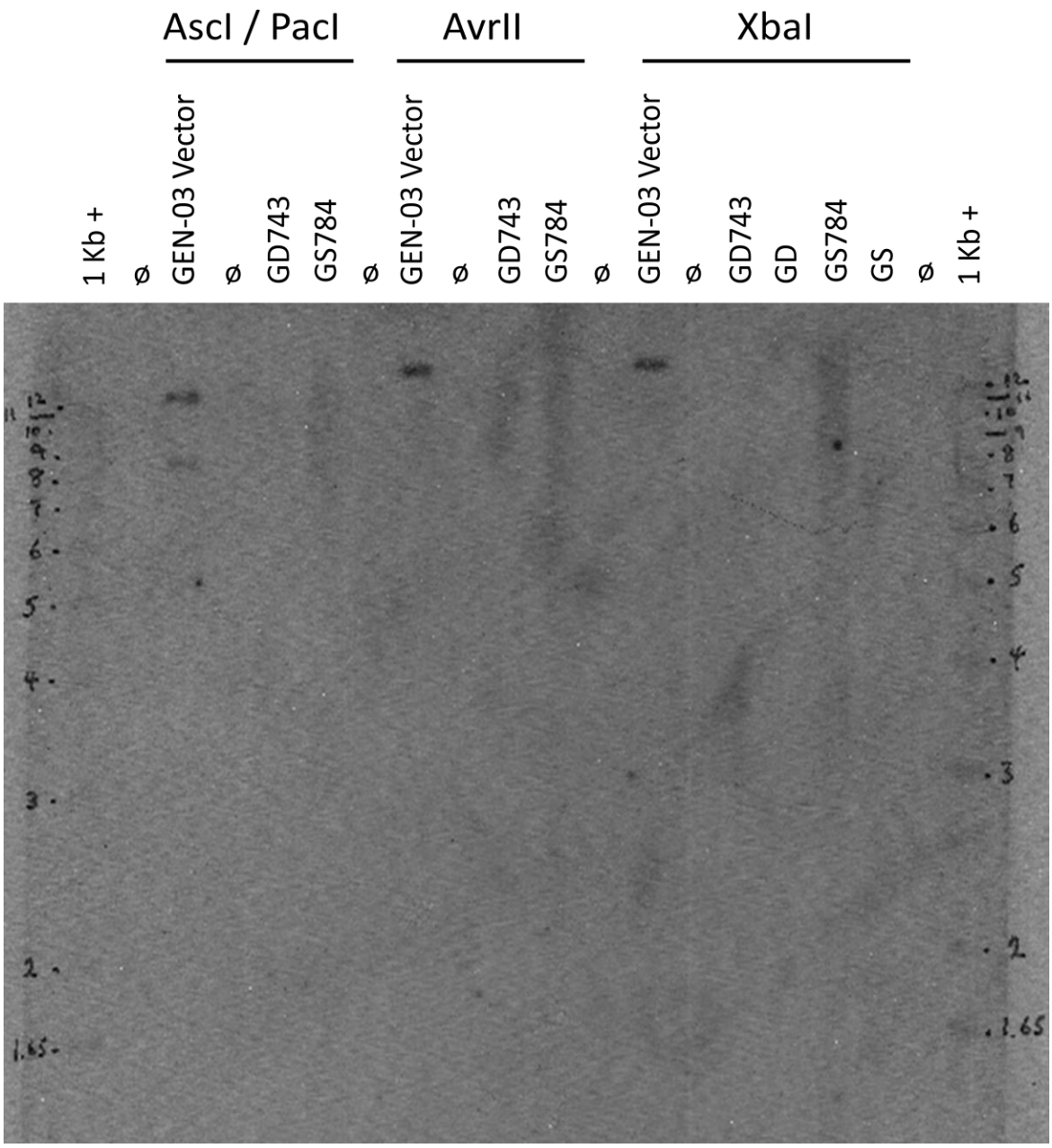


Figure 6: Southern Blot - Backbone Probe

Southern blot analysis of transgenic GD743 and GS784 and respective control cultivars GD and GS. The plant transformation vector GEN-03 was also resolved on the same gel. The 12 kb *Asc*I-*Pac*I fragment detected in the GEN-03 vector represents the entire plant transformation vector (less the PPO suppression transgene). The >13,000 bp *Avr*II and *XbaI* fragments detected in the GEN-03 vector represents the entire plant transformation vector (show that the probe functions). No bands were detected in any of the restriction enzyme digests of GD743 or GS784 indicating that backbone is not present in either.

Absence of Vector Backbone

To determine the presence or absence of vector backbone, the Southern blot (previously hybridized to the Transgene Probe) was stripped and re-probed with a Backbone Probe (see Figure 6: Southern Blot - Backbone Probe).

The 12 kb *Asc*I-*Pac*I fragment detected in the GEN-03 vector represents the entire plant transformation vector (less the PPO suppression transgene). The >13,000 bp *Avr*II and *XbaI* fragments detected in the GEN-03 vector represents the entire plant transformation vector. No bands were detected in any of the restriction enzyme digests of GD743 or GS784 indicating that backbone is not present in either.

5.1.3 Reverse Primer PCR (rpPCR)

In a study of transgenic aspen it was determined that 1 in 4 transgenic lines assessed contained some form of T-DNA repeat (Kumar and Fladung, 2000). To determine if the multiple transgene inserts detected in GS784 were associated with transgene repeat structures, genomic DNA of GS784 was subjected to PCR with primers specifically designed to detect the presence of tandem or inverted repeats. The PCR method, primer sequences, and primer location upon a theoretical tandem transgene repeat structure are described in detail below.

A PCR technique known as reverse primer PCR (rpPCR) was used to probe the structure of the T-DNA insertion in GS784 (Kumar and Fladung, 2000). This method uses primer pairs in which the primers are oriented in the opposite direction so that amplification cannot occur if only a single insertion is present. However, if the transgene is inserted in the form of tandem or inverted repeats, amplification products are possible.

The sequences of the PCR primers (Table 8), their orientation in a theoretical tandem or inverted insertion structures (Figure 7) and the amplification products expected (Table 9) are shown. The size of the amplification products provided reflects integration products with precise border junctions. Repeat structures are often accompanied by filler DNA (Kumar and Fladung, 2000) which would increase the size of the amplification product.

Primer pair 4/6 is used as a PCR positive control (Figure 7). Primer 4 and primer 6 are oriented towards each other and would be expected to produce a PCR product in the presence of a single GEN-03 T-DNA insertion. The primer 4 / primer 6 pair produced a PCR product of expected size (1517 bp) in all genomic DNA samples of GD743 and GS784 tested.

Table 8: Primer Sequences for Reverse Primer PCR (rpPCR)

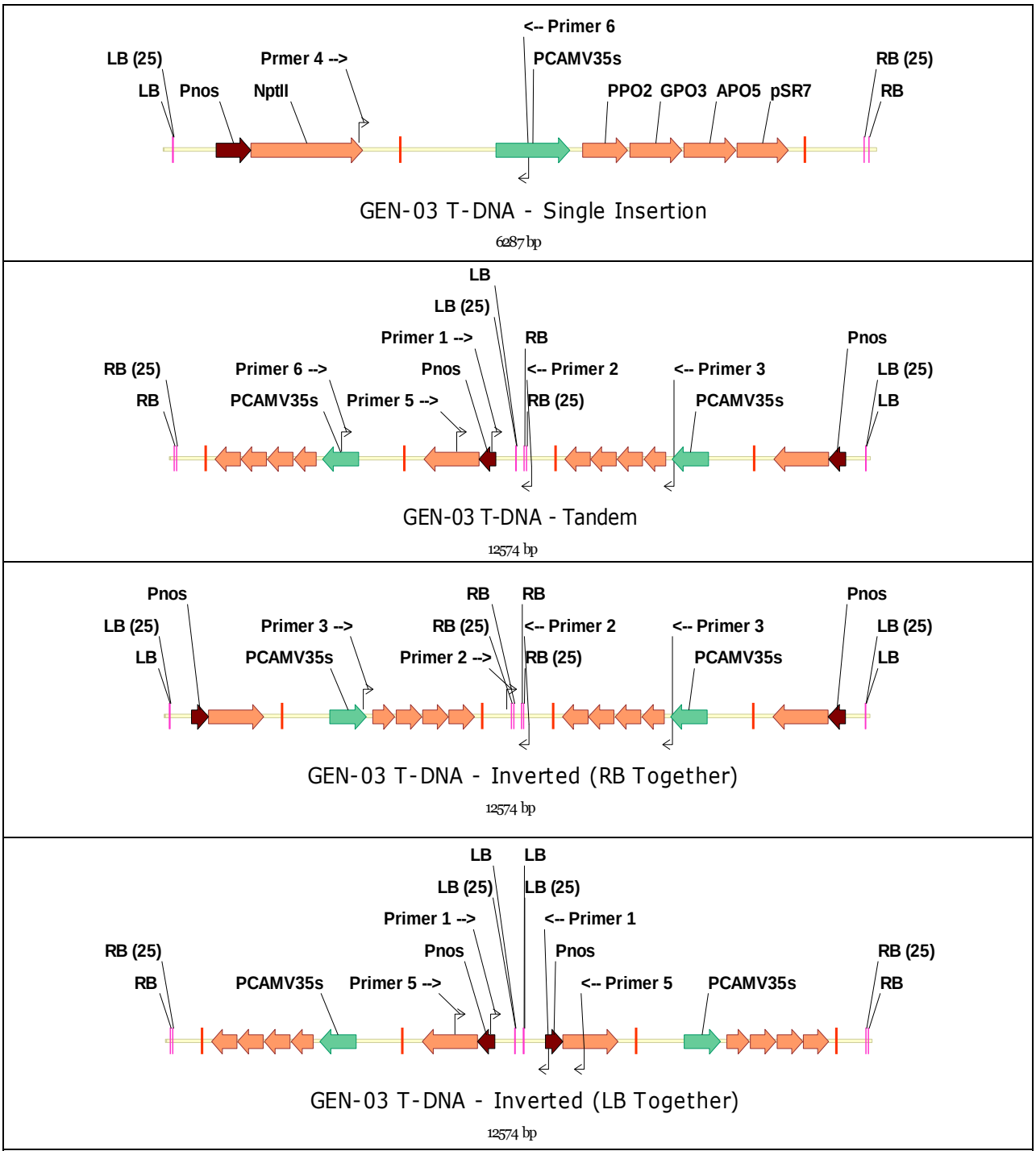
Primer Name	Sequence	Target Region (bp)	Direction
Primer 1	CCAAACGTAAAACGGCTTGT	Pnos (307)	→
Primer 2	TTTCCCCGTCAAGCTCTAAA	Region between RB-Tnos (356)	←
Primer 3	TTCGCAAGACCCTTCCTCTA	PCAMV35s (650)	←
Primer 4	TCTCATGCTGGAGTTCTTCG	*nptII* (984)	←
Primer 5	GTAGCCGGATCAAGCGTATG	*nptII* (984)	→
Primer 6	CAGATCGGACCATCACATCA	PCAMV35s (650)	→

Table 9: Primer Pairs for the Detection of Tandem or Inverted Repeats

Left Primer	Right Primer	Fragment Size (bp)1	Structure[2]
Primer 1	Primer 2	735	Tandem Insertion
Primer 1	Primer 3	3294	Tandem Insertion
Primer 2	Primer 5	1368	Tandem Insertion
Primer 3	Primer 5	3927	Tandem Insertion
Primer 2	Primer 6	3436	Tandem Insertion
Primer 1	n/a	1054	Inverted Repeat – LB Together
Primer 5	n/a	2320	Inverted Repeat – LB Together
Primer 2	n/a	416	Inverted Repeat – RB Together
Primer 3	n/a	5534	Inverted Repeat – RB Together

[1] Approximate fragment size, depending on the precision of RB and LB excision and the presence of filler sequence between the RB and LB repeat.

[2] Potential insertion structures are shown in Figure 7.



LB = Left Border; RB = Right Border; LB (25) = Left Border 25 base pair repeat; RB (25) = Right Border 25 base pair repeat.

Figure 7: Potential T-DNA Insertion Structures and Primer Locations

Screening for the presence of tandem insertions (Primer Pairs: 1/2, 1/3, 2/5, 3/5, 2/6), inverted repeats - LB together (Single Primer 1 or Primer 5) and inverted repeats - RB together (Single primer 2 or Primer 3) was completed. No PCR products were detected using any of the selected primer pairs indicating that multiple insertions at a single site, in the form of simple tandem or inverted repeats, had not occurred in the GS784 event.

This information is consistent with each of the four copies of the transgene being inserted at a different location in the genome in GS784.

5.2 Inheritance and Stability of the Insert

5.2.1 Mendelian Inheritance

In the New York field trial, trees are covered with insect-proof netting. Thus for fruit production, hand pollination is required. Trees of GD743 and GS784 and their respective controls are hand-pollinated with a non-transgenic pollen (apple cultivar, Idared) each year and apple fruit was harvested at maturity. For each event and control, a total of 100 seeds were collected. Fifty seeds from each event were subjected to genomic DNA extraction and PCR testing using primers specific for *nptII* or PPO suppression transgene (Table 10). The other fifty seeds were stratified to induce germination. Shoots were grown out to produce enough material for testing by NptII ELISA. Once the seeds had germinated, shoots were collected, individually packaged in Ziploc^{TM} bags and shipped overnight to Agdia (Elkhart, IN) for NptII ELISA (PathoScreen kit for neomycin phosphotransferase II, Catalog number: PSP 7300) testing.

Table 10: Primers for Determining Mendelian Inheritance

Target	Primer Name	Primer Sequence	Product (bp)
nptII	NptII-F	GAACAAGATGGATTGCACGCAG	483
	NptII-R	CTGATGCTCTTCGTCCAGATCA	
5' Transgene	Transgene Forward	CGCACAATCCCACTATCCTT	229
	Transgene Reverse	GCGTCCCAGTTCCAGAAG	

Method for PCR Screening GD743 x Idared and GS784 x Idared Seeds

Seeds (with seed coat removed) were frozen in liquid nitrogen and then ground individually in 1.5 ml microfuge tubes with a plastic pestle. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Samples were eluted in a final volume of 200 µl. PCR was done in a 25 µl reaction containing 1 x PCR buffer, 1 mM $MgCl_2$, 200 µM each dNTP, 0.4 µM forward primer. 0.4 µM reverse primer, 2 U Taq DNA polymerase, and 10 ng DNA. PCR reactions were cycled: 35 x 94^oC for 1 min, 55^oC for 1 min, 72^oC for 2.5 min, followed by 1 x 72^oC for 7 min. DNA loading buffer (1.6 µl) was added to 8 µl of the PCR reaction and 8 µl of this was run on 1% TBE-agarose for 50 minutes at 70V. Gels

were stained with ethidium bromide, rinsed and photographed. Seeds that carry either the selection marker or the PPO suppression transgene were considered to be transgenic.

In apple seeds, the seed coat is maternal and there was some concern that this would lead to false positive arising from PCR of seed material, even though considerable effort was made to remove the seed coat prior to genomic DNA extraction. This is why some of the seeds were germinated and sent for ELISA screening.

The results and statistical analysis is provided in Table 11. If the GD743 was expressing NptII from two unlinked insertion sites then the expected segregation for the cross should be 3:1 (NptII Positive to NptII Negative). If the GS784 was expressing NptII from four unlinked insertion sites then the expected segregation for the cross should be 15:1 (NptII Positive to NptII Negative).

Table 11: Mendelian Inheritance

Cross	**Total Seeds**	**NptII Positive (PCR)**	**Segregation**	**Chi square**	**P[1]**
GD743 x Idared	50	41	3:1	1.3	P > 0.2
GS784 x Idared	50	47	15:1	0.005	P > 0.95
Cross	**Total Seeds**	**NptII Positive (ELISA)**	**Segregation**	**Chi square**	**P[1]**
GD743 x Idared	50	42	3:1	2.2	P > 0.1
GS784 x Idared	33[2]	31	15:1	0.002	P > 0.95

[1] The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. In this case, P > 0.05 indicates that the portion of total seeds that are NptII positive is statistically consistent with the segregation ratio.

[2] There were 50 seeds of GS784 that were to be screened by NptII ELISA. Rodent damage resulted in the loss of several GS784 shoots, while they were being sprouted, leaving only 33 seeds to be screened by NptII ELISA

5.2.2 Clonal Stability

Given apples are vegetatively propagated, the stability of any new apple clone is very important. The growers, packers, processors and retailers will expect the tree and fruit to remain stable from year to year, as well as from graft to graft over multiple generations. The clonal stability of events GD743 and GS784 has been assessed through a number of experiments, as well as seven years of field observation.

Apple Leaf Samples

Apple leaf samples were harvested from trees of GD743, GS784 and their respective controls from both field trials. Each sample is a pooled leaf sample (several leaves) from one tree, comprising approximately 5 grams of leaf tissue per sample. Leaves were picked into Ziploc™ bags and sent to Agdia (Elkhart, IN) for testing. Samples were tested for NptII (ELISA) and

subjected to genomic DNA extraction. Genomic DNA was then subject to PCR using primers specific for NptII.

Genomic DNA Extraction and PCR

Genomic DNA was isolated from apple leaf tissue by Agdia (Elkhart, IN) using a DNeasy Plant Mini Kit (Qiagen). PCR was done at OSF (Saskatoon) on genomic DNA isolated by Agdia. PCR was done using Taq Polymerase (Invitrogen) and NptII-specific primers. PCR was done in a 25 µl reaction containing 1 x PCR buffer, 1 mM $MgCl_2$, 200 µM each dNTP, 0.4 µM forward primer. 0.4 µM reverse primer, 2 U Taq DNA polymerase, and 10 ng DNA. PCR reactions were cycled: 35 x 94°C for 1 min, 55°C for 1 min, 72°C for 2.5 min, followed by 1 x 72°C for 7 min. DNA loading buffer (1.6 µl) was added to 8 µl of the PCR reaction and 8 µl of this was run on 1% TBE-agarose for 50 minutes at 70V. Gels were stained with ethidium bromide, rinsed and photographed. Seeds that carry the nptII were considered to be transgenic.

PCR was used to show that *nptII* was present in leaf tissue of all samples of GD743 and GS784 trees and absent from the respective GD and GS Control trees (Table 12). Similarly, NptII ELISA confirmed the presence of NptII protein in all leaf samples of GD743 and GS784 and absence from the respective GD and GS control trees (Table 13). Based on these results we can conclude that transgene insertion in both GD743 and GS784 is stable in vegetatively propagated trees.

Table 12: Detection of the *nptII* gene in Apple Leaf Tissue by PCR

Group	Trees Tested[1]	*nptII* Positive (PCR)[2]
Arctic™ Apple GD743	14	14
GD Control	4	0
Arctic™ Apple GS784	14	14
GS Control	4	0

[1] For GD743 and GS784 there were 9 trees from the WA trial and 5 trees from the NY trial tested. For GD and GS there were 2 trees tested from the WA and NY field trials.

[2] PCR was done by OSF (Saskatoon) on apple leaf DNA samples extracted by Agdia (Elkhart, IN) with primers specific for *nptII* (NptII-F GAACAAGATGGATTGCACGCAG / NptII-R CCTGGATCTGGTTCAGTGC).

Table 13: Detection of NptII Protein in Apple Leaf Tissue by ELISA

Group	Trees Tested[1]	NptII Positive (ELISA)[2]
Arctic™ Apple GD743	14	14
GD Control	4	0
Arctic™ Apple GS784	14	13[3]
GS Control	4	0

[1] For GD743 and GS784 there were 9 trees from the WA trial and 5 trees from the NY trial tested. For GD and GS there were 2 trees tested from the WA and NY field trials

[2] NptII ELISA results are classified as Negative (< 0.5), Questionable (0.5 to < 1.0), Elevated (1.0 to < 5.0) or Positive (≥ 5.0) by Agdia. OSF has considered samples identified as Elevated or Positive to be NptII Positive by ELISA.

[3] One sample of GS784 from Washington State had NptII levels slightly greater than control but was considered to be questionable by Agdia.

5.3 Basis for Resistance to Enzymatic Browning in GD743 and GS784

The intended consequence of insertion of the PPO suppression transgene into apple was to suppress expression of the PPO gene family. Reduced gene expression should lead to lower total PPO activity, resulting in a nonbrowning phenotype. To confirm this result, we compared the total PPO activity (see Section 5.3.1), and bruise response (Section 5.3.2) in GD743 and GS784 relative to their untransformed control cultivars Golden Delicious (GD) and Granny Smith (GS).

5.3.1 PPO Enzyme Activity

In apple, browning is related to polyphenol content or polyphenol oxidase activity, depending on the cultivar (Coseteng and Lee, 1987). In events GD743 and GS784, in which the amount of polyphenol oxidase has been specifically limited through suppression of the PPO gene family, bruising will be related to PPO activity only. Therefore, to further validate success of the transformation of GD743 and GS784, various apple tissues were subjected to PPO activity assessment. PPO specific activity experimental results from tissue culture leaves, greenhouse leaves, field leaves, immature fruit and mature fruit can be found in the following subsections.

Method for Measuring PPO Activity in Apple

PPO activity was measured using a modification of the method of Broothaerts (Broothaerts et al., 2000) in which the assay portion of the procedure was adapted to a microtitre plate. In the modification, leaf tissue, whole immature fruit or mature fruit skin were ground in a mortar and pestle under liquid nitrogen. Samples of ground tissue (50 mg) were extracted in 1 ml of extraction buffer (0.1 M sodium phosphate, 2% Triton X-100, 1 % PVPP, pH 6.0). After centrifugation, extracts of leaf or mature fruit skin were diluted 5 times, and extracts of immature fruit skin were diluted 50 times. PPO activity is measured using 4-methyl catechol as substrate and protein content was measured using bicinchoninic acid (BCA) (Thermo Scientific Pierce). PPO activity was reported as specific activity (U/mg protein), in an assay scaled down

proportionally to fit into a microtitre plate format. The Unit Definition of enzyme activity is 1 U = 0.001 A400 / min.

Tissue Culture Leaves

Leaves from early shoot cultures of Golden Delicious or Granny Smith transformed with the GEN-03 vector were collected from each plantlet. Leaf tissue was snap frozen in liquid nitrogen and stored at -80^{o}C until processing.

PPO screening of transformed events went on from October 30, 2002 until December 28, 2004. Event GD743 was first identified as PPO suppressed on December 20, 2003 and GS784 was first identified as PPO suppressed on March 5, 2003. The tissue culture Events of GD743 and GS784 were tested for PPO Activity on 2 successive subcultures. This work was part of a larger screening effort that tested 176 different GEN-03 transgenic events of four different cultivars and resulted in the identification of 16 – 20 highly PPO suppressed events.

PPO activity was reduced 77% in GD743 and 87% in GS784 relative to their respective controls (Table 14). Statistical analysis of results was not performed.

Table 14: PPO Activity in GD743 and GS784 - Tissue Culture Leaves

Event	Mean SpActivity[1]	n[2]	PPO Suppression[3]
GD743	593	2	77 %
GD	2561	6	
GS784	289	2	87 %
GS	2166	4	

[1] SpActivity = Specific Activity of PPO.

[2] n = number of pooled tissue culture leaf samples per event.

[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Greenhouse Leaves

Transgenic, PPO suppressed events GD743 and GS784 were grafted onto M9 rootstocks and grown under greenhouse conditions. Leaves of greenhouse plants were collected, snap frozen in liquid nitrogen and stored at -80^{o}C until processing. Since apple trees are clonally propagated, only a subset of the plants propagated were tested. PPO Screening of greenhouse plants occurred between May 20, 2003 and January 8, 2004.

PPO activity was reduced 93% (GD743 and GS784) relative to control levels (Table 15). Statistical analysis of results was not performed.

Table 15: PPO Activity in GD743 and GS784 - Greenhouse Leaves

Event	Mean SpActivity[1]	n[2]	PPO Suppression[3]
GD743	150	6	93 %
GD	2248	10	
GS784	338	3	93 %
GS	4978	2	

[1] SpActivity = Specific Activity of PPO.

[2] n = number of pooled greenhouse leaf samples per event. In the greenhouse, generally, only one pooled leaf sample was taken from each tree. Therefore, the number of samples approximately equals the number of trees sampled.

[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Field Leaves

Transgenic, PPO Suppressed events GD743 and GS784 were grafted onto M9 rootstocks and grown under greenhouse conditions before being sent to field trials. Mature leaves of field-grown plants were collected from the Washington field trial in the fall of 2005 (September 24, 2005), snap frozen in liquid nitrogen and stored at -80^{o}C until processing. Since apples are clonally propagated, only a subset of the plants that went to the field was tested.

Field plants of events GD743 and GS784 showed a marked reduction of PPO activity in both GD743 (82%) and GS784 (76%), relative to their respective control GD and GS cultivars (Table 16). The reduction of PPO activity in field leaves was not as dramatic as previously reported in greenhouse leaves (93%). However, this appeared to be due to a decrease in the reported value for PPO activity in leaves from field-grown control GD and GD. The absolute level of PPO activity in field-grown, greenhouse-grown and tissue culture leaf tissue was similar for GD743 and GS784.

Table 16: PPO Activity in GD743 and GS784 - Field Leaves

Event	Mean SpActivity[1]	*S*	n[2]	PPO Suppression[3]
GD743	207	104	14	82 %
GD	1165	390	6	
GS784	315	95	10	76 %
GS	1297	245	8	

[1] SpActivity = Specific Activity of PPO.

[2] n = number of pooled field leaf samples per event. In the field, generally, two pooled leaf samples were taken from each tree. Therefore, the number of samples approximately equals twice the number of trees sampled.

[3] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Independent sample t-tests of average PPO specific activity in GD743 versus GD Control and GS784 versus GS Control were performed using SOFA Statistics v0.9.22 (Paton-Simpson, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant effect for the Arctic™ Apple trait, $t(18) = -8.78$, $p = 0.0$, with GD743 having lower PPO specific activity than GD. Similarly, there was a significant effect for the Arctic™ Apple trait, $t(16) = -11.71$, $p = 0.0$, with GS784 having lower PPO specific activity than GS.

Immature Fruit

Immature fruit, approximately 10 mm in length, was harvested from the Washington field trial in the spring of 2005 (June 10, 2005). A small amount of fruit was available from GD743, GD and GS (but not GS784). Fruit of GS784 was limited due to poor fruit set after a spring frost; therefore, it was not possible to determine PPO activity or PPO suppression in GS784 immature fruit (GS784 fruit that was available in 2005 was reserved for Controlled Bruising analysis). Since PPO is produced in early fruit development, immature fruit of field grown GEN-03 plants and their respective control were collected, snap frozen in liquid nitrogen and stored at -80°C until processing.

PPO activity was reduced 94% in GD743 relative to its control (Table 17).

Table 17: PPO Activity in GD743 and GS784 - Immature Fruit

Event	Mean SpActivity[1]	*S*	n	PPO Suppression[2]
GD743	4519	3703	4	94 %
GD	75160	43329	8	
GS784	-	-	-	Not determined
GS	68171	26351	11	

[1] SpActivity = Specific Activity of PPO.

[2] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Independent sample t-tests of average PPO Specific Activity in Arctic™ Apple GD743 versus GD Control was performed using SOFA Statistics v0.9.22 (Paton-Simpson, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant effect for the Arctic™ Apple trait, $t(10) = -3.18$, $p = 0.01$, with GD743 having lower PPO specific activity than GD.

Mature Fruit

Mature apple fruit were harvested from five trees of GD743 and GS784, and their respective controls. Fruit was harvested from the Washington field trial (WA2003) in 2007, when the trees

were in their fifth growing year. Two apples were harvested from each tree. Apples were stored at 4°C until processing.

PPO activity was reduced 91% in GD743 and 90% in GS784 relative to their respective controls (Table 18).

Table 18: PPO Activity in GD743 and GS784 - Mature Fruit

Event	Mean SpActivity[1]	*S*	n	PPO Suppression[2]
GD743	294	173	10	91 %
GD	3176	1235	10	
GS784	520	259	10	90 %
GS	5390	2341	10	

[1] SpActivity = Specific Activity of PPO.

[2] PPO Suppression = ((Mean SpActivity of Control – Mean SpActivity of Event) / Mean SpActivity of Control)*100

Independent sample t-tests of average PPO Specific Activity in Arctic™ Apple GD743 versus GD Control and Arctic™ Apple GS784 versus GS Control were performed using SOFA Statistics v0.9.22 (Paton-Simpson, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant effect for the Arctic™ Apple trait, $t(18) = -7.31$, $p = 0.0$, with GD743 having lower PPO specific activity than GD. Similarly, there was a significant effect for the Arctic™ Apple trait, $t(18) = -6.54$, $p = 0.0$, with GS784 having lower PPO specific activity than GS.

Notably, it is observed that specific activity of PPO was considerably higher in immature fruit tissue than in mature fruit tissue. This is consistent with the fact that PPO is produced in immature fruit and is then dispersed throughout the apple (diluted) as the apple grows in size.

5.3.2 Controlled Bruising of Apple

The ultimate goal and predicted outcome of genetic modification in the GD743 and GS784 events, is to develop an apple cultivar that would not brown when bruised or cut. Therefore, to further validate the success of the transformation, mature apples were harvested and subjected to controlled bruising.

Mature fruit was harvested from the Washington state field trial. Fruit was sampled in 2005 and 2006, when trees were in their third and fourth growing year. The bruise response to controlled mechanical damage was measured as described in detail below. Briefly, the unpeeled apple is subjected to a controlled impact. After allowing a specified amount of time to pass to permit for bruises and their resultant color changes to develop, the apples are peeled and the color of the

apple flesh is compared on and off the bruise location. Bruising is reported as change in Lightness (ΔL*) or Color (ΔE*).

Controlled bruising experimental data, analysis and results are presented below.

Method for Measuring Bruising Response in Apples

A special bruising apparatus was designed to deliver a controlled bruise to the apple with minimal destruction to the tissue. Apples are bruised in a consistent manner using the improvised Impact Device. Bruise response is reported as Change in Lightness (ΔL), or Total Change in Color (ΔE*) between the bruised and non-bruised tissue as measured using a Minolta Chroma Meter.*

The Impact Device

The Impact Device consists of the Impact Device itself, plus a shallow container of glass beads into which the apple is set, prior to being bruised. The Impact Device consists of a wooden block with a rounded impact surface that can be dropped from a consistent and adjustable height. A bruise, as delivered by the Impact Device could, alternatively, be produced by dropping a marble or steel ball down a tube, of a specific length, which is placed on the surface of the apple. A shallow dish full of glass beads, into which the apple is placed, provides a cushion to prevent damage to the underside of the apple during impact to the top side of the apple.

The Minolta Color Meter

A Konica Minolta CR-300 Chroma Meter with DP-301 Data Processor was used to measure flesh color (Figure 8).

Figure 8: The Konica Minolta CR-300 Chroma Meter

Procedure

Apples are removed from storage and allowed to come to room temperature for 2 hours. Positions of the bruises are marked with a felt pen on the apple skin. Each apple is bruised 5 times and allowed to sit at room temperature for 3 hours for the bruise to form. The apples are peeled over the bruised areas (careful not to remove the pen marking or to go too deep with the peeling). Each peeled area is measured on the non-bruised area adjacent to the bruise (trt 1) and directly on the bruised area (trt 2). Bruising, or Change in Lightness (ΔL) and Total Change in Color (ΔE*) are calculated as:*

$$\Delta L^* = L^*_{trt2} - L^*_{trt1}$$

$$\Delta a^* = a^*_{trt2} - a^*_{trt1}$$

$$\Delta b^* = b^*_{trt2} - b^*_{trt1}$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where:

L* = Lightness
a* = Position between red/magenta and green
b* = Position between yellow and blue

L* a* b* are defined in greater detail below.

2005 Results

Apple fruit samples were harvested from trees of GD743 and GS784, and their respective GD and GS control trees, at maturity, from the Washington field trial. For each transgenic event and control, three (3) trees were sampled. Due to limited fruit, only one (1) apple was selected from each tree. Apples were stored at 2^{o}C prior to testing.

Each apple was bruised in a controlled manner and the bruising /browning reaction was assessed, as follows: The apples are brought to room temperature and left to sit for 2 hours at room temperature (18^{o}C). Each apple is labeled, marked and bruised 5 times, in 5 different locations around the mid-section. The apples are left to sit for 3 hours at room temperature to allow bruise development. An apple peeler is used to expose an area that is larger than the bruised area. The peeled area is measured using a colorimeter 'off' and 'on' the bruise, which represents the 'trt2' and 'trt1' treatments.

The measured variable is lightness (L*) described in the CIE 1976 (L*, a*, b*) color space, where L* = 0 yields black and L* = 100 indicated diffuse white. A negative change in lightness (negative ΔL*), indicates a darkening of the apple tissue.

The average ΔL* for GD (-11.65) and GS (-9.08) control apples produced visible brown bruising of the apple flesh. Conversely, the average ΔL* for GD743 (-1.37) and GS784 (-1.04) did not produce visible bruising of the apple flesh (Table 19).

Table 19: Controlled Bruising of GD743 and GS784 (18°C) - 2005

Event	ΔL*	*S*	n	temp[1]
GD743	-1.37	1.02	15	18
GD	-11.65	3.16	15	18
GS784	-1.04	0.78	15	18
GS	-9.08	2.79	15	18
[1] Bruising done while apple flesh temperature was 18°C (room temperature).				

Independent samples t-test of average ΔL* for GD743 versus GD Control and GS784 versus GS Control were performed using SOFA Statistics v0.9.22 (Paton-Simpson, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant effect for the Arctic™ Apple trait, $t(28) = 11.97$, $p = 0.0$, with GD743 having lower ΔL* than GD. Similarly, there was a significant effect for the Arctic™ Apple trait, $t(28) = 10.76$, $p = 0.0$, with GS784 having lower ΔL* than GS.

2006 Results

Apple fruit samples were harvested from trees of GD743 and GS784, and their respective GD and GS control trees, at maturity from the Washington field trial. For each transgenic event and control, five (5) trees were sampled. Apples were stored at 2°C prior to testing.

Each apple was bruised in a controlled manner and the bruising /browning reaction was assessed, as follows: The apples are brought to room temperature and left to sit for 2 hours (18°C) or bruised straight out of cold storage (2°C). Two (2) apples were randomly selected from each tree for each temperature. Each apple is labeled, marked and bruised 5 times, in 5 different locations on the mid-section of the apple. The apples are left to sit for 3 hours at room temperature to allow bruise development. An apple peeler is used to expose an area that is larger than the bruised area. The peeled area is measured using a colorimeter 'off' and 'on' the bruise.

The measured variables are lightness (L* = 0 yields black and L* = 100 indicated diffuse white), its position between red/magenta and green (a*, negative value indicate green while positive values indicate magenta), and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow) as described in the CIE 1976 (L*, a*, b*) color space. The reported variable is change in color (ΔE*), calculated from the measured variables. The change in color (ΔE*) is a positive number and represents the difference (distance) between two colors. A larger ΔE* represents a larger color difference.

For Golden Delicious, change in color (ΔE*) associated with bruising is caused by a darkening of the tissue (negative ΔL*), shift from green to magenta (positive Δa*), and deepening of the yellow color (positive Δb*). For Granny Smith, change in color (ΔE*) associated with bruising is caused by a darkening of the tissue (negative ΔL*), shift from green to magenta (positive Δa*), but no shift in blue / yellow color. The change in color (ΔE*) is a positive number.

Independent samples t-test of average ΔE* for GD743 versus GD Control and GS784 versus GS Control were performed using SOFA Statistics v0.9.22 (Paton-Simpson, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level.

Bruising at 18°C

The average ΔE* for GD (13.43) and GS (18.26) control apples produced visible brown bruising of the apple flesh. Conversely, the average ΔE* for GD743 (2.41) and GS784 (4.11) did not produced visible bruising of the apple flesh (Table 20).

Table 20: Controlled Bruising of GD743 and GS784 (18°C) - 2006

EventID	ΔE*	*S*	n	temp[1]
GD743	2.41	1.18	50	18
GD	13.43	5.66	50	18
GS784	4.11	1.90	50	18
GS	18.26	3.08	50	18
[1] Bruising done while apple flesh temperature was 18°C (room temperature).				

There was a significant effect for the Arctic™ Apple trait, $t(98) = -13.49$, $p = 0.0$, with GD743 having lower ΔE* than GD. Similarly, there was a significant effect for the Arctic™ Apple trait, $t(98) = -27.96$, $p = 0.0$, with GS784 having lower ΔE* than GS.

Bruising at 2°C

The average ΔE* for GD (13.56) and GS (12.01) control apples produced visible brown bruising of the apple flesh. Conversely, the average ΔE* for GD743 (2.39) and GS784 (3.18) did not produced visible bruising of the apple flesh (Table 21).

Table 21: Controlled Bruising of GD743 and GS784 (2°C)

EventID	ΔE*	*S*	n	Temp[1]
GD743	2.39	1.09	50	2
GD	13.56	3.40	50	2
GS784	3.18	2.67	50	2
GS	12.01	6.65	50	2
[1] Bruising done while apple flesh temperature was 2°C.				

There was a significant effect for the Arctic™ Apple trait, $t(98) = -22.11$, $p = 0.0$, with GD743 having lower ΔE* than GD. Similarly, there was a significant effect for the Arctic™ Apple trait, $t(98) = -8.71$, $p = 0.0$, with GS784 having lower ΔE* than GS.

Images and Other Data

Both GD743 and GS784 events showed markedly reduced browning response relative to their untransformed parent cultivars, further demonstrating success of the genetic transformation and that a nonbrowning phenotype had in fact resulted. Indeed, bruises yielding ΔE* values of less than 2-4 were invisible to the naked eye. Images of the bruise response are included in the photographic record provided in Appendix 1 (Figure 14).

Other forms of mechanical damage, such as slicing or juicing, have consistently yielded the same dramatic nonbrowning phenotype for GD743 and GS784 (Appendix 1, Figure 15 and Figure 16). Also, in an independent study of events GS784 and others, Cornell University scientists established a clear relationship between the nonbruising phenotype and total PPO activity. High PPO activity and high browning was observed in bruised tissue of conventional apples studied, contrasted with almost no PPO activity and no changes of color in the transformed apples (data not shown).

5.4 Conclusions

The following conclusions can be made concerning genotypic and phenotypic changes made to create the events GD743 and GS784, demonstrating that OSF has successfully developed a nonbrowning apple:

(a) PCR confirms the presence of both the 3' end of the PPO suppression transgene and the NptII selection marker.

(b) Southern blot analysis confirms the presence of an intact copy of the PPO suppression portion of the insert in both GD743 and GS784. Southern data, combined with Mendelian Inheritance data indicates that GD743 arises from two unlinked insertions, while GS784 arises from four unlinked insertions. Southern blots also confirm that there is no evidence for inclusion of a complete copy of the vector backbone in either GD743 or GS784;

(c) Reverse Primer PCR confirms that the multiple copies of the insert in GS784 do not arise from tandem or inverted repeat structure, suggesting instead that GS784 has four copies of the insert at four independent locations;

(d) PPO activity is reduced by more than 75 percent in field leaves and more than 90 percent in greenhouse leaves and mature fruit of both GD743 and GS784 events; and

(e) Suppression of PPO is sufficient to induce a nonbrowning apple phenotype.

6 AGRONOMIC EVALUATION

6.1 Description of Field Trials

6.1.1 Site Selection

OSF has two field trial sites in the USA, covered by USDA APHIS Notifications and Permits detailed in Table 22 and Table 23. These trials are located in New York and Washington states, and represent conditions found in the two largest apple-growing areas in the country. The field trials also encompass two distinct agronomic zones, with the principal apple growing area of Washington State being a dry, arid to semi-arid region with low insect and very low fungal pressure, and New York being a much wetter and humid region with higher insect and disease pressure.

Table 22: Washington State Field Trial Notifications and Permits

Notification or Permit No.	Effective Date	Date Expires	Reporting Status
03-073-07n	April 13, 2003	Dec. 31, 2006	Annual report submitted
04-097-02n	May 6, 2004	Dec. 31, 2007	Annual report submitted
06-087-10n	May 1, 2006	May 1, 2007	Annual report submitted
06-087-09n	May 1, 2006	May 1, 2007	Annual report submitted
07-086-111n	April 27, 2007	April 27, 2008	Annual report submitted, extended by ePermit
07-086-110n	April 27, 2007	April 27, 2008	Annual report submitted, extended by ePermit
07-086-106n	May 30, 2007	May 30, 2008	Annual report submitted, extended by ePermit
07-086-107n	May 31, 2007	May 31, 2008	Annual report submitted, extended by ePermit
07-348-101r	April 1, 2008	March 31, 2011	Annual report submitted
11-056-102r	April 1, 2011	April 1, 2014	New in 2011

Table 23: New York State Field Trial Notifications and Permits

Notification or Permit No.	Date Issued	Date Expires	Reporting Status
05-046-14n	March 24, 2005	March 24, 2009	Annual report submitted, extended by ePermit
07-355-101r	April 1, 2008	April 1,2011	Annual report submitted
11-067-105r	March 31, 2011	March 31, 2014	New in 2011

The Washington trial is located in the dry Pacific Northwest agronomic zone 5, which is characterized by 700-1,000 growth degree days (base 50^{o}F) per year, silty clay loam soils over 40 inches deep and annual rainfall ranging from 10-14 inches (Douglas *et al.*, 1990). The New

York field trial is located in the New York Central Plains agronomic region, which is strongly influenced by Lake Ontario providing 1000-1250 growing degree days (base 50^{o}F) and 100-200 frost free days from late April to early October (Dethier and Vittum, 1967). Annual rainfall averages approximately 35 inches, with 14-20 inches of rain during the growing season. Soils are shallow with low water-holding capacity, largely derived from glacial activity. Mean maximum temperatures in July range from 80-86° F, and mean minimum temperatures of 5-14° F occur in January (Fick, 1995).

The first Washington field trial relevant to this petition was planted in 2003 (WA2003) (See Appendix 2, Table 49: Map of the Washington Field Trial – 2003 Block). It consisted of 220 trees planted in a single row approximately 528 feet long. Trees were on commercially-popular Malling 9 rootstocks and were planted as in a commercial orchard, with tree spacing 24 inches apart. This 0.14 acre row contained trees representing 14 different GEN-03 transgenic events of the apple cultivars GD, and GS, plus untransformed controls. OSF events GD743 and GS784 covered in this petition are dispersed within this row. In the spring of 2005, trees were flagged in the WA2003 block. These included 3 GD743 (743-8, 743-8, 743-14), 3 GD (1000-1, 1000-2, 1000-9), 3 GS784 (784-3 784-4, 784-5), and 3 GS (1001-1, 1001-4, 1001-17). Throughout the period that the WA2003 trial was active, trees were removed for a variety of reasons (rodent damage, winter kill), including 743-8, 1001-17, 784-4, 1000-2, and 1000-1.No new trees were added to the block. The entire WA2003 block was removed at the end of the 2007 growing season to make way for a new more homogenous demonstration block containing only selected GEN-03 events plus controls (renamed WA2008).

The Washington field trial relevant to this petition was planted in 2004 (WA2004) (See Appendix 2, Table 51: Map of the Washington Field Trial - 2004 Block). It consists of 242 trees planted in a single row approximately 580 feet long. Trees are on commercially-popular Malling 9 rootstocks and are planted as in a commercial orchard, with tree spacing 24 inches apart. This 0.16 acre row contains trees representing 28 different GEN-03 transgenic events of the apple cultivars GD, GS, Fuji and Gala plus untransformed controls. OSF events GD743 and GS784 covered in this petition are dispersed within this row. In the spring of 2005, 3 trees of selected GEN-03 events represented in the row, plus 3 trees of each control cultivar were randomly chosen and flagged for data collection. In the spring of 2008, a new and expanded subset of trees was flagged, which included 4 trees of selected GEN-03 events, plus 4 trees of each control. For some events, insufficient numbers of trees were available, so fewer than 4 trees were chosen. Throughout the period that the WA2004 trial was active, considering the GD743, GD, GS784 and GS trees that are subject of this petition, one control tree (1001-37) died due to rodent damage and was removed. No new trees were added to the block.

An expansion of the Washington field trial was planted in 2008 (WA2008) (see Appendix 2, Table 52: Map of the Washington Field Trial - 2008 Block). It consists of 146 trees planted in a single row approximately 300 feet long, placed within an existing trial block of unrelated trees. Trees are on commercially-popular Malling 9 rootstocks and are planted as in a commercial orchard, with tree spacing 24 inches apart. This 0.10 acre row contains trees representing 4 different GEN-03 transgenic events of the apple cultivars GD and GS plus untransformed controls. OSF events GD743 and GS784 covered in this petition are planted in blocks within this row. In the spring of 2009, 10 trees of each GEN-03 event represented in the row, plus 10 trees

of each control cultivar were chosen and flagged for data collection. Only one tree (1001-128) has been removed from this block. No new trees have been added.

The New York field trial was planted in 2005 (NY2005) and consists of 119 trees covering an area of 0.15 acres, made up of two rows 15 feet apart and trees rows 225 feet long with trees placed at 4 feet on center. The trees are also on Malling 9 rootstock. In this trial, there are a total of five GD743 trees and six GS784 trees located randomly in the two rows (see Appendix 2, Table 50: Map of the New York Field Trial – 2005 Block). The trial also contains five GD and six GS control trees. The balance of the trial consists of control trees and other events

6.1.2 Horticultural Management

OSF operates these field trials with the assistance of local collaborators who provide the day-to-day onsite horticulture management. OSF staff visit the trials on a regular basis to ensure management and regulatory integrity.

It is essential to manage apple field trials in a manner consistent with commercial apple cultivation methods, adhering to integrated pest management (IPM) and Good Agricultural Practices (GAP) approaches. By assuring this type of management, the data collected from the trials is both reproducible and can be extrapolated with confidence to a commercial setting. OSF field trial Standard Operating Procedures (SOPs) include, but are not limited to: soil preparation and testing, tree planting, tree fertility, pest management, disease management, irrigation scheduling, crop load management (i.e., pruning and thinning), insect monitoring and data collection, crop spraying and reporting, harvest, postharvest fertility, rodent and wildlife control, and disposal of transgenic trees.

A commercial apple orchard is a highly-managed agricultural production environment. This is particularly the case in modern high-density orchards (which average more than 1,000 trees/acre) using dwarf rootstock. Careful monitoring of tree vigor, insect pressure and disease allows for timely optimization of tree growth, production and yield. For this reason, little pest and disease pressure is tolerated or observed in commercial apple orchards.

6.1.3 Data Collection

In Washington state, OSF engaged an independent horticultural consultant and IPM specialist to monitor the trial, place insect pheromone traps for that region's leading insect pests – codling moth (*Cydia pomonella),* oblique-banded leafroller (*Choristoneura rosaceana)* and Pandemis moth (aka barred fruit-tree tortrix, *Pandemis cerasana)* – and collect relevant field data. This consultant visited the trial approximately every 10 days to do a general inspection of tree vigor, insect and disease pressure and trap counts. These visits began in April and ended in late October. Regulatory compliance data was collected from flagged trees of events GD743 and GS784, as well as controls and other trees of interest. The data collected represented the dominant insect and disease problems found in a commercial orchard and, although not exhaustive, are representative of how these transgenic trees compare to controls in a commercial setting.

In 2010 we changed the process for monitoring the WA field trial. Up until that time, we had been using an adjacent block of Pink Lady as an early indicator to forewarn us of pest and/ or

disease problems. Beginning in the early spring of 2010 we began using a control block of trees within the trial to monitor for pest problems (See Appendix 2, Table 51: Map of the Washington Field Trial - 2004 Block, Positions 76 – 92). Within this block is a representative sample of GD/GD743 and GS/GS784 trees. The control block was monitored on a weekly basis by the field manager. If pests or diseases were present in the control block, then data was collected from tagged trees from within the WA2004 (Appendix 2, Table 51) and WA2008 (see Appendix 2, Table 52: Map of the Washington Field Trial - 2008 Block) trial blocks.

In New York state, OSF's collaborator provided the independent horticultural support and data collection service similar to those engaged in at the Washington trial. This collaborator has considerable experience in running tree fruit field trials and is capable of handling this task. The data collected from this trial was consistent with the Washington trial; however, data was also collected regarding damage due to Japanese beetle (*Popillia japonica*), a new pest that is specific to this area.

6.2 Agronomic Performance

Agronomic performance data described in this section is consolidated in Table 24 and Table 25 for the New York and Washington state field trials. Given many of the trees planted were preceded by year-round tissue culture and greenhouse activities, the trees planted in these trials were a wide range of sizes. At planting, tree height ranged from 20 to72" and tree caliper ranged from ¼" to ¾". Getting trees established outdoors was always a priority as they perform better in this environment. Planting trees in this manner likely increased the variability within the agronomic data being collected. Figure 9 illustrates how this variability tended to work itself out over time and how all trees, once they filled their space (height and volume-wise), had performance consistent with the controls.

Table 24: Agronomic Performance - New York

Sample Description		Tree Height (cm)			TCA[2] (cm2)			Flower Clusters			Fruit Number at Harvest		
Event	**Leaf**	**Average**	***S***	**n**	**Average**	***S***	**n**	**Average**	***S***	**n**	**Average**	***S***	**n**
GD743	3rd	177	22	5	265	63	5	25	18	5	16	4	5
GD	3rd	186	24	5	328	52	5	4	3	5	10	11	5
GS784	3rd	178	23	6	289	98	6	6	3	6	3	2	6
GS	3rd	197	11	6	354	75	6	46	38	6	9	9	6
GD743	4th	232	28	5	512	103	5	13	12	5	10	8	5
GD	4th	225	26	5	706	105	5	25	8	5	26	10	5
GS784	4th	235	22	6	663	161	6	28	14	6	24	11	6
GS	4th	242	14	6	685	90	6	39	15	6	27	9	6

[1] Results are a compilation of data from 2007 and 2008 from Rows 101 and 102 planted in 2005 (NY2005).

[2] TCA = trunk cross-sectional area.

Table 25: Agronomic Performance - Washington

Sample Description		Tree Height (cm)[5]			TCA[2] (cm2)			Flower Clusters[5]			Fruit Number at Harvest		
Event	**Leaf**	**Average**	***S***	**n**	**Average**	***S***	**n**	**Average**	***S***	**n**	**Average**	***S***	**n**
GD743	3rd				58	12	3				14	7	3
GD	3rd				40	1	2[3]				27	8	3
GS784	3rd				59	16	3				16	2	3
GS	3rd				41	3	3				18	10	3
GD743	4th				87	9	4[4]				14	7	4
GD	4th				72	1	2				1	1	2
GS784	4th				82	23	4				14	9	4
GS	4th				71	14	4				24	10	4

[1] Results are a compilation of data from 2005 and 2006 from Row 14 planted in 2003 (WA 2003).

[2] TCA = trunk cross-sectional area.

[3] In 2005, one GD control tree was removed after fruit harvest and before fall measurement of trunk cross-sectional area (TCA) .

[4] In 2006, additional trees were added to the dataset.

[5] Data not collected.

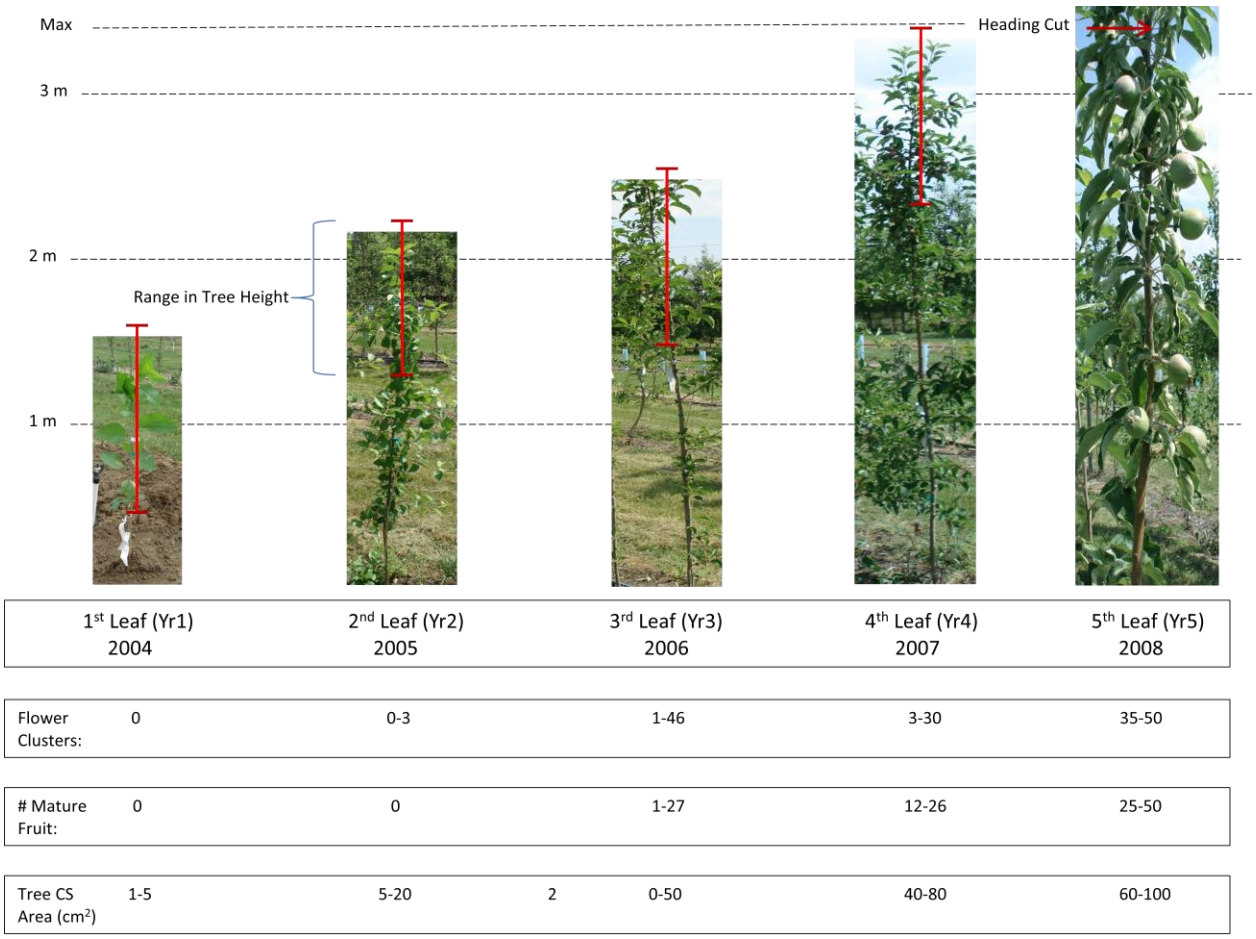


Figure 9: Agronomic Performance Overview
This figure pictorially represents the establishment of the WA2004 field block. Year round micrografting to produce trees, followed by greenhouse and screenhouse activities resulted in a high variability in size of trees that were planted in the spring of 2004. This figure also shows the approximate range of flower clusters, mature fruit and tree (trunk) cross sectional area that would be found in the years following planting. This is true in subsequent years of planting in Washington, as well. Numbers for flower clusters, mature fruit and tree cross sectional area is different in New York, where tree spacing is greater (4 feet).

6.2.1 Tree Growth Rate

To determine the relative size and growth rate of events GD743 and GS784 compared to the controls, growth components were measured, including tree height and trunk cross-sectional area (TCA), at the end of every season of the trial. TCA is recognized by the American Society for Horticultural Science as a descriptor of tree size in young trees that have not yet been pruned to control size; TCA is correlated with height and tree volume.

Data from the end of the third and forth growing season was judged to be the most appropriate illustrator of size, as this is before trees have been top pruned to manage height. We did not observe any differences in tree height or TCA between GD743 and GS784 events and their corresponding controls.

6.2.2 Flower Cluster Number

More prolific flowering in younger trees is usually regarded as a favorable trait in apples. Study of this trait allows us to measure the precocity of the GD743 and GS784 events compared to their untransformed controls. The number of flower clusters per tree was counted in each season of the trial to give some indication of this component of yield.

SF evaluated the average flower cluster number of the events vs. controls in the New York state trial in trees in their third and fourth growing seasons. We did not observe any differences in this agronomic trait between GD743 and GS784 events and their corresponding controls.

6.2.3 Fruit Number at Harvest Time

Fruit number is an important component of yield, as a low fruit number might indicate a degree of sterility, or lack of precocity.

OSF's measurements of fruit number shows the average fruit number of the events vs. controls in both the Washington State and New York state trials in the third and fourth year of the trials. We did not observe any differences in this agronomic trait between GD743 and GS784 events and their corresponding controls.

6.3 Pest and Disease Characteristics

OSF evaluated how GD743 and GS784 events performed in the field with respect to control fruit. Pest and disease characteristics were monitored, and data was collected that would help to analyze if these events were less, equal or more susceptible to pest and diseases than control fruit. As described in section 6.1, OSF engaged a horticulturalist to monitor both field trials and to report incidents of pest and disease and the related data associated with these incidents. Figure 10 illustrates typical field trial monitoring over the growing season. From this we can see tree growth stage, degree day accumulation, monitoring frequency, incident reporting and field interventions over the April through October growing season. Appendix 3 provides data specific to relevant pest and disease incidents as these occurred in the two field trials over the multiple years of evaluation. These appendices track incidents by pest and summarize the data collected, provide an incident summary and the interpretation and statistics on this data used to establish if there was a significant variance between the GD743 and GS784 events and the control cultivars.

Pest and disease data referred to in Section 6.3 can be found in Appendix 3.

Statistical calculations used the Chi-Square test of independence. Yates' correction for continuity is employed where the frequency is less than 5 in at least one of the cells. The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level.

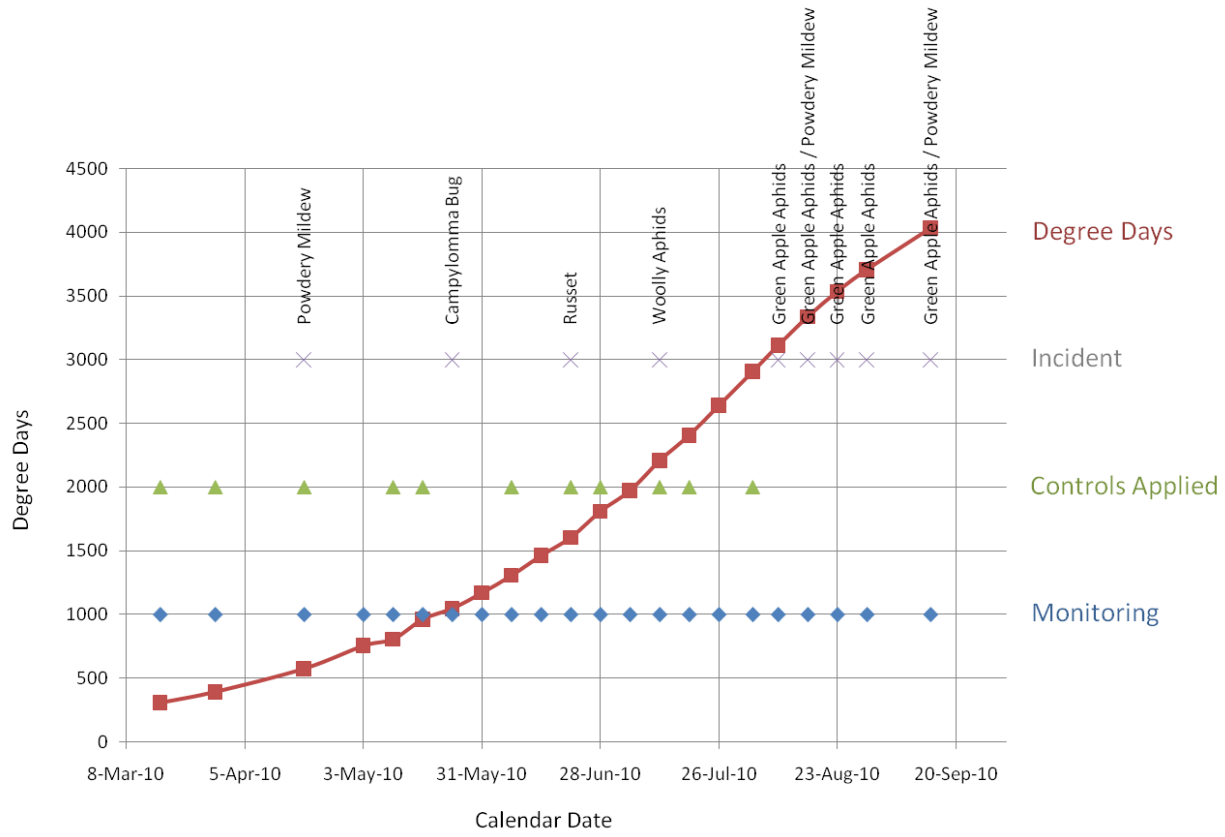


Figure 10: Pest and Disease Monitoring (Washington 2010)
This figure is a pictorial overview of the pest and disease monitoring at the Washington field trial for the 2010 growing season. The figure is described in more detail in Section 6.3 Pest and Disease Characteristics.

6.3.1 Scab (*Venturia inaequalis)*

This fungal disease infects both apple fruit and leaves. It is a serious pest of concern to commercial apple growers particularly in wet and humid areas, less so in drier areas such as Washington State.

This disease was monitored in both the Washington and New York field trials in each year of the trials. No incidences of this disease were detected in GD743, GS784 or controls, nor were any infection sites observed. This result was probably the consequence of a normal commercial scab spray-control program. Controlled inoculations without chemical control were not carried out.

Typically, apple cultivars that are sensitive to Powdery Mildew, such as Golden Delicious and Granny Smith are sprayed with fungicide every two weeks. As a consequence, scab is controlled on these cultivars. Consistent with this, very few instances of scab have been reported from either field trial.

In 2006, scab lesions in the fruit were reported in the NY field trial, but there were very few trees or fruit affected. None of the trees were the subject of this petition. In NY there were no other

reported instances of scab between 2007 and 2011. The WA field trial has never reported an instance of scab.

6.3.2 Mildew (*Podosphara leucotricha)*

This fungal disease primarily infects apple leaves around blossom time; it can also infect fruit, which results in russeting of the skin that is apparent by harvest time. Powdery Mildew (PM) is a common disease of concern to commercial growers in both humid and drier growing areas. Appendix 3 (Table 53, Table 54 and Table 55) summarize PM incidents in both field trials.

The Washington Field trial site reported incidences of PM almost every year from 2004 to 2010. Representative data is presented from the WA2008 field block from the spring (Table 53) and fall (Table 55) of 2010. Data is reported as mildew infested shoots in 10 shoots of each tree monitored. The New York field trial reported only a single incidence of PM in 2008 (Table 54). PM was very light affecting only GS events. In New York, PM infection was assessed according to a rating scale where 1 < 10%, 2 = 10 – 50%, and 3 > 50%. The percentages refer to the percent (%) of shoot tips colonized by PM. Since the rate of infection was similar (<10%) in the PM infected trees, the number of trees infected is reported.

In the spring of 2010, PM was present in the WA2008 block (Table 53). PM appeared to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (7.5%) than Granny Smith (GS + GS784) (3.5%). However, Chi-square test revealed that the percentage of PM affected shoots did not significantly differ by cultivar (X^2(1, N=400) =3.08, P>0.05). PM infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GD743 and GD (X^2(1, N=200) =0.65, P>0.3). Similarly, Chi-square test (Yates) revealed that the percentage of PM infected shoots did not significantly differ between GS784 and GS (X^2(1, N=200) =0.59, P>0.03).

In the fall of 2010, PM was present in the WA2008 block (Table 55). PM did not appear to be cultivar specific. Chi-square test revealed that the percentage of PM affected shoots did not significantly differ by cultivar (X^2(1, N=400) =0.23, P>0.5). PM infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GD743 and GD (X^2(1, N=200) =2.61, P>0.1). Similarly, Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GS784 and GS (X^2(1, N=200) =3.03, P>0.05).

In the summer of 2008, PM was present in the NY field trial block (Table 54). PM was very light affecting only GS events. However, Chi-square (Yates) test revealed that the percentage of PM affected shoots did not significantly differ by cultivar (X^2(1, N=22) =0.37, P>0.5). PM infection was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly unaffected by PM. Similarly, Chi-square (Yates) test revealed that the percentage of PM infected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0.6, P>0.3).

6.3.3 Fireblight (*Erwinia amylovora*)

Fireblight (FB) is a contagious disease which affects apples, pears, and other members of the Rosaceae family. FB is of concern in all apple growing regions of North America, because under

optimal conditions, it can destroy entire orchards in a single growing season.

FB and FB management has been reviewed extensively. In brief, *Erwinia amylovora* can overwinter only in the blight strikes (cankers) from the previous season. Most of the bacteria in these cankers will die, but a portion of them may reactivate around blossom time allowing the infection to spread to other parts of the orchard. If the temperature is warm (greater than 70^{o}F) cankers will ooze bacteria to their surface. From here, bacteria are spread by insects that feed on the ooze followed by the nectar of apple. If the weather remains warm, bacterial growth will continue and can be spread by water into the flower's nectary. If successful at attacking the fruitlet, the bacteria will travel through the phloem of the plant into sensitive tissue (tender parts of the plant), killing nearby host tree structures. Symptoms of fireblight will be easily visible 5 – 14 days after infection (Smith, 2010).

With respect to a PPO inhibited apple and the concern that a FB infected tree might be less visible, our Washington field manager stated the following:

"We see first, limp leaves that are olive color (not the right color of green). These will ooze, and then start to crook (wilt). They will continue to wilt, then dry, (look as if touched by an acetylene torch), and the fruit will pygmy up (shrivel)." (Field Manager – Washington Field Trial)

Symptoms of FB are distinct and clearly visible and have been detected in both PPO inhibited GEN-03 trees, and their respective control trees.

OSF monitored for FB at both trial sites. In Washington there were 12 trees from the WA2003 and WA2004 block affected by FB (Table 26). Two of these were in trees of GD743, while a further six were in trees of related, PPO-suppressed, GEN-03 x GD events (702, 703, 705, and 707). In addition to the two GD743 trees affected by FB, OSF offers these trees to augment the fireblight dataset. These latter events were established as moderately (702, 703) or highly (705, 707) PPO-suppressed.

There were no incidences of FB reported for the New York trial.

Table 26: Record of Fireblight Incidences in OSF's Washington Field Trial

Plant Name[1]	Vector Name	Cultivar	Rootstock	Year Planted	Fireblight	Date
743-19	GEN-03	GD	M9	2003	FB Hits Detected	May 30, 2005
1000-1	Control	GD	M9	2003	FB Hits Detected	May 30, 2005
707-7	GEN-03	GD	M9	2003	Shoot Tip Infection (ooze)	July 1, 2006
743-41	GEN-03	GD	M9	2003	FB (Tree Removed)	September 25, 2007
705-24	GEN-03	GD	M9	2003	FB (Tree Removed)	September 25, 2007
705-42	GEN-03	GD	M9	2003	FB (Tree Removed)	September 25, 2007
1003-3	Control	PG	M9	2004	FB Hits Detected	May 30, 2005
707-17	GEN-03	GD	M9	2004	FB Hits Detected	June 11, 2006
872-13	GEN-03	NF	M9	2004	FB (Tree Removed)	September 25, 2007
707-17	GEN-03	GD	M9	2004	FB (Tree Removed)	September 25, 2007
702-35	GEN-03	GD	M9	2004	FB (Tree Removed)	September 25, 2007
1000-33	Control	GD	M9	2004	FB (Tree Removed)	September 25, 2007
703-48	GEN-03	GD	M9	2004	FB (Tree Removed)	September 25, 2007

[1] Notably, in addition to GD743 and GS784, there are other GEN-03 events in the Washington field trial are referred to in this petition in the context of fireblight susceptibility. The additional events are GD702, GD703, GD705, and GD707. These events were established as moderately (702, 703) or highly (705, 707) PPO-suppressed and yield low-browning or non-browning fruit, respectively.

FB was detected in 2005, 2006 and 2007 in trees from the WA2003 and WA2004 field blocks (Table 56). FB was detected in at a higher rate in Golden Delicious (GD702, GD703, GD705, GD707, GD743 and GD) (4% of trees) than in Granny Smith (GS784 and GS) (0% of trees) However, Chi-square (Yates) test revealed that the percentage of FB affected trees did not significantly differ by cultivar ($X^2(1, N=327) =1.61, P>0.2$). FB was not influenced by incorporation of the GEN-03. Chi-square (Yates) test revealed that the percentage of FB affected trees did not significantly differ between the PPO suppressed GEN-03 events (GD702, GD703, GD705, GD707 and GD743) and the non-transgenic control cultivar GD ($X^2(1, N=258) =0.34$ $P>0.5$). Both GS784 and GS were similarly unaffected by fireblight.

6.3.4 Aphids: Green Apple Aphid *(Aphis pomi)*, Woolly Apple Aphid *(Eriosoma lanigerum)*, Rosy Apple Aphid *(Dysaphis plantaginea)*

Aphids are sucking insects that feed on tender new growth; they are a concern in commercial orchards from time to time, and are a noteworthy pest in fruit tree nurseries. Appendix 3 (Table 57, Table 58, Table 59 and Table 60) provide details with respect to aphid incidents.

The Washington Field trial site reported incidences of aphids, Green Apple Aphid (GAA) or Woolly Aphid, most every year from 2004 to 2010. Representative data is presented from the WA2004 field block for GAA (Table 58 and Table 59) and Woolly Aphid (Table 60) in 2010.

Data is reported as the number of aphid infested shoots in 10 upper portion of tree, rapidly growing shoots collected from each tree.

The New York field trial has reported only a single incidence of aphids in 2009 (Table 57). Aphid infection was assessed according to a rating scale where 1 < 10%, 2 = 10 – 50%, and 3 > 50%. The percentages refer to the percent (%) of shoot tips colonized by aphids. Aphid infection was rated at > 50% in 21/22 trees assessed (Arctic™ Apple and Controls). Therefore, the number of trees infected is reported.

Woolly Aphid was identified in the WA2004 field trial block in 2010 (Table 60). The populations were relatively low. Woolly Aphid appeared to be cultivar specific with a higher rate of incidence in Golden Delicious (GD + GD743) (11.3%) than in Granny Smith (GS + GS784) (1.4%). Chi-square (Yates) test revealed that the percentage of Woolly Aphid affected shoots significantly differed by cultivar ($X^2(1, N=150) =4.32, P<0.05$). Woolly aphid infection was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Woolly Aphid infected shoots did not significantly differ between GD743 and GD ($X^2(1, N=80) =0, P>0.95$). Similarly, Chi-square (Yates) test revealed that the percentage of Woolly Aphid infected shoots did not significantly differ between GS784 and GS ($X^2(1, N=70) =0.02, P>0.8$).

Green Apple Aphid (GAA) was reported on August 9, 2010 (Table 58) and again on August 16, 2010 (Table 59). GAA infected primarily the WA2004 block.

On August 9, GAA appeared to be cultivar specific with a higher rate of GAA incidence in Golden Delicious (GD + GD743) (22.5%) than in Granny Smith (GS + GS784) (14.3%) (Table 58). However, Chi-square test revealed that the percentage of GAA affected shoots did not significantly differ by cultivar ($X^2(1, N=150) =2.14, P>0.1$). GAA infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of GAA infected shoots did not significantly differ between GD743 and GD ($X^2(1, N=80) =0.62, P>0.3$). Similarly, Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GS784 and GS ($X^2(1, N=70) =0.70, P>0.3$).

On August 16, 2010, GAA was not cultivar specific with a similar rate of GAA incidence in Golden Delicious (GD + GD743) (6.3%) and Granny Smith (GS + GS784) (4.3%) (Table 59). Chi-square (Yates) test revealed that the percentage of GAA affected shoots did not significantly differ by cultivar ($X^2(1, N=150) =0.03, P>0.8$). GAA infection was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GD743 and GD ($X^2(1, N=80) =0, P>0.95$). Similarly, Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GS784 and GS ($X^2(1, N=70) =0.88, P>0.3$).

Aphids were present throughout the NY field trial in the fall of 2009 (Table 57). Aphid infection was rated at > 50% in 21/22 trees assessed (Arctic™ Apple and Control). Since the rate of infection was similar (>50%) in the infected trees, the number of trees infected was compared. Since the rate of GAA infection was 100% in all cultivars (GD743, GD, GS784 and GS) it is indicated that GAA infection was not influenced by cultivar or PPO suppression.

6.3.5 Mites: McDaniel Spider Mite *(Tetranychus McDanieli)*, Two-spotted Spider Mite *(Tetranychus urtica)*, Apple Rust Mite *(Aculus schlechtendal)*

Mites can sometimes be a significant pest of apples, more commonly in dry regions than wet ones. Infestations are often the result of pesticides disrupting the balance between the pest mites and their predators.

OSF monitored mite infestation at both trial sites. Only a single mite incident was recorded and this was in the NY trial in fall 2009. No trees among the events or controls covered by this petition were involved. No differences in the incidence of this arachnid were detected between GD743 and GS784 events and controls, and few mites were apparent. This result was probably the consequence of normal commercial chemical-control measures. Controlled challenges without chemical control were not carried out.

6.3.6 Japanese Beetle *(Popillia japonica)*

This newly-emerging apple insect pest was monitored in the New York State field trial but not in Washington State. The New York field trial site reported incidences of Japanese Beetle (JB) in 2008 and 2010 (See Appendix 3, Table 61 and Table 62). JB monitoring involves an inspection of each leaf looking for the presence of Japanese Beetles and Leaf Skeletonization (LS). LS is assessed as 1 = light (1-5%), 2 = moderate (6-10%) and 3 = severe (>10%). The rate of infection was similar (light) in the infected trees (data not shown). Therefore, the number of trees infected is reported.

In 2008, JB was not cultivar specific with a similar level of incidence in Golden Delicious (GD + GD743) (30%) and Granny Smith (GS + GS784) (25%) (Table 61). Chi-square (Yates) test revealed that the percentage of JB affected trees did not significantly differ by cultivar ($X^2(1, N=22) =0.05$, $P>0.8$). JB was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GD743 and GD ($X^2(1, N=10) =0$, $P>0.95$). Similarly, Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0$, $P>0.95$).

In 2010, JB appeared to be cultivar specific with lower level of incidence in Golden Delicious (GD + GD743) (0%) than in Granny Smith (GS + GS784) (17%) (Table 62). However, Chi-square (Yates) test revealed that the percentage of JB affected trees did not significantly differ by cultivar ($X^2(1, N=22) =0.37$, $P>0.5$). JB was not influenced by incorporation of the GEN-03 transgene. JB was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly unaffected by JB. Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0.6$, $P>0.3$).

6.3.7 Codling Moth *(Lapeyresia pomonella)*

This lepidopteron fruit worm is a key insect pest of apple and can attain very high infestation rates, especially in dry areas such as the Washington state trial region.

The Washington trial pest monitoring protocol included pheromone traps to monitor emergence and populations. In addition, fruit was examined for damage caused by the insect's burrowing. In

each year of the Washington field trial, codling moth was persistent throughout the growing season, with trap counts high enough to invoke control applications. Despite this, few incidences of codling moth were reported. These rarely involved trees of GS784, and never involved trees of GD743. These incidences are summarized below.

In the fall of 2007, the WA2004 block reported low level codling moth damage. Trees of GD743, GS784 or their respective controls were not involved. At the same time, the WA2003 block field trial also reported low level codling moth damage. There was a single sting reported on GS784-3 (GS784, tree # 3), and none on any of the corresponding GS control trees.

In the fall of 2008, the WA2004 block reported low level codling moth damage. Included in the report were a single sting on GS784-60, and three stings on a GS control tree (1001-15). Codling moth was not detected in the WA2003 block.

In the fall of 2011, WA2004 low level of codling moth damage, not involving trees of GD743, GS784 or their respective controls. No codling moth damage was detected in WA2008.

No differences in the incidence of this insect were detected between GD743 and GS784 events and controls, and few fruit entry points were apparent. This result was probably the consequence of normal commercial chemical-control measures. Controlled challenges without chemical control were not carried out.

In New York, there was no evidence of codling moth damage in the 2010 – 2011 growing season, despite the continual presence of codling moth in New York (Scaffolds, 2011). Codling moth damage was not seen in previous years either. Normal protectant schedule kept the codling moth at bay, and therefore there was no unusual susceptibility observed.

6.3.8 Tentiform Leaf Miner *(Phyllonorycter blancardella)*

Introduced from Europe, this insect pest is now found in most of the Midwestern and northwestern USA and eastern Canada. The larvae mine between the layers of leaves, thereby reducing the photosynthetic area. Heavy infestations may affect fruit sizing, and may result in reduced vegetative growth and/or premature fruit drop which can affect winter hardiness.

OSF monitored for Tentiform Leaf Miner (TLM) each year. The Washington field trial site reported a single incidence of Tentiform Leaf Miner in 2005 (see Appendix 3, Table 63). Data is reported as TLM infected leaves in 25 leaves.

TLM was not cultivar specific. Chi-square test revealed that the percentage of TLM affected trees did not significantly differ by cultivar ($X^2(1, N=250) =0.70, P>0.3$). TLM was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of TLM infected leaves did not significantly differ between GD743 and GD ($X^2(1, N=100) =0.03, P>=0.8$). Conversely, Chi-square test revealed that the percentage of infected leaves significantly differed between GS784 and GS ($X^2(1, N=150) =5.33, P<0.05$), with a higher incidence of TLM in GS784 (21%) than in GS (8%).

6.3.9 Burr Knot

Apple "Burr Knot" occurs where adventitious roots are trying to develop. A few apple varieties appear particularly prone to the development of the burr knots, as do some rootstocks such as MM111. No one knows exactly what causes it although it has been associated with woolly aphid feeding injury. There is no control (Turner, 2005). Burr Knot was identified throughout the NY field trial in 2006 (see Appendix 3, Table 64). The presence (+) or absence (-) of Burr Knot is assessed. The number of Burr Knot affected trees is reported.

Burr Knot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (70%) than Granny Smith (GS + GS784) (25%). However, Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ by cultivar ($X^2(1, N=22) =2.83, P>0.05$). Burr Knot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ between GD743 and GD ($X^2(1, N=10) =1.91, P>0.1$). Similarly, Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0, P>0.95$).

In the WA field trial, Burr Knot was considered a normal condition, not associated with disease, and it therefore was not reported. Burr Knot occurs when the rootstock is planted a little high, and is most often associated with rootstocks that are sensitive to the condition, such as Malling 9 (M9) and Malling 26 (M26) (OMAFRA, 2011).

6.3.10 Leaf Spot

Leaf Spot was identified throughout the NY field trial in 2006 (See Appendix 3, Table 65). Data is reported as trees affected (+) or not affected (-) with Leaf Spot. The incidences of Leaf Spot never resulted in severe symptoms and were difficult to accurately diagnose. The majority are probably due to *Alternaria mali* infections, although a small proportion may be due to black rot (*Botryosphaeria obtusa*) infections (Jones and Aldwinckle, 1990).

Leaf Spot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (100%) than Granny Smith (GS + GS784) (17%). Chi-square (Yates) test revealed that the percentage of Leaf Spot affected trees significantly differed by cultivar ($X^2(1, N=22) =12.10, P<0.001$). Leaf Spot was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly affected by Leaf Spot (100% in both). Similarly, Chi-square (Yates) test revealed that the percentage of Leaf Spot affected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0.60, P>0.3$).

Leaf Spot due to fungal infection was not observed in the WA field trial. Leaf Spot, as described at our WA field trial is not associated with fungal infection, but is generally associated with aphid damage, or as a result of herbicide drift. In WA, Leaf Spot is not considered a condition of importance.

6.3.11 Russet

Russeting on apples is a particular type of skin, slightly rough, usually with a greenish-brown to yellowish-brown color. The amount of russeting can be affected by various factors including,

weather, disease or pest damage and agrochemical applications (*e.g.*, insecticides, fungicides and growth regulators). Most fruit russetting is the consequence of injury to rapidly dividing epidermal cells early in fruit development (Teviotdale *et al.*, 1997).

The monitoring and reporting of russet is different in the New York and Washington field trials. In New York, russet is assessed while the apples are still on the trees. Therefore, the number of russet-affected trees is reported for NY. In Washington, russet is assessed after the fruit is harvested. Russet is assessed on a fruit by fruit basis. Therefore, the number of russet-affected fruit is reported in WA.

In NY in 2009, russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (80%) than Granny Smith (GS + GS784) (8%) (Table 66). Chi-square (Yates) test revealed that the percentage of russet affected trees significantly differed by cultivar ($X^2(1, N=22) =8.81, P<0.01$). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GD743 and GD ($X^2(1, N=10) =0.63, P>0.3$). Similarly, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0, P>0.95$).

In the WA2004 block in 2010, russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (91%) than Granny Smith (GS + GS784) (23%) (Table 67). Chi-square test revealed that the percentage of russet affected fruit significantly differed by cultivar ($X^2(1, N=150) = 72.37, P<0.001$). Russet was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected fruit significantly differed between GD743 and GD ($X^2(1, N=80) =5.64, P<0.05$) with a higher incidence of russet in GD (100%) than GD743 (82.5%) fruit. Conversely, Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GS784 and GS ($X^2(1, N=70) =1.14, P>0.2$).

In the WA2008 block in 2010, russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (86%) than Granny Smith (GS + GS784) (20%) (Table 68). Chi-square test revealed that the percentage of russet affected fruit significantly differed by cultivar ($X^2(1, N=323) =139.3, P<0.001$). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GD743 and GD ($X^2(1, N=197) =0.10, P>0.7$). Similarly, Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GS784 and GS ($X^2(1, N=126) =3.05, P>0.05$).

In NY in 2010, russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD7443) (70%) than Granny Smith (GS + GS784) (8%) (Table 69). Chi-square test (with Yates correction) revealed that the percentage of russet affected trees significantly differed by cultivar ($X^2(1, N=22) =6.50, P<0.05$). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GD743 and GD ($X^2(1, N=10) =0, P>0.95$). Similarly, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0, P>0.95$).

6.3.12 Campylomma (Mullein Bug) *Campylomma verbasci* (Meyer)

Campylomma was identified in the WA field trial in 2010 (see Appendix 3, Table 70 and Table 71). Data is reported as Campylomma affected fruit in 10 fruit.

Within the WA2004 tested fruit, Campylomma appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (16%) than Granny Smith (GS + GS784) (4%) (Table 70). Chi-square (Yates) test revealed that the percentage of Campylomma affected fruit significantly differed by cultivar ($X^2(1, N=150) =4.42, P<0.05$). Campylomma Bug was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit differed significantly between GD743 and GD ($X^2(1, N=80) =5.88, P<0.05$) with a higher incidence of Campylomma in GD (38%) than GD743 (5%). Conversely, Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit did not significantly differ between GS784 and GS ($X^2(1, N=70) =2.10, P>0.1$), although there was a higher incidence of Campylomma in GS (10%) than GS784 (0%).

Within the WA2008 tested fruit, Campylomma was not cultivar specific (Table 71). Chi-square (Yates) test revealed that the percentage of Campylomma affected fruit did not significantly differ by cultivar ($X^2(1, N=400) =3.24, P>0.05$). Campylomma Bug was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit did not significantly differ between GD743 and GD ($X^2(1, N=200) =3.28, P>0.05$). Both GS784 and GS were similarly unaffected by Campylomma.

Campylomma Bug was not detected in the NY field trial.

6.3.13 Fruit Rot after Storage

Fruit rot after storage was monitored in 2008 NY fruit and in 2009 NY fruit (see Appendix 3, Table 72 and Table 73). Fruit rot after storage was reported as the number of fruit showing tan or black rot and the total number of fruit examined. Black rot is caused by *Botryosphaeria obtuse*. Tan rot is probably mostly caused by *Colletotrichum* spp. However, we didn't culture from any of these fruits, so we can't be 100% certain what caused each rot.

In 2008, Tan Rot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (20%) than Granny Smith (GS + GS784) (2%) (Table 72). Chi-square test revealed that the percentage of Tan Rot affected fruit significantly differed by cultivar ($X^2(1, N=469) =46.19, P<0.001$). Tan Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GD743 and GD ($X^2(1, N=156) =0.13, P>0.7$). Similarly, Chi-square (Yates) test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GS784 and GS ($X^2(1, N=313) =3.44, P>0.05$).

In 2008, Black Rot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (9%) than Granny Smith (GS + GS784) (1%) (Table 72). Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit significantly differed by cultivar ($X^2(1, N=469) =19.50, P<0.001$). Black Rot was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of

Tan Rot affected fruit differed significantly between GD743 and GD (X^2(1, N=156) =7.51, P<0.01) with higher incidence of Black Rot in GD (15%) than in GD743 (1%). Conversely, Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not significantly differ between GS784 and GS (X^2(1, N=313) =0.35, P>0.5).

In 2009, Tan Rot did not appear to be cultivar specific with a similar rate of incidence in Golden Delicious (GD + GD743) (3%) than Granny Smith (GS + GS784) (3%) (Table 73). Chi-square test revealed that the percentage of Tan Rot affected fruit did not significantly differ by cultivar (X^2(1, N=372) =0.04, P>0.8). Tan Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GD743 and GD (X^2(1, N=176) =0.17, P>0.5). Similarly, Chi-square (Yates) test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GS784 and GS (X^2(1, N=196) =3.35, P>0.05).

In 2009, Black Rot appears to be cultivar specific with a higher rate of incidence in Golden Delicious (GD + GD743) (2.3%) than Granny Smith (GS + GS784) (0%) (Table 73). However, Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not significantly differ by cultivar (X^2(1, N=372) =2.62, P>0.1). Black Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not significantly differ between GD743 and GD (X^2(1, N=176) =0.26, P>0.5). Both GS784 and GS were similarly unaffected by Black Rot in 2009 fruit after storage.

6.3.14 Pest and Disease Summary

In a commercial apple orchard there is constant pressure from codling moth and scab. However, there is a also zero tolerance for the damage caused by these pests and diseases and so they are highly managed. As a consequence, there are very few incidences of codling moth and scab reported from either field trial. Notably, management of codling moth and scab at both field trial locations was consistent with normal commercial production of Golden Delicious and Granny Smith cultivars. Normal management procedures were sufficient to prevent these pests and diseases in GD743 and GS784 and no additional control measures were required. This indicates that GS743 and GS784 are not significantly more susceptible to codling moth or scab, under conditions of a managed orchard.

Some pests and diseases, including aphids and powdery mildew, tend to be endemic. Damage caused by these organisms is tolerable, at a low level. Controls are routinely applied, but these pests and diseases may persist. In the Washington state field trial, aphids and powdery mildew were reported in almost every year of the trial. A few datasets are reported here as examples, and these datasets show clearly that there is no enhanced susceptibility to aphids or mildew associated with the Arctic™ Apple trait.

Other pests, diseases and affections of apple, such as Campylomma, Japanese Beatle, Tentiform leaf miner, Fireblight, burr knot, leaf spot and russet occur sporadically when the field conditions are right.

A summary of effects identified following from Chi Square statistical analysis of pest and disease data is summarized in Table 27.

Golden Delicious is well known for its tendency to russet. Data collected from our Washington and New York field trials is consistent with the known cultivar sensitivity of Golden Delicious to russet, relative to the russet resistant Granny Smith. Additionally, Golden Delicious has been shown to be more sensitive than Granny Smith to other pests and diseases, including Campylomma (Cockfield and Beers, 2004). In 2010, the WA2004 field trial reported significantly more Campylomma in Golden Delicious than in Granny Smith. In that same year, the WA2008 field block also recorded more Campylomma in GD, although the effect was not significant. These observations show that the field trials are capable of identifying known cultivar-specific issues.

If PPO were involved in pest and disease resistance, through a role in the hypersensitivity response, then one might expect a systematic increase in the sensitivity of PPO suppressed GD743 and GS784 to a wide variety of agents. In fact, we do not observe such a change in phenotype in our field observation of GD743 and GS784. Indeed, there are only four instances in which a statistically significant difference was detected between the Arctic™ Apple cultivar and its respective control and in three of these cases (black rot, Campylomma and russet) there was a lower rate of incidence of the particular pest / disease in the GD743 event than in GD.

There was only one case in which we found an increased susceptibility to pest or disease in an Arctic™ Apple cultivar. In 2005, there was an increased incidence of Tentiform Leaf Miner (TLM) in GS784 compared to GS. The same effect was not seen in GD743 versus GD, which suggests there is no systematic increase in susceptibility to TLM associated with the Arctic™ Apple trait. There could perhaps be a trait x cultivar effect specifically increasing TLM susceptibility in Granny Smith. However, the Washington field trial blocks have been observed for the presence of TLM each year since planting with relatively little TLM detected GD743m GS784 or their respective controls. TLM has been of little consequence.

Table 27: Effect of Cultivar or Arctic™ Apple Trait on Pest and Disease Resistance

Bacterial Disease							
Disease[1]	**Test Block**	**Date**	**Table**	**Cultivar**		**Arctic™ Apple Trait**	
				Effect Present	**Type of Effect[2]**	**Effect Present**	**Type of Effect**
FB	WA2003 WA2004	All Years	Table 56	No		No	
Fungal Disease							
Disease[1]	**Test Block**	**Date**	**Table**	**Cultivar**		**Arctic™ Apple Trait**	
				Effect Present	**Type of Effect[2]**	**Effect Present**	**Type of Effect**
PM	WA2008	April 19. 2010	Table 53	No		No	
	WA2008	August 16, 2010	Table 55	No		No	
	NY2005	July 21, 2008	Table 54	No		No	
Leaf Spot	NY2005	August 24, 2006	Table 65	Yes	GD > GS	No	
Tan Rot	NY2005	June 30, 2009	Table 72	Yes	GD > GS	No	
	NY2005	December 30, 2010	Table 73	No		No	
Black Rot	NY2005	June 30, 2009	Table 72	Yes	GD > GS	Yes	GD > GD743
	NY2005	December 30, 2010	Table 73	No		No	
Insect Pest							
Pest	**Test Block**	**Date**	**Table**	**Cultivar**		**Arctic™ Apple Trait**	
				Effect Present	**Type of Effect[2]**	**Effect Present**	**Type of Effect**
Aphids	NY2005	November 19. 2009	Table 57	No		No	
GAA	WA2004	August 9, 2010	Table 58	No		No	
GAA	WA2004	August 16, 2010	Table 59	No		No	
Woolly Aphids	WA2004	July 12, 2010	Table 60	Yes	GD > GS	No	
JB	NY2005	July 21, 2008	Table 61	No		No	
JB	NY2005	July 21, 2010	Table 62	No		No	
Campylomma	WA2004	May 24, 2010	Table 70	Yes	GD > GS	Yes	GD > GD743
Campylomma	WA2008	May 24, 2010	Table 71	No		No	
TLM	WA2004	August 23, 2005	Table 63	No		Yes	GS784 > GS
Other Conditions							
Condition	**Test Block**	**Date**	**Table**	**Cultivar**		**Arctic™ Apple Trait**	
				Effect Present	**Type of Effect[2]**	**Effect Present**	**Type of Effect**
Burr Knot	NY2005	August 26, 2006	Table 64	No		No	
Russet	NY2005	November 19, 2009	Table 66	Yes	GD > GS	No	
Russet	WA2004	June 21, 2010	Table 67	Yes	GD > GS	Yes	GD > GD743
Russet	WA2008	June 21, 2010	Table 68	Yes	GD > GS	No	
Russet	NY2005	July 21, 2010	Table 69	Yes	GD > GS	No	

[1] FB = Fire Blight; TLM = Tentiform Leaf Miner; PM = Powdery Mildew; GAA = Green Apple Aphid; JB = Japanese Beatle

[2] Type of Effect - Where the incidence of the named pest or disease is higher in GD than in GS.

6.4 Nutrition and Compositional Analysis

The main nutrients in apple are sugar, dietary fiber, potassium, phenolic antioxidants and, to a lesser extent, vitamin C. To establish that the new cultivars are nutritionally equivalent to their parent cultivars, apples from events GD743 and GS784 and the control Golden Delicious (GD) and Granny Smith (GS) were subjected to nutritional and proximate analysis, and measured for total phenolic and water-soluble oxygen radical absorbance capacity (ORAC). The results of this study were also compared to the published data for apple (NDB09003) provided by the USDA.

The USDA nutrient values for apples, raw with skin (NDB09003) are based on data for Red Delicious, Golden Delicious, Gala, Granny Smith, and Fuji cultivars of apple. These are the five most popular apple cultivars in the US, representing almost 70% of US production (Table 46). Data is compiled from a variety of sources (USDA, 2009). It is not possible from the data provided, to determine the specific growing region the apples are from, or any specifics regarding the individual apple samples or the contribution of the different apple cultivars to the final values provided by the USDA. It is obvious however, that only a limited number of apple samples are included in the final numbers provided. As such, this data provides an approximation of nutrient composition that might be expected in the most commonly consumed apple cultivars grown under a variety of conditions.

Mature fruit was harvested in the fall of 2009 from the Washington and New York field trials. For each event (GD743 and GS784) and control (GD and GS), fruit was harvested from 3 trees in Washington and 3 trees in New York (n = 6). Golden Delicious apples were harvested approximately one month prior to Granny Smith in both Washington and New York, and were stored at 2^{o}C. Immediately after the Granny Smith harvest, all apples were sampled and sent for proximate and phenolic analysis. Composite samples were created by combining one-quarter slices from four apples from one tree, providing, in total, one whole apple equivalent. Samples were cut, cored and placed in a Ziploc^{TM} bag. The samples were packed in a cooler on wet ice and sent overnight to Exova for the proximate analysis and Brunswick Laboratories for the ORAC and total phenolics analysis. One sample of GD743 from New York was discarded because the sample was mislabeled.

Results of the composite analysis of fruit from New York (Table 28) and Washington (Table 29) are presented. A detailed discussion of the data for proximates and phenolic antioxidants follows.

Table 28: Apple Fruit Compositional Analysis - New York

Description	Units	GD743	GD	GS784	GS	Apple (NDB09003)		
						Average	Min	Max
Fat	%	ND	ND	ND	ND	0.17	0.05	0.31
Protein	%	0.35	0.23	0.40	0.33	0.26	0.17	0.57
Moisture	%	81.85	82.60	83.47	83.10	85.56	82.4	87.5
Ash	%	0.20	0.23	0.20	0.23	0.19	0.07	0.48
Carbohydrates	%	17.60	16.93	15.93	16.33	13.81		
Calories	cal / 100 g	72	69	65	67	52		
Sugar Profile	%	12.85	11.14	10.67	10.27	10.39	8.77	12.0
Dietary Fiber	%	2.4	2.6	2.9	2.8	2.4	1.4	3.5
Potassium	mg / 100 g	90	89	96	105	107	88	136
Vitamin C	mg / 100 g	14.1	ND	9.7	ND	4.6	4	5.5
ORAC	µmol TE/100g	3000	567	3133	967	3056	1661	4811
Phenolics	mg GAE/100g	209	70	241	103	262	165	396
Number of Composite Samples =		n = 2	n = 3	n = 3	n = 3			

ND = None Detected (Method Reporting Limit: Fat = 0.1%)

Proximate analysis was completed by Exova (Portland, OR) per AOAC, AACC, APHA/AWWA, ASTM, BAM, PAM, USDA, EPA or other testing procedures as deemed applicable. Proximates for NDB09003: USDA National Nutrient Database for Standard Reference – Release 22 (USDA, 2009), Prepared by Nutrient Data Laboratory, Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA)

ORAC and total phenolics was completed by Brunswick Laboratories (Norton, MA). The acceptable precision is <15% relative standard deviation. The ORAC is expressed as micromole Trolox equivalency (µmol TE). The phenolic result is expressed as milligram gallic acid equivalency. Antioxidant capacity and phenolics for NDB09033: Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods 2007, Prepared by Nutrient Data Laboratory, Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA).

Table 29: Apple Fruit Compositional Analysis - Washington

Description	Units	GD743	GD	GS784	GS	Apple (NDB09003)		
						Average	Min	Max
Fat	%	ND	ND	ND	ND	0.17	0.05	0.31
Protein	%	0.20	0.30	0.30	0.30	0.26	0.17	0.57
Moisture	%	84.17	84.30	85.07	85.10	85.56	82.4	87.5
Ash	%	0.30	0.30	0.30	0.37	0.19	0.07	0.48
Carbohydrates	%	15.33	15.10	14.33	14.23	13.81		
Calories	cal / 100 g	62	62	58	58	52		
Sugar Profile	%	10.90	11.50	8.53	9.45	10.39	8.77	12.0
Dietary Fiber	%	2.2	2.4	2.7	2.5	2.4	1.4	3.5
Potassium	mg / 100 g	97	96	111	110	107	88	136
Vitamin C	mg / 100 g	2.6	1.4	2.5	1.4	4.6	4	5.5
ORAC	µmol TE/100g	1700	757	1800	667	3056	1661	4811
Phenolics	mg GAE/100g	155	80	147	78	262	165	396
Number of Composite Samples =		n = 3	n = 3	n = 3	n = 3			

ND = None Detected (Method Reporting Limit: Fat = 0.1%)

Proximate analysis was completed by Exova (Portland, OR) per AOAC, AACC, APHA/AWWA, ASTM, BAM, PAM, USDA, EPA or other testing procedures as deemed applicable. Proximates for NDB09003: USDA National Nutrient Database for Standard Reference – Release 22 (USDA, 2009), Prepared by Nutrient Data Laboratory, Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA)

ORAC and total phenolics was completed by Brunswick Laboratories (Norton, MA). The acceptable precision is <15% relative standard deviation. The ORAC is expressed as micromole Trolox equivalency (µmol TE). The phenolic result is expressed as milligram gallic acid equivalency. Antioxidant capacity and phenolics for NDB09033: Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods 2007, Prepared by Nutrient Data Laboratory, Beltsville Human Nutrition Research Center (BHNRC), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA).

Proximate Data

This section discusses proximate data which includes fat, protein, moisture, carbohydrates, calories, sugar, fiber and potassium. Proximate data for GD743, GD, GS784 and GS from both the NY and WA field trials falls within the range of, or closely approximates the published data for apple, raw with skin (NDB09003) provided by the USDA.

Statistical analysis reveals that there are significant effects of both cultivar and field trial for proximate data including protein, moisture, carbohydrate, calorie, sugar and fiber content of the apples. Granny Smith had higher water (Table 30), protein (Table 31) and fiber (Table 34) content, and correspondingly lower carbohydrate (Table 32), calorie (Table 33), and sugar (Table 35) content than Golden Delicious. Apples grown in Washington had higher moisture content

(Table 30), and correspondingly lower protein (Table 31), carbohydrate (Table 32), calories (Table 33), sugar (Table 35), and fiber (Table 34) content than apples grown in New York.

Granny Smith apples had significantly more potassium than Golden Delicious (Table 36).

With respect to proximates, there was no effect of the Arctic™ Apple trait on the composition of the apples. For both Washington and New York apples, statistical analysis found no significant differences in proximate between events GD743 or GS784 and their respective controls.

Table 30: Moisture% in GD743 and GS784

Vector	**Trial**	Moisture%		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	82.6	83.1	82.9
	WA	84.3	85.1	84.7
	Mean	83.5	84.1	83.8
GEN-03	NY	81.9	83.5	82.7
	WA	84.2	85.1	84.7
	Mean	83.1	84.3	83.7
Column Mean		83.3	84.2	83.7

Three-way ANOVA of average Moisture% was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 33.84$, P = 0.000, with the average moisture content being significantly higher for WA ($M = 84.7$, $S = 0.9$) than NY ($M = 82.8$, $S = 0.7$). There was a significant main effect for cultivar, $F(1,23) = 8.49$, P = 0.011, with the average moisture content being significantly higher for GS ($M = 84.2$, $S = 1.2$) than GD ($M = 83.4$, $S = 1.2$). The main effect of vector was not significant, $F(1,23) = 0.10$, P = 0.75.

Table 31: Protein% in GD743 and GS784

Vector	Trial	Protein%		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	0.23	0.33	0.28
	WA	0.30	0.30	0.30
	Mean	0.27	0.32	0.29
GEN-03	NY	0.35	0.40	0.38
	WA	0.20	0.30	0.25
	Mean	0.28	0.35	0.31
Column Mean		0.27	0.33	0.30

Three-way ANOVA of average Protein% was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 6.14$, P = 0.026, with the average protein content being significantly higher for NY ($M = 0.33$, $S = 0.08$) than WA ($M = 0.28$, $S = 0.06$). There was a significant main effect for cultivar, $F(1,23) = 10.20$, P = 0.006, with the average protein content being significantly higher for GS ($M = 0.33$, $S = 0.05$) than GD ($M = 0.26$, $S = 0.08$). The main effect of vector was not significant, $F(1,23) = 0.62$, P = 0.44.

Table 32: Carbohydrate% in GD743 and GS784

Vector	Trial	Carbohydrate%		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	16.9	16.3	16.6
	WA	15.1	14.2	14.7
	Mean	16.0	15.3	15.6
GEN-03	NY	17.6	15.9	16.8
	WA	15.3	14.3	14.8
	Mean	16.5	15.1	15.8
Column Mean		16.2	15.2	15.7

Three-way ANOVA of average Carbohydrate% was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 32.66$, P = 0.000, with the average carbohydrate content being significantly higher for NY ($M = 16.6$, $S = 0.8$) than WA ($M = 14.8$, $S = 0.9$). There was a significant main effect for cultivar, $F(1,23) = 9.28$, P = 0.008, with the average carbohydrate content being significantly higher for GD ($M = 16.1$, $S = 1.2$) than GS ($M = 15.2$, $S = 1.2$). The main effect of vector was not significant, $F(1,23) = 0.13$, P = 0.72.

Table 33: Calories per 100g in GD743 and GS784

Vector	Trial	Calories per 100g		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	68.7	67.0	67.9
	WA	61.7	58.0	59.9
	Mean	65.2	62.5	63.9
GEN-03	NY	71.5	65.3	68.4
	WA	62.0	58.3	60.2
	Mean	66.8	61.8	64.3
Column Mean		66.0	62.2	64.1

Three-way ANOVA of average Calories per 100g was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 33.91$, P = 0.000, with the average calorie content being significantly higher for NY ($M = 67.8$, $S = 3.0$) than WA ($M = 60.0$, $S = 3.8$). There was a significant main effect for cultivar, $F(1,23) = 7.38$, P = 0.016, with the average calorie content being significantly higher for GD ($M = 65.5$, $S = 4.9$) than GS ($M = 62.2$, $S = 5.2$). The main effect of vector was not significant, $F(1,23) = 0.06$, P = 0.81.

Table 34: Fiber% in GD743 and GS784

Vector	Trial	Fiber%		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	2.6	2.8	2.7
	WA	2.4	2.5	2.5
	Mean	2.5	2.7	2.6
GEN-03	NY	2.4	2.9	2.7
	WA	2.2	2.7	2.4
	Mean	2.3	2.8	2.5
Column Mean		2.4	2.7	2.6

Three-way ANOVA of average Fiber% was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 6.50$, P = 0.022, with the average fiber content being significantly higher for NY ($M = 2.7$, $S = 0.3$) than WA ($M = 2.4$, $S = 0.3$). There was a significant main effect for cultivar, $F(1,23) = 8.86$, P = 0.009, with the average fiber content being significantly higher for GS ($M = 2.7$, $S = 0.3$) than GD ($M = 2.4$, $S = 0.3$). The main effect of vector was not significant, $F(1,23) = 0.05$, P = 0.83.

Table 35: Sugar% in GD743 and GS784

Vector	Trial	Sugar%		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	11.1	10.3	10.7
	WA	11.5	9.5	10.5
	Mean	11.3	9.9	10.6
GEN-03	NY	12.9	10.7	11.8
	WA	10.9	8.5	9.7
	Mean	11.9	9.6	10.7
Column Mean		11.6	9.7	10.7

Three-way ANOVA of average Sugar% was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 4.86$, P = 0.044, with the average sugar content being significantly higher for NY ($M = 11.1$, $S = 1.2$) than WA ($M = 10.1$, $S = 1.6$). There was a significant main effect for cultivar, $F(1,23) = 16.24$, P = 0.001, with the average sugar content being significantly higher for GD ($M = 11.5$, $S = 1.2$) than GS ($M = 9.7$, $S = 1.2$). The main effect of vector was not significant, $F(1,23) = 0.05$, P = 0.83.

Table 36: Potassium mg per 100 g in GD743 and GS784

Vector	Trial	Potassium mg per 100 g		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	88.7	105.3	97.0
	WA	96.0	109.7	102.9
	Mean	92.4	107.5	99.9
GEN-03	NY	90.0	95.7	92.9
	WA	97.0	111.0	104.0
	Mean	93.5	103.4	98.4
Column Mean		92.9	105.4	99.2

Three-way ANOVA of average Potassium mg per 100g was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. The main effect for trial was not significant, $F(1,23) = 2.05$, P = 0.173. There was a significant main effect for cultivar, $F(1,23) = 5.03$, P = 0.041, with the average potassium content being significantly higher for GS ($M = 105$, $S = 13$) than GD ($M = 93$, $S = 11$). The main effect of vector was not significant, $F(1,23) = 0.09$, P = 0.77.

Vitamin C, ORAC, Total Phenolics

This section discusses vitamin C and phenolic antioxidant content of the apples. Vitamin C and phenolic antioxidant content of Arctic™ Apple events GD743 andGS784 from both the NY and WA field trials falls within the range for apple, raw with skin (NDB09003) provided by the USDA. Conversely, vitamin C and phenolic antioxidant content of the control cultivars GD and GS from both the NY and WA field trials falls well below the published norms for apple.

Statistical analysis reveals that there are significant effects of field trial and vector and an interaction of field trial and vector for ORAC, total phenolics and vitamin C. Arctic™ Apple events GD743 and GS784 had significantly higher ORAC (Table 37), total phenolics (Table 38) and vitamin C (Table 39) than the control cultivars GD and GS. This effect was more pronounced in the NY field trial location.

ORAC, total phenolic and vitamin C levels in events GD743 and GS784 fell within, or very close to, the published range for apple (NDB09003). This indicates that Arctic™ Apple cultivars GD743 and GS784 are, in all aspects (proximates, phenolics antioxidants and vitamin C), nutritionally equivalent to the published norms for apple. By contrast, it is the GD and GS control values that fell well below the minimum values established for apple. The differences were more extreme for the New York samples, where the ORAC values were only 19 percent (GD) and 31 percent (GS) of GD743 and GS784, respectively.

Table 37: ORAC in GD743 and GS784

Vector	Trial	ORAC (µmol TE/100g)		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	567	967	767
	WA	767	667	717
	Mean	667	817	742
GEN-03	NY	3000	3133	3067
	WA	1700	1800	1750
	Mean	2350	2467	2408
Column Mean		1509	1642	1575

Three-way ANOVA of average ORAC was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 17.81$, P = 0.001, with the average ORAC being significantly higher for NY (M = 1818, S = 1258) than WA (M = 1233, S = 596). The main effect for cultivar was not significant, $F(1,23) = 3.06$, P = 0.100. There was a significant main effect for vector, $F(1,23) = 138.59$, P = 0.000, with the average ORAC being significantly higher for GEN-03 (M = 2355, S = 749) than Control (M = 742, S = 326). The interaction effect of trial and vector was significant, $F(1,23) = 20.20$, P = 0.000, indicating that effect of cultivar was greater in NY.

Table 38: Total Phenolics in GD743 and GS784

Vector	Trial	Total Phenolics (mg GAE / 100 g)		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	70	103	87
	WA	80	78	79
	Mean	75	90	83
GEN-03	NY	209	241	225
	WA	155	147	151
	Mean	182	194	188
Column Mean		128	142	135

Three-way ANOVA of average Total Phenolics was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 6.48$, P = 0.022, with the average phenolic content being significantly higher for NY (M = 151, S = 86) than WA (M = 115, S = 40). The main effect for cultivar was not significant, $F(1,23) = 1.92$, P = 0.186. There was a significant main effect for vector, $F(1,23) = 54.11$, P = 0.000, with the average phenolic content being significantly higher for GEN-03 (M = 186, S = 53) than Control (M = 83, S = 29). The interaction effect of trial and vector was significant, $F(1,23) = 5.44$, P = 0.034, indicating that effect of cultivar was greater in NY.

Table 39: Vitamin C in GD743 and GS784

Vector	Trial	Vitamin C (mg / 100 g)		
		Golden Delicious	**Granny Smith**	**Row Mean**
Control	NY	0.0	0.0	0.0
	WA	1.4	1.4	1.4
	Mean	0.7	0.7	0.7
GEN-03	NY	14.1	9.7	11.9
	WA	2.6	2.5	2.5
	Mean	8.3	6.1	7.2
Column Mean		4.5	3.4	4.0

Three-way ANOVA of average Vitamin C was calculated using R Statistics v2.11.1 (Ihaka and Gentleman, 2010). The nominal alpha criterion level is = 0.05. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level. There was a significant main effect for trial, $F(1,23) = 43.18$, P = 0.000, with the average vitamin C content being significantly higher for NY ($M = 5.21$, $S = 6.33$) than WA ($M = 1.97$, $S = 0.71$). The main effect for cultivar was not significant, $F(1,23) = 0.71$, P = 0.412. There was a significant main effect for vector, $F(1,23) = 150.98$, P = 0.000, with the average vitamin C content being significantly higher for GEN-03 ($M = 6.58$, $S = 5.11$) than Control ($M = 0.71$, $S = 0.81$). The interaction effect of trial and vector was significant, $F(1,23) = 113.66$, P = 0.000, indicating that effect of cultivar was greater in NY.

In trying to understand why ORAC, total phenolics and vitamin C were lower in the control fruit the following rational is presented. The fruit tested was harvested, samples prepared and then shipped to the lab for testing. This protocol likely varied from the USDA methodology (NDB09003); given that fruit samples were cut put in Ziploc™ bags and put on ice, leaving the fruit flesh exposed for as long as 24 hours prior to testing. This resulted in some PPO-driven fruit browning within the control fruit. Given that phenols and vitamin C are substrates for the browning reaction they were partially consumed during the 24 hour transit period and prior to testing. Since GD743 and GS784 have very low levels of PPO this reaction did not take place and these substrates were conserved and available when tested.

PPO is involved in the degradation of vitamin C, catalyzing the reversible conversion of reduced ascorbic acid (RAA) to dehydroascorbic acid (DHA). Subsequently, DHA is irreversibly converted to diketogulonic acid (DGA) (Erdman and Klein, 1982). According to Brunswick Laboratories, the Vitamin C assay detects vitamin C only in its reduced form (RAA). RAA is a substrate for PPO, so presence of PPO would result in the conversion of RAA to DHA. Since DHA and RAA have the same activity, what is reported as loss of vitamin C in control GD and GS, may in fact only be conversion of RAA to DHA and not true loss of vitamin C activity.

Evidence provided here is consistent with the concept that Arctic™ Apple cultivars GS743 and GS784 are nutritionally equivalent with their parent cultivars, prior to slicing. While after slicing, GD743 and GS784 retain their original phenolic content, whereas GD and GS suffer the loss of phenolic compounds, and possibly vitamin C, through the action of PPO.

6.5 Expression of NptII in Mature Fruit

As stated in Section 4.4, the GEN-03 vector used to generate Events GD743 and GS784 contains the *nptII* gene as a selectable marker. As such, an NptII-specific ELISA was used to measure the accumulation of NptII protein in leaf and mature fruit samples. This work was done as part of a study of clonal stability of the GEN-03 transgene.

Apple leaf and fruit samples were collected from trees of events GD743 and GS784, and their respective control trees from each field trial. Each sample is a composite from one tree. Samples were collected, packed on ice and tested for NptII (ELISA) within 24 to 48 hours.

In leaf, expression levels for the NptII enzyme were found to range from 2.6 to 8.4 ng/g fresh tissue in GD743 with an average of 5.0 ng/g and to range from 0.7 to 8.4 ng/g fresh tissue in GS784 with an average of 3.8 ng/g (Table 40).

In fruit, expression levels for the NptII enzyme were found to range from 0.0 to 0.4 ng/g fresh tissue in GD743 with an average of 0.1 ng/g and to range from 0.0 to 0.5 ng/g fresh tissue in GS784 with an average of 0.1 ng/g (Table 41). The NptII protein expressed in mature fruit of GD743 and GS784 fall within the range of the controls. Stated another way, the *nptII* gene under control of the nopaline synthase promoter (P_{NOS}), does not result in accumulation of detectable amounts of NptII protein in mature apple fruit.

Table 40: Presence of NptII Protein in Leaves of GD743 and GS784 (ELISA)

Group	NptII[2]	*S*	n
Arctic™ Apple GD743	5.0	1.9	14
GD Control	0.0	0.1	4
Arctic™ Apple GS784	3.8	2.4	14
GS Control	0.1	0.1	4

[1] Apple leaf samples were tested for the presence of NptII by Agdia (Elkhart, IN) in two batches on August 26, 2010 and August 30, 2010. Tissue samples were sampled, weighed, and tested in duplicate for the presence of NptII.

[2] NptII = Amount of NptII protein present expressed as ng NptII / g fresh weight.

Table 41: Presence of NptII Protein in Mature Fruit of GD743 and GS784 (ELISA)

Group	NptII (ng/mg)[2]	*S*	n
Arctic™ Apple GD743	0.1	0.1	14
GD Control	0.1	0.1	4
Arctic™ Apple GS784	0.1	0.1	15
GS Control	0.1	0.1	4

[1] Apple fruit samples were tested for the presence of NptII by Agdia (Elkhart, IN) in three batches on September 28, 2010, October 22, 2010 and November 2, 2010. Tissue samples were sampled, weighed, and tested in duplicate for the presence of NptII.

[2] NptII = Amount of NptII protein present expressed as ng NptII / g fresh weight.

6.6 Conclusions

The following statements support the conclusion that events GD743 and GS784 are equivalent to their parent cultivars in cultivation and do not pose a plant pest risk:

(a) Field trials, maintained and observed by independent horticultural consultants, have been established that represent the two major apple growing areas in the USA;

(b) Primary components of agronomic performance, including tree height, trunk cross-section area, flower cluster number and fruit yield show that GD743 and GS784 are equivalent to their parent cultivars. The trees planted initially varied considerably given they came from the greenhouse and ranged in age, but once planted they settled down and behaved as expected;

(c) In a commercial orchard setting, based on extensive monitoring and a multitude of pest and disease incidents, GD743 and GS784 events were not systematically more or less susceptible to plant pests and disease;

(d) GD743 and GS784 events are nutritionally equivalent to the published norms for apple and may have improved phenolic compound stability;

(e) GD743 and GS784 events did not demonstrate any level of increased weediness that the control trees. However, in a highly managed orchard block with excellent weed control, weediness is difficult to assess:

(f) GD743 and GS784 events are stable both from year-to-year and after multiple years of grafting; and

(g) Mature fruit of GD743 and GS784 does not contain detectable levels of NptII protein.

7 ENVIRONMENTAL CONSEQUENCES OF INTRODUCTION OF THE TRANSFORMED CULTIVAR

7.1 Importance of Apple

7.1.1 Description

Apple is the pomaceous fruit of the apple tree, species *Malus x domestica*, in the rose family, Rosaceae. It is one of the most widely-cultivated tree fruit in temperate growing areas worldwide. The tree is small and deciduous, reaching 9-20 feet tall, with a broad, often densely twiggy crown. The leaves are alternately arranged simple ovals 1-4 inches long and 0.75-2 inches broad, having a petiole with an acute tip, serrated margin and a slightly downy underside. Blossoms are produced in spring simultaneously with budding of the leaves. The flowers are white with a pink tinge that gradually fades, are five petaled, and 1 inch in diameter. The fruit matures in autumn, and is typically 2-3.5 inches in diameter. The center of the fruit contains five carpels arranged in a five-point star, each carpel containing 1-3 seeds.

7.1.2 Production and Origin

The apple tree originated in Central Asia, where its wild ancestor is still found today. There are more than 8,000 known cultivars of apples resulting in a range of desired characteristics (Smith, 1971). Cultivars vary in yield and ultimate tree size, even when grown on the same rootstock.

At least 69 million tons of apples were harvested worldwide in 2008, with a farm-gate value of about $11 billion. China produced about 35 percent of this total. The United States is the world's second-leading producer, with more than 7.5 percent of the world production. Poland, Iran, Turkey, Italy, and India are also among the leading apple producers (Table 42).

Table 42: Top Ten Apple Producers (2008)

Country	Production (Tonnes)
People's Republic of China	29,851,163
United States	4,358,710
Poland	2,830,870
Iran	2,718,775
Turkey	2,504,490
Italy	2,208,227
India	1,985,000
France	1,940,200
Russia	1,467,000
Chile	1,370,000
World	69,819,324
Source: Based on FAOSTAT, accessed April 2011	

7.1.3 Apple Cultivars

Different cultivars are available for temperate and subtropical climates. Most of these cultivars are bred for eating fresh, though some are cultivated specifically for cooking or producing cider. Cider apple cultivars are typically too tart and astringent to eat fresh, but they give the beverage a rich flavor that fresh-market varieties cannot.

Commercially-popular fresh market apple cultivars are crisp, juicy, not too soft, a varying blend of tartness and sweetness and not too heavy skinned. Other desired qualities in modern commercial apple breeding are a colorful skin, absence of russeting, ease of shipping, lengthy storageability, high yields, disease resistance, good shape, long stems and a consistent popular flavor. Modern apples are generally sweeter than older cultivars, as popular tastes in apples have varied over time. Most North Americans and Europeans favor sweet, subacid apples, but tart apples have a strong minority following. Extremely sweet apples with barely any acid flavor are popular in most parts of Asia and India.

Old cultivars are often poorly shaped, russeted, and have a variety of textures and colors. Some consumers find them to have better flavor than modern cultivators, but they may have other problems which make them commercially unviable, such as low yields, high susceptibility to disease, or poor storage and transport qualities. A few old cultivars are still commercially produced on a large scale, and many others have been kept alive by home gardeners and farmers who sell directly to local markets.

Although only approximately 15 varieties make up the majority of commercial production worldwide, many unusual and locally important cultivars with their own unique taste and appearance exist. In the United Kingdom, old cultivars such as Cox's Orange Pippin and

Egremont Russet are still commercially important, even though by modern standards they are low yielding and disease prone.

7.1.4 Apple Uses

Apples can be utilized in many ways, including fresh, canned or juiced. They are pressed to produce both single-strength fresh apple juice as well as apple juice concentrate. The juice can be used to make sweet (nonalcoholic) apple cider, or fermented to make hard (alcoholic) cider and cider vinegar. Through distillation various alcoholic beverages are produced such as applejack and Calvados. Apple wine can also be made. Pectin is also produced.

Apples are an important ingredient in many desserts, such as apple pie, apple crumble, apple crisp and apple cake. They are often eaten baked or stewed; dehydrated/dried apples can be eaten as such or used in various applications, or can be reconstituted (soaked in water, alcohol or some other liquid) for later use. Puréed apples are generally known as apple sauce. Apples are also made into apple butter and apple jelly.

Fresh-cut apple slices are gaining popularity as a healthy snack food. Sliced or bruised apples turn brown due to the conversion of natural phenolic substances into melanin. Different cultivars differ in their propensity to brown after slicing. Sliced fruit can be treated with various antioxidant treatments (dips) to prevent this effect (see Section 7.2 for more details).

7.1.5 Apple Nutrition

The proverb "An apple a day keeps the doctor away," addressing the health effects of the fruit dates from 19th century Wales (Philips, 1866). Recent research suggests that apples may promote better health and help maintain a healthy weight. For example, apple consumption may reduce the risk of cancers, including of the colon, prostate and lung.

Compared to many other fruits and vegetables, apples contain relatively low amounts of Vitamin C, but are a rich source of other antioxidant compounds (Boyer and Liu, 1994). Apples are an excellent source of dietary fiber, which helps regulate bowel movements and may reduce the risk of colon cancer, help prevent heart disease and promote weight loss. Apples are also cholesterol-free, and their high fiber content helps control high cholesterol levels by preventing cholesterol absorption, and are nutrient dense for their low calorie content like most fruits and vegetables (Sharma, 2005). There is *in vitro* evidence that phenolic compounds in apples may be cancer-protective and demonstrate antioxidant activity (Lee *et al.*, 2004). The predominant phenolic phytochemicals in apples are quercetin, epicatechin and procyanidin B2 (Lee *et al.*, 2003).

Apple juice concentrate has been found in animal studies involving mice to increase production of the neurotransmitter acetylcholine, providing a potential mechanism for preventing decline in cognitive performance that accompanies dietary and genetic deficiencies of aging. Other studies have shown consuming apple juice may alleviate oxidative damage and cognitive decline in mice (Chan *et al.*, 2006).

The common nutritional composition of apple is as presented in Table 43.

Table 43: Nutritional Composition of Apple, Raw with Skin (NDB09003)

Energy 50 kcal 220 kJ		
Nutrient	**Units**	**Value per 100 g (3.5 oz.)**
Carbohydrates	g	13.81
- Sugars	g	10.39
- Dietary Fiber	g	2.4
Fat	g	0.17
Protein	g	0.26
Vitamin A equivalents	µg	3
Thiamine (Vitamin B1)	mg	0.017
Riboflavin (Vitamin B2)	mg	0.026
Niacin (Vitamin B3)	mg	0.091
Pantothenic Acid (Vitamin B5)	mg	0.061
Vitamin B6	mg	0.041
Folate (Vitamin B9)	µg	3
Vitamin C	mg	4.6
Calcium	mg	6
Iron	mg	0.12
Magnesium	mg	5
Phosphorous	mg	11
Potassium	mg	107
Zinc	mg	0.04
Source: USDA National Nutrient Database for Standard Reference – Release 22 (USDA, 2009)		

7.2 Current Methods for Inhibiting Browning in Apple

Various approaches to control vegetable and fruit browning have been documented and resulted in mixed success, due to a variety of reasons, including cost and amount of handling. For a general review of strategies for reducing fruit browning, see e.g., (Friedman and Bautista, 1991), (Iyengar and McEvily, 1992), (McEvily *et al.*, 1992), (Whitaker and Lee, 1995a), (Sapers, 1993), (Weemaes *et al.*, 1998), (Martinez and Whitaker, 1995) and (Brushett and Lacasse, 2006).

U.S. patents 5,939,117 (Chen *et al.*, 1999) and U.S. 5,925,395 (Chen, 1999) describe an antibrowning/antioxidant dip treatment. Fresh-cut apple slices which have been treated with an antibrowning/antioxidant dip are described as having reduced browning. However, the off-flavoring and high cost of the antibrowning/antioxidant dip solution has limited their commercial success. Furthermore, antioxidant dip solutions do not effectively address secondary browning that result from slicing knife injury and skin deformation prior to cutting or other secondary

browning reactions which can cause a thin brown line under the skin of the apple slice or other market detracting results.

Other approaches to control browning have been described, including but not limited to: cultivation in low oxygen atmosphere and low temperature (Heimdal *et al.*, 1995); treatment with calcium ascorbate, glutathione, cysteine and citrate (Jiang and Fu, 1998); treatment with sulfites and suboptimal pH and high-pressure carbon dioxide (Chen *et al.*, 1992); treatment of fresh-cut apple slices with natural products (Buta *et al.*, 1999); and a treatment with a 10 percent solution of honey (Oszmianski and Lee, 1990).

Murata (Murata *et al.*, 2000) (Murata *et al.*, 2001) reports that by suppressing a single PPO gene homologous to the apple PPO gene APO5, they obtained apple shoots and callus of reduced PPO activity which exhibit low browning potential *in vitro*. However, these references do not disclose a reduced browning fruit-producing plant or reduced-browning apple, nor whether suppression of a single PPO gene homologous to APO5 would be sufficient to obtain such a reduced-browning fruit-producing plant or reduced-browning apple. At OSF, an antisense APO5 transgene was not sufficient to reduce total PPO activity (OSF unpublished results).

7.3 Impact of Enzymatic Browning in Fresh and Processed Apple Products

Browning of apples and other fruit from damage that disrupts cell membranes – such as cuts, bruises, slicing, juicing or cell death, – is believed to be caused by enzymatic reaction catalyzed by PPO. The resulting brown pigment is a polymer formed from the nonenzymatic condensation of quinones, with lesser amounts of amino acids and proteins, into lignin-like compounds. The quinones are synthesized from diphenols in an enzymatic reaction catalyzed by PPO (Whitaker and Lee, 1995a). Most PPOs also have monophenolase activity, converting monophenols to diphenols (Mar - Sojo *et al.*, 1998).

The cause of browning has been understood for some time, yet the solution to reducing browning remains an on-going problem for industry, resulting in significant market loss. Browning reduces the quality of fresh-cut fruit and its resulting products by causing detrimental flavor and nutritional changes (Eskin, 1990). With the explosive growth of the fresh-cut produce sector, it has become increasingly evident that, for instance, browning limits the widespread introduction and use of apple and other fruits in commercial fresh-cut produce products such as prepared apple slices. Browning is also a major consideration in the manufacture of juice. Browning renders apple juice unsuitable for some blending applications because it colors the blended product in an unacceptable way, limiting its market potential.

Brown bruises in fresh-market apples – such as those caused incidentally during harvest and packing – are a significant cause of reduced grade for growers, resulting in revenue losses for producers as well as their retail and institutional processor customers (such as restaurants, hospitals, etc.). All segments of the fresh-market supply chain have been forced to accept these losses, or try to minimize them by implementing improved handling practices or otherwise compensate for them.

7.4 Expected Economic Impact of Arctic™ Apple

7.4.1 USA Apple Production Environment

As of 2008 the USA had approximately 450,000 acres of apple trees planted and produced 225 million boxes of fresh fruit (4.75 million tons). Table 44 illustrates apple production and average selling prices from 1997-2006; this period is dominated by large production and price variations from year to year, with higher price returns during low-volume years. Apples are commercially produced in more than 20 U.S. states; Washington state dominates production (58 percent), followed by New York and Michigan. Table 45 summarizes apple production in the top 10 states over the period 2002 to 2007.

Table 44: Past 10 Years Annual Apple Production vs. Average Farmgate Prices Received

Year	Production (000 boxes)	All Sales (cents/lb)
1997	245,805	15.4
1998	277,295	12.2
1999	253,112	15.0
2000	251,993	12.8
2001	224,357	15.8
2002	202,950	18.9
2003	209,360	20.9
2004	248,586	13.5
2005	231,069	17.4
2006	236,469	22.4
Source: USDA ERS Fruit & Tree Nut Outlook 2008		

Table 45: USA Apple Production, By Top 10 States (000 boxes)

State	2002	2003	2004	2005	2006	2007
Washington	121,429	108,333	146,429	135,714	134,524	128,571
New York	16,190	25,476	30,476	24,881	29,762	30,714
Michigan	12,381	21,190	17,381	18,095	20,238	18,810
Pennsylvania	8,810	10,524	9,643	11,905	11,190	10,833
California	11,190	10,714	8,452	8,452	8,452	8,095
Virginia	5,952	6,429	7,143	5,952	5,238	4,762
Oregon	4,810	3,167	3,881	3,452	3,571	3,452
West Virginia	2,262	2,071	1,929	2,071	2,143	1,905
Illinois	1,024	1,250	1,345	1,167	1,250	238
North Carolina	3,810	3,214	3,690	3,095	4,119	1,190
US Totals	**202,950**	**209,360**	**248,586**	**231,069**	**236,469**	**221,064**
Source: USDA 2008						

7.4.2 New Cultivars and Marketing Dynamics

The U.S. apple industry is currently undergoing dramatic changes. Downward price pressure from export markets, consolidation of produce wholesale and retail markets, advent of "Big Box" grocery stores and changing consumer demands have led to significant acreage being planted or removed and replanted. This has led producers to introduce apple cultivars having stronger market demand, better returns and offering niche market opportunities.

Table 46 presents the top 15 apple cultivars and their production 2002-2007. These new cultivars, or clonal improvements of existing cultivars, are being planted in new, higher-density orchard management and production systems that deliver higher yields and better fruit quality. These systems also lend themselves to more efficient use of mechanization, reducing labor costs. The U.S. apple nursery tree business has seen an increase in demand, as can be seen by nursery tree sales in Washington state 2004-2008 (Table 47). Extrapolating these figures, it can be estimated that over 7 million nursery trees were sold in the U.S. market in 2008. Given favorable economic returns, it is expected this trend will continue and will drive the revitalization of this important industry.

Table 46: USA Apple Production, By Top 15 Cultivars (000 boxes)

Cultivar	2002	2003	2004	2005	2006	2007
Red Delicious	63,232	58,350	69,578	64,968	61,101	53,692
Gala	18,810	20,634	25,807	23,975	28,904	28,519
Golden Delicious	27,766	26,317	31,810	30,014	28,283	24,635
Granny Smith	19,265	18,101	21,884	20,531	22,314	23,021
Fuji	20,357	15,332	22,570	21,000	20,218	18,164
McIntosh	7,866	11,057	12,019	9,913	10,065	10,136
Rome	7,979	10,183	10,463	9,822	8,428	7,082
Empire	2,820	4,498	4,965	4,281	6,553	6,473
Braeburn	3,056	2,955	5,337	4,945	4,330	5,024
Idared	3,225	5,165	4,964	4,677	4,838	4,670
York	3,724	4,186	4,096	4,395	4,090	3,857
Cripps Pink	1,448	1,969	3,602	3,342	2,915	3,322
Cameo	1,005	1,303	2,236	2,071	1,969	1,682
Jonagold	1,388	1,347	1,860	1,723	1,601	1,588
Totals	**202,950**	**209,360**	**248,586**	**231,069**	**236,469**	**221,064**
Source: USDA 2008						

Table 47: Washington Nursery Tree Sales (Number of Trees Sold)

Top Cultivars	2004	2006	2008
Red Fuji	225,990	490,210	567,840
Gala (various)	767,250	579,040	524,160
Honey Crisp	292,950	500.080	513,240
Early Fuji	111,600	223,720	218,400
Granny Smith	172,980	210,560	262,080
Golden Delicious	312,480	161,210	69,160
Cripps Pink	97,650	85,540	101,920
Other	809,100	1,039,640	1,384,200
Total	2,790,000	3,290,000	3,640,000
Source: TreeTop Inc. 2008 Apple and Pear Production and Planting Trends in Washington State			

7.4.3 Market Impact of Arctic™ Apples

It is anticipated that the Arctic™ apple events covered under this petition, including nonbrowning apple cultivars Arctic™ Golden and Arctic™ Granny Smith apples, will be considered a success in the market place if they:

- are widely used in fresh-cut apple slice processing and the product finds retail and institutional sales success;
- are sold fresh at the retail level, stimulating a demand for apples and competing successfully on price and value with current apple cultivars;
- are able to benefit apple growers through lower cullage rates and better prices;
- are able to offer fruit packers the benefit of fewer scuff marks and a more efficient packing regime; and
- are embraced by the foodservice industry and Arctic™ Apples are widely used in a broad range of new and existing products.

The target market during the initial sales period (Year 1-3) of Arctic™ Apples is the fresh-cut processor and foodservice businesses, with a limited number of fresh-cut apples going to retail. There are sufficient prospects in this market segment to establish the product and work through the various consumer issues that may arise given this is a genetically modified (GM) food. It is anticipated that during the initial sales period, culls and low-grade Arctic™ fruit will go to juice.

During Years 3-5, Arctic™ Apple production will ramp up and the product will achieve mainstream success in selected niche markets. There will still not be enough fruit to distribute these apples into all regions; distribution will be targeted to regions where retailers have shown the most interest and consumers are ready to embrace the product. Point-of-sale literature, in-store demonstrations, and brand labeling will be used to ensure all buyers are aware of the nonbrowning trait benefit and the fact that this represents a new generation of products.

From Year 6 on, we see Arctic™ Apples replacing regular apples at the retail level. Nonbrowning Arctic™ Golden Delicious and Granny Smith would have their own displays, sell for a price premium and take market share away from traditional products. Furthermore, Arctic™ Apples used in fresh-cut processing would become the fresh-market industry standard, now able to meet the high product specifications used in snack foods and specialty products at both existing and new retail outlets.

The market penetration target for OSF's Arctic™ Apples is forecast as outlined in Table 48. Given the nature of the product, the current consumer attitude about GM crops in general and the fact that Arctic™ apples have few, if any, comparables in the food business, this table outlines a "best guess" estimate for market penetration. Box totals have been calculated according to industry norms for bins of fruit per acre and packed boxes per bin.

Table 48: Target Market Penetration (1 box = 42 pounds)

Region[1]	Units	YR1	YR2	YR3	YR4	YR5	YR6	YR7	YR8	YR9	YR10	Totals
PNW	Acres	10	50	100	200	300	500	600	700	800	900	4,160
	Boxes		800	5,920	21,600	61,600	140,800	280,000	494,400	793,600	1,180,800	2,979,520
GLR	Acres	5	25	50	100	200	250	300	350	400	400	2,080
	Boxes		400	2,960	10,800	30,800	74,400	149,600	267,200	428,800	622,400	1,587,360
NER	Acres	5	25	50	100	200	250	300	350	400	400	2,080
	Boxes		400	2,960	10,800	30,800	74,400	149,600	267,200	428,800	622,400	1,587,360
CDN	Acres			10	20	50	100	200	250	300	350	1,280
	Boxes		-	-	800	3,520	11,840	32,000	74,400	149,600	267,200	539,360
Total	Acres	20	110	220	450	800	1,200	1,450	1,700	1,950	2,100	9,600
	Boxes	-	1,600	12,640	46,720	135,040	321,600	653,600	1,178,400	1,918,400	2,854,400	6,693,600

Source: OSF's Arctic™ Apple Marketing Report
Regions: PNW=Pacific Northwest Region, GLR = Great Lake Region, NER = Northeast Region, CDN = Canada

OSF has estimated at total planted area of slightly less than 10,000 acres or about 2 percent of total U.S. apple plantings over the first 10 years. This estimate includes additional Arctic™ Apple cultivars not included under this petition but anticipated over this 10-year period. The estimated total planted area for the 2 events covered by this petition is 4,000 acres over 10 years. As additional cultivars are released, this total may increase somewhat; however, it is not anticipated that Arctic™ Apples will ever achieve the large market share enjoyed by GM field crops (i.e., corn and soybean). We anticipate that apples, being a perennial crop of 20 or more years before replanting, will have a much slower adoption and introduction curve than annual crops.

Utilizing market penetration data found in Table 48 and based on an average return of $20 per box, the economic impact of Arctic™ Apple cultivars will approximate $120 million in the first 10 years.

7.5 Gene Flow

7.5.1 Potential recipients of transgenes from cultivated apple

An experimental data set has been developed describing pollen gene flow as a result of bee pollination in apple, using a model predicting gene flow when donor and recipient block vary in size, shape and orientation. The model was developed by monitoring the beta-glucuronidase (GUS) reporter transgene in an isolated test block, and collecting seeds from transects up to 200 meter from the row of donor plants.

Empirical results show that the majority of bee-carried pollen travels less than 100 feet. The model derived from the empirical data set may be useful for predicting out-crossing rates of transgenic orchards with nearby conventional orchards and the commercial cultivars they contain. The model will also be useful to help define isolation distances and contamination rates

at different locations in neighbouring orchards so that conditions imposed on cultivation of transgenic cultivars can be prescribed. Further details on the data set, experimental design, discussion and conclusions are available in two papers concerning gene flow in apple (Tyson *et al.*, 2010) (Tyson *et al.*, 2011).

In addition to being pollinated by other cultivars, apple can also be pollinated by crab apple (and vice versa); indeed, a number of cultivars of crab apple are routinely used as the pollinizers in commercial apple orchards. Of the 25 or so species of crab apple known worldwide, five are native to North America and two imported species have become naturalized after escaping from cultivation.

Hybrids between apple and native crabs have been noted growing in uncultivated areas, but have not become widespread nor weedy despite growing in proximity since apple was introduced by pioneers to America 300 or so years ago. Apple is thought to cross with the native crabs of the *Malus* section *Chloromeles* when they are grown together. Many of the crab species native to eastern and central Asia have been imported into the USA; they are used either as ornamentals themselves or as breeding parents for new ornamental cultivars.

The chromosome number of these crab species is often unknown. Even if cross-pollination between apple and crab species occurs and is followed by seed set, progeny developing from the seed may be aneuploid because crabs differ in chromosome number; this would result in poorly-growing and likely sterile progeny, or deficiencies of other kinds. Some are known to be apomictic (that is, capable of asexual reproduction), but may still produce viable pollen. Neither escaped, nor naturalized plants of these Asian origin species nor their hybrids with apple have been documented, though they may occur as persistent plants in some unknown locations.

7.5.2 Routes of gene flow in addition to bee pollination

Apples are commonly discarded along travel routes and the seeds from this fruit can germinate and develop into a tree. This would manifest itself mostly as persistent trees that do not spread, although naturalized populations have been documented (Little, 1979). They also can germinate in rubbish and compost piles. Apples float, so they can be distributed via in waterways. Several mammal species eat apples and hence could carry away the seeds notably bears, mice and squirrels. Birds feed on the fruit but aren't thought to carry away seed. The risk of gene outflow from these uncommon and low frequency events is considered low, demonstrated by the low number of persistent apple trees.

7.6 Stewardship of Arctic™ Apple Gene Flow

Particulars specific to Arctic™ Apples will contribute to the ability to monitor and control Arctic™ Apple gene flow.

Similar to most new apple cultivars being released, Arctic™ Apple cultivars will be royalty-bearing. For this reason the propagation, planting area, cultivation, packing and selling of these cultivars and their trees will be carefully monitored and tracked. Furthermore, it is OSF's intent to license these cultivars to a very limited number of integrated producer/packer/sellers, to create a managed marketing environment. It is our intent that Arctic™ Apple trees will not be available for widespread commercial distribution, nor for backyard and small-scale plantings.

Furthermore, the use of Arctic™ Apple cultivars in commercial breeding programs will be limited, and any resulting cultivars of commercial merit will be managed in a similar manner as their Arctic™ parent.

All Arctic™ Apple cultivars will be sold under the Arctic™ brand name, which along with the logo has been trademarked. This brand name will be utilized in a range of venues – including point-of-sale literature, price look-up code stickers on the apples and all forms of retail packaging – to identify Arctic™ fruit. The Arctic™ name will be used to inform buyers, as well as to allow traceability of the product.

The apple industry has the advantage over the field crop industry in the fact that cultivars are already segregated and packed in lots. It is OSF's intent that traceability will be maintained of all Arctic™ Apple cultivars from field to retail and foodservice outlet. Further, Arctic™ Apples can be identified at all steps along the value chain, through a simple bioassay or a PCR test, as well as by DNA fingerprinting.

The risk of trait out-crossing and pollen gene flow is well recognized. However, literature and our own research shows that this risk is low and can be readily managed. Arctic™ Apple growers will be provided with stewardship guidelines as part of their licensing requirements, and these will be monitored for compliance by OSF. Two key stewardship obligations will include: 1) hives used for pollination will not be transferred to another apple block upon completion, they will be used on field crops (i.e., alfalfa) or fed for a suitable period of time before re-entering an apple orchard; and 2) suitable isolation distances between Arctic™ Apple blocks and conventional blocks will be maintained. Isolation distances from organic apple blocks will likely be greater. Other ways to mitigate pollen gene flow (i.e. border rows) will be discussed and adopted as required.

One must remember that if out-crossing does take place, only the seeds will carry some component of the transgene; the apple cortex and the maternal tissue it contains would remain non-transgenic. Further, in a commercial setting, it is very uncommon for a seed to develop into a viable, commercially harvested apple tree.

8 ADVERSE CONSEQUENCES OF INTRODUCTION

Apples are propagated vegetatively by grafting, therefore the silenced PPO Arctic™ Apple cultivars are clonal improvements of well-established, commercially proven cultivars. Field testing to date indicates that growth and fruiting is very similar to the parent cultivar, except that the silenced PPO gene results in a nonbrowning phenotype. Consequences that may result from the introduction of the cultivar follow:

1) Increased consumption of apples, particularly apples and fresh-cut apple slices. This is regarded as primarily a positive consequence, though consumer reaction may be adverse should the public react negatively to the method of origin of the new clones and avoid apples in general – reducing consumption nationally or through reduced international trade. Given OSF's commitment for transparency and labelling this adverse reaction is considered unlikely.

2) Adverse environmental consequences are not anticipated because the new clones should not hybridize, persist nor naturalize more than has occurred historically with their conventional brethren. Further, it can be argued that the silenced PPO gene is likely a hindrance rather than benefit to wild trees, as such a trait would have previously become established through natural mutation if were a beneficial trait. Therefore, the PPO gene could be considered a benefit to apple outside of the commercial setting.

3) An adverse agro-ecological consequence is the potential for contamination of seeds in conventional or organic apple crops with the PPO transgene as a result of pollination flow from transgenic trees. However, only the seed would be transgenic, not the maternal tissue in the apple cortex. Cortex tissue, the portion of the apple that is eaten, would remain nontransgenic.

4) The amount of transgene-containing seed that might develop in neighboring orchards depends on a number of factors, including coincidence of bloom time so that flowers are receptive at the time when pollen is available, the distance between the orchards, and the presence or absence of buffer rows. Another important influencing factor is competition from conventional pollen that any transgenic pollen might encounter to effectively pollinate in a conventional orchard. The larger the conventional block, the greater the likelihood that a flower will be pollinated first by the overwhelming predominance of conventional pollen present. However, trap plant experiments – by which plants are arranged so that seeds can develop only from transgenic pollen because no competing pollen is available to them – have demonstrated bees can transport pollen for distances of more than a mile. However, this situation is very rare in a commercial orchard setting because of the competition from nearby conventional pollen.

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Appendix 1: Photographic Record of the Development of GD743 and GS784

Figure 11: Micrografting ArcticTM Apple Shoot to M9 Rootstocks

These images were taken during the micrografting process. Tissue culture shoots of transgenic, PPO suppressed events are collected by our tissue culture technicians and grafted directly onto established rootstock in our greenhouse. The rate of grafting success can be >90% depending on the time of year. Within about 3 months, this process can generate a whip tree that is ready for transfer to the screenhouse to condition it for the field.

2004

2007

Figure 12: Trial Block - Washington

These images were taken at the Washington Field trial. They show the planting of the GEN-03 trees that was done in 2004 and how the tree appeared in 2007 (4th leaf). This row contains GD743 and GS784, as well as many other non-browning events arising from the GEN-03 transformations, plus controls.

GD743 

GS784 

Figure 13: Whole Apple Images of GD743 and GS784

These images were taken as part of a marketing package produced in 2008. They show apple fruit that is typical of the GD743 and GS784.

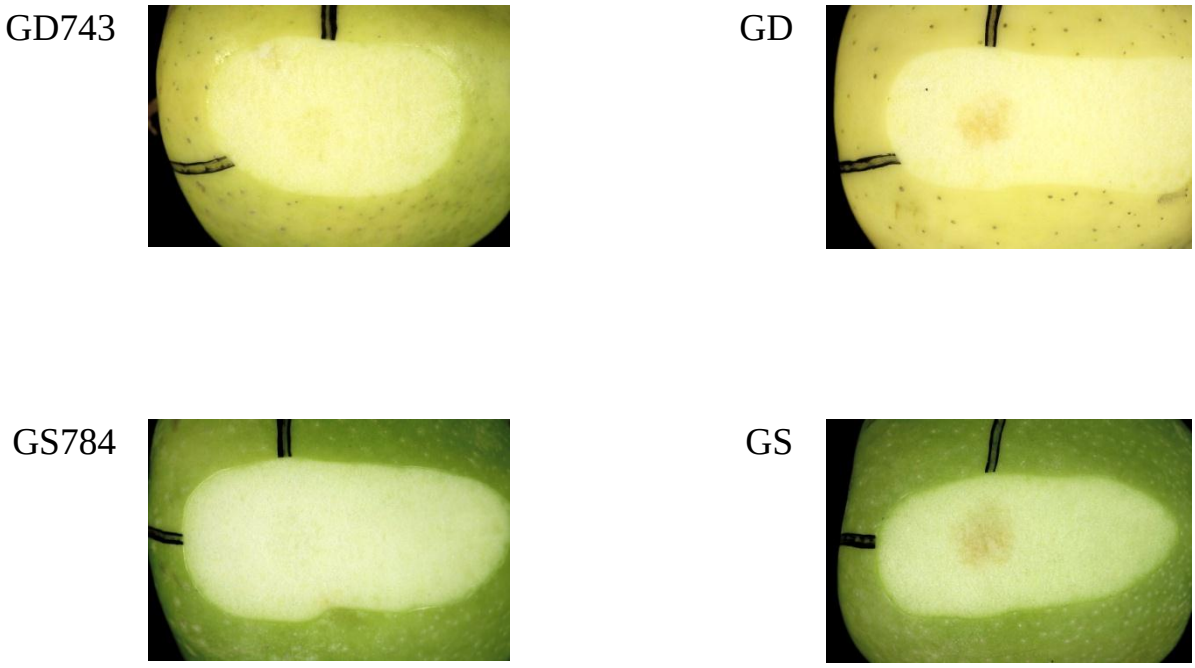
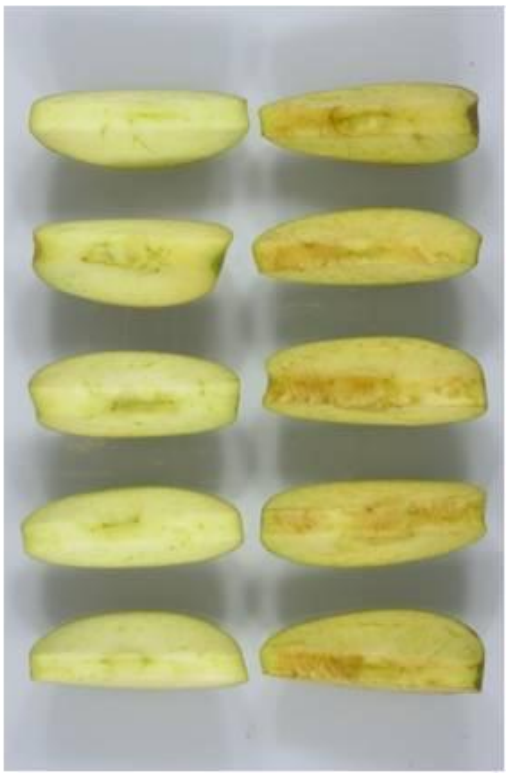


Figure 14: Bruise Response in GD743 and GS784

These images were taken during controlled bruising tests. GD743 and GS784 plus untransformed controls were subjected to impact bruising designed to mimic scrabble bruising that might be incurred during packing line handling. The bruises are allowed to develop for 2 hours and then the apples are peeled to reveal the flesh color changes. The intersection of the black lines on the apple show where the bruise was made. Note that for GD732 and GS784, no bruising is visible.



GD743 GD

Figure 15: Apple Slices of GD743 and GD Control

These images were taken during apple slice tests. The apples were sliced, washed and stored in 5^{o}C storage for 3 weeks in ZipLoc^{TM} bags, prior to photographing.

GD743  GD

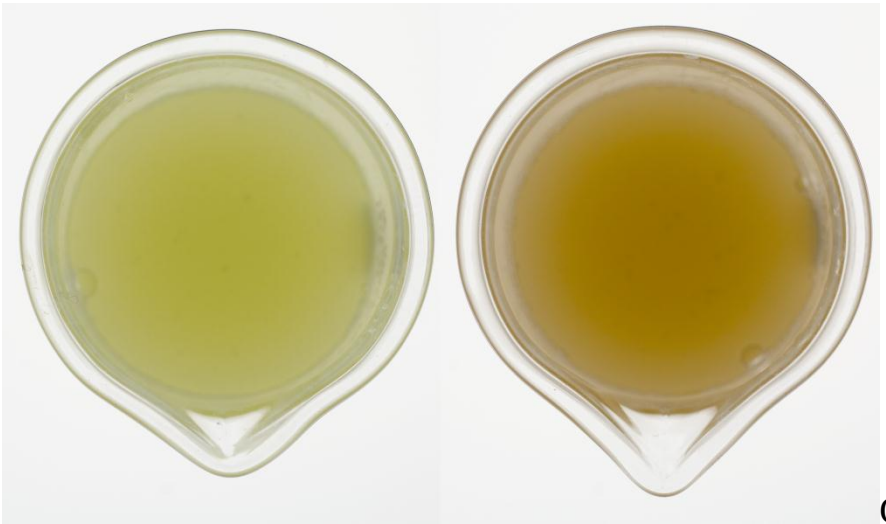
GS784  GS

Figure 16: Apple Juice Made From GD743 and GS784

The following images were taken during apple juice tests. The apples were sliced, cored and juiced (with skin). The resulting apple juice was left overnight at room temperature for color development. Notably, full color development occurred in the untransformed parents within minutes of juicing.

Appendix 2: Field Maps

Table 49: Map of the Washington Field Trial – 2003 Block

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA - 14	1	784-5	GEN-03	GS	PPO Suppression	2003	
Yes	WA - 14	2	801-7	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	3	784-3	GEN-03	GS	PPO Suppression	2003	
Yes	WA - 14	4	801-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	5	801-45	GEN-03	GD	PPO Suppression	2003	
	WA - 14	6	705-21	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	7	743-8	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	8	1001-17	Control	GS	Control	2003	Other
Yes	WA - 14	9	784-4	GEN-03	GS	PPO Suppression	2003	Other
	WA - 14	10	705-25	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	11	743-14	GEN-03	GD	PPO Suppression	2003	
	WA - 14	12	743-23	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	13	743-7	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	14	705-31	GEN-03	GD	PPO Suppression	2003	
	WA - 14	15	705-37	GEN-03	GD	PPO Suppression	2003	
	WA - 14	16	784-2	GEN-03	GS	PPO Suppression	2003	
Yes	WA - 14	17	801-8	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	18	709-1	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	19	784-6	GEN-03	GS	PPO Suppression	2003	
	WA - 14	20	801-44	GEN-03	GD	PPO Suppression	2003	
	WA - 14	21	705-22	GEN-03	GD	PPO Suppression	2003	
	WA - 14	22	707-14	GEN-03	GD	PPO Suppression	2003	
	WA - 14	23	743-16	GEN-03	GD	PPO Suppression	2003	
	WA - 14	24	743-11	GEN-03	GD	PPO Suppression	2003	
	WA - 14	25	743-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	26	1001-12	Control	GS	Control	2003	Other
Yes	WA - 14	27	705-32	GEN-03	GD	PPO Suppression	2003	
	WA - 14	28	801-26	GEN-03	GD	PPO Suppression	2003	
	WA - 14	29	801-39	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	30	1001-1	Control	GS	Control	2003	
Yes	WA - 14	31	773-8	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	32	773-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	33	773-2	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	34	705-30	GEN-03	GD	PPO Suppression	2003	
	WA - 14	35	743-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	36	707-13	GEN-03	GD	PPO Suppression	2003	

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	37	705-40	GEN-03	GD	PPO Suppression	2003	
	WA - 14	38	705-34	GEN-03	GD	PPO Suppression	2003	
	WA - 14	39	707-10	GEN-03	GD	PPO Suppression	2003	
	WA - 14	40	784-13	GEN-03	GS	PPO Suppression	2003	
	WA - 14	41	784-14	GEN-03	GS	PPO Suppression	2003	
Yes	WA - 14	42	1001-4	Control	GS	Control	2003	
	WA - 14	43	801-46	GEN-03	GD	PPO Suppression	2003	
	WA - 14	44	705-20	GEN-03	GD	PPO Suppression	2003	
	WA - 14	45	1001-6	Control	GS	Control	2003	
	WA - 14	46	801-47	GEN-03	GD	PPO Suppression	2003	
	WA - 14	47	743-41	GEN-03	GD	PPO Suppression	2003	FB
	WA - 14	48	705-24	GEN-03	GD	PPO Suppression	2003	FB
	WA - 14	49	784-9	GEN-03	GS	PPO Suppression	2003	
	WA - 14	50	1001-5	Control	GS	Control	2003	
	WA - 14	51	743-19	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	52	707-23	GEN-03	GD	PPO Suppression	2003	
	WA - 14	53	784-7	GEN-03	GS	PPO Suppression	2003	
	WA - 14	54	1001-10	Control	GS	Control	2003	
	WA - 14	55	1001-3	Control	GS	Control	2003	
	WA - 14	56	801-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	57	743-12	GEN-03	GD	PPO Suppression	2003	
	WA - 14	58	743-15	GEN-03	GD	PPO Suppression	2003	
	WA - 14	59	705-43	GEN-03	GD	PPO Suppression	2003	
	WA - 14	60	705-29	GEN-03	GD	PPO Suppression	2003	
	WA - 14	61	707-24	GEN-03	GD	PPO Suppression	2003	
	WA - 14	62	707-16	GEN-03	GD	PPO Suppression	2003	
	WA - 14	63	705-41	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	64	707-22	GEN-03	GD	PPO Suppression	2003	
	WA - 14	65	801-35	GEN-03	GD	PPO Suppression	2003	
	WA - 14	66	801-41	GEN-03	GD	PPO Suppression	2003	
	WA - 14	67	1000-25	Control	GD	Control	2003	
	WA - 14	68	801-37	GEN-03	GD	PPO Suppression	2003	
	WA - 14	69	784-12	GEN-03	GS	PPO Suppression	2003	
	WA - 14	70	705-23	GEN-03	GD	PPO Suppression	2003	
	WA - 14	71	743-21	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	72	707-18	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	73	707-15	GEN-03	GD	PPO Suppression	2003	
	WA - 14	74	707-19	GEN-03	GD	PPO Suppression	2003	
	WA - 14	75	801-42	GEN-03	GD	PPO Suppression	2003	

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	76	801-38	GEN-03	GD	PPO Suppression	2003	
	WA - 14	77	743-10	GEN-03	GD	PPO Suppression	2003	
	WA - 14	78	801-33	GEN-03	GD	PPO Suppression	2003	
	WA - 14	79	773-7	GEN-03	GD	PPO Suppression	2003	
	WA - 14	80	773-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	81	1001-2	Control	GS	Control	2003	
	WA - 14	82	1001-9	Control	GS	Control	2003	
	WA - 14	83	792-1	GEN-03	GD	PPO Suppression	2003	
	WA - 14	84	709-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	85	709-10	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	86	773-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	87	773-1	GEN-03	GD	PPO Suppression	2003	
	WA - 14	88	773-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	89	709-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	90	773-10	GEN-03	GD	PPO Suppression	2003	
	WA - 14	91	709-7	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	92	792-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	93	792-3	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	94	709-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	95	705-38	GEN-03	GD	PPO Suppression	2003	
	WA - 14	96	1001-11	Control	GS	Control	2003	
	WA - 14	97	705-35	GEN-03	GD	PPO Suppression	2003	
	WA - 14	98	705-36	GEN-03	GD	PPO Suppression	2003	
	WA - 14	99	801-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	100	707-11	GEN-03	GD	PPO Suppression	2003	
	WA - 14	101	707-26	GEN-03	GD	PPO Suppression	2003	
	WA - 14	102	705-28	GEN-03	GD	PPO Suppression	2003	
	WA - 14	103	784-10	GEN-03	GS	PPO Suppression	2003	
	WA - 14	104	707-12	GEN-03	GD	PPO Suppression	2003	
	WA - 14	105	707-27	GEN-03	GD	PPO Suppression	2003	
	WA - 14	106	743-43	GEN-03	GD	PPO Suppression	2003	
	WA - 14	107	705-42	GEN-03	GD	PPO Suppression	2003	FB
	WA - 14	108	801-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	109	743-20	GEN-03	GD	PPO Suppression	2003	
	WA - 14	110	784-16	GEN-03	GS	PPO Suppression	2003	
	WA - 14	111	703-15	GEN-03	GD	PPO Suppression	2003	
	WA - 14	112	743-1	GEN-03	GD	PPO Suppression	2003	
	WA - 14	113	801-27	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	114	1000-2	Control	GD	Control	2003	Other

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA - 14	115	1000-1	Control	GD	Control	2003	Other
	WA - 14	116	707-6	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	117	1000-9	Control	GD	Control	2003	
	WA - 14	118	743-45	GEN-03	GD	PPO Suppression	2003	
	WA - 14	119	707-30	GEN-03	GD	PPO Suppression	2003	
	WA - 14	120	705-44	GEN-03	GD	PPO Suppression	2003	
	WA - 14	121	707-8	GEN-03	GD	PPO Suppression	2003	
	WA - 14	122	703-13	GEN-03	GD	PPO Suppression	2003	
	WA - 14	123	705-45	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	124	703-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	125	703-17	GEN-03	GD	PPO Suppression	2003	
	WA - 14	126	743-44	GEN-03	GD	PPO Suppression	2003	
	WA - 14	127	705-7	GEN-03	GD	PPO Suppression	2003	
	WA - 14	128	707-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	129	703-18	GEN-03	GD	PPO Suppression	2003	
	WA - 14	130	705-18	GEN-03	GD	PPO Suppression	2003	
	WA - 14	131	784-17	GEN-03	GS	PPO Suppression	2003	
	WA - 14	132	801-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	133	703-8	GEN-03	GD	PPO Suppression	2003	
	WA - 14	134	703-16	GEN-03	GD	PPO Suppression	2003	
	WA - 14	135	703-12	GEN-03	GD	PPO Suppression	2003	
	WA - 14	136	703-14	GEN-03	GD	PPO Suppression	2003	
	WA - 14	137	705-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	138	705-47	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	139	703-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	140	705-1	GEN-03	GD	PPO Suppression	2003	
	WA - 14	141	801-28	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	142	792-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	143	703-10	GEN-03	GD	PPO Suppression	2003	
	WA - 14	144	703-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	145	784-15	GEN-03	GS	PPO Suppression	2003	
	WA - 14	146	703-19	GEN-03	GD	PPO Suppression	2003	
	WA - 14	147	792-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	148	784-18	GEN-03	GS	PPO Suppression	2003	
	WA - 14	149	792-8	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	150	792-7	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	151	792-4	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	152	792-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	153	784-19	GEN-03	GS	PPO Suppression	2003	Other

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	154	705-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	155	707-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	156	707-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	157	707-31	GEN-03	GD	PPO Suppression	2003	
	WA - 14	158	784-8	GEN-03	GS	PPO Suppression	2003	
	WA - 14	159	707-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	160	705-19	GEN-03	GD	PPO Suppression	2003	
	WA - 14	161	743-13	GEN-03	GD	PPO Suppression	2003	
	WA - 14	162	743-17	GEN-03	GD	PPO Suppression	2003	
	WA - 14	163	743-18	GEN-03	GD	PPO Suppression	2003	
	WA - 14	164	784-11	GEN-03	GS	PPO Suppression	2003	
	WA - 14	165	743-5	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	166	702-2	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	167	707-29	GEN-03	GD	PPO Suppression	2003	
	WA - 14	168	705-46	GEN-03	GD	PPO Suppression	2003	
	WA - 14	169	702-8	GEN-03	GD	PPO Suppression	2003	
	WA - 14	170	703-7	GEN-03	GD	PPO Suppression	2003	
	WA - 14	171	707-32	GEN-03	GD	PPO Suppression	2003	
	WA - 14	172	746-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	173	703-11	GEN-03	GD	PPO Suppression	2003	
	WA - 14	174	705-12	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	175	702-7	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	176	746-1	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	177	702-5	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	178	739-1	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	179	752-2	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	180	752-1	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	181	745-2	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	182	739-3	GEN-03	GD	PPO Suppression	2003	Other
Yes	WA - 14	183	745-1	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	184	705-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	185	707-1	GEN-03	GD	PPO Suppression	2003	
	WA - 14	186	703-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	187	702-3	GEN-03	GD	PPO Suppression	2003	
Yes	WA - 14	188	703-1	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	189	702-1	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	190	702-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	191	703-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	192	1000-4	Control	GD	Control	2003	

Washington (2003 Block)[2]								
Flagged	**Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	193	739-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	194	1000-3	Control	GD	Control	2003	
Yes	WA - 14	195	739-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	196	1000-6	Control	GD	Control	2003	Other
	WA - 14	197	705-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	198	1000-7	Control	GD	Control	2003	
	WA - 14	199	1000-5	Control	GD	Control	2003	
	WA - 14	200	1000-8	Control	GD	Control	2003	
	WA - 14	201	703-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	202	739-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	203	743-3	GEN-03	GD	PPO Suppression	2003	
	WA - 14	204	705-15	GEN-03	GD	PPO Suppression	2003	
	WA - 14	205	743-2	GEN-03	GD	PPO Suppression	2003	
	WA - 14	206	702-6	GEN-03	GD	PPO Suppression	2003	
	WA - 14	207	705-13	GEN-03	GD	PPO Suppression	2003	
	WA - 14	208	705-8	GEN-03	GD	PPO Suppression	2003	
	WA - 14	209	705-11	GEN-03	GD	PPO Suppression	2003	
	WA - 14	210	705-17	GEN-03	GD	PPO Suppression	2003	
	WA - 14	211	705-2	GEN-03	GD	PPO Suppression	2003	Other
	WA - 14	212	705-16	GEN-03	GD	PPO Suppression	2003	
	WA - 14	213	707-5	GEN-03	GD	PPO Suppression	2003	
	WA - 14	214	784-1	GEN-03	GS	PPO Suppression	2003	
	WA - 14	215	707-7	GEN-03	GD	PPO Suppression	2003	
	WA - 14	216	705-9	GEN-03	GD	PPO Suppression	2003	
	WA - 14	217	705-10	GEN-03	GD	PPO Suppression	2003	
	WA - 14	218	705-14	GEN-03	GD	PPO Suppression	2003	
	WA - 14	219	743-4	GEN-03	GD	PPO Suppression	2003	
	WA - 14	220	801-1	GEN-03	GD	PPO Suppression	2003	

[1] FB: Removed due to fireblight on September 25, 2007; Other: Trees are removed during the growing season for a variety of reasons, mostly commonly transplant mortality, rodent or mechanical damage. The trees noted here as 'other' were removed at the end of 2006 or beginning of the 2007 field season

[2] The WA2003 Block was removed at the end of the 2007 growing season to make way for the WA2008 demonstration block.

[3] The plant name is a unique identifier for each tree, where the number preceding the dash is the transgenic event name and the number following the dash is the tree number (event name 1000 = GD control; event name 1001 = GS control).

Table 50: Map of the New York Field Trial – 2005 Block

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
NY - 101	1	1001-49	Control	GS	Control	2005
NY - 101	2	703-65	GEN-03	GD	PPO Suppression	2005
NY - 101	3	707-68	GEN-03	GD	PPO Suppression	2005
NY - 101	4	831-8	GEN-03	GD	PPO Suppression	2005
NY - 101	5	707-60	GEN-03	GD	PPO Suppression	2005
NY - 101	6	707-67	GEN-03	GD	PPO Suppression	2005
NY - 101	7	846-5	GEN-03	GD	PPO Suppression	2005
NY - 101	8	773-11	GEN-03	GD	PPO Suppression	2005
NY - 101	9	1001-46	Control	GS	Control	2005
NY - 101	10	705-94	GEN-03	GD	PPO Suppression	2005
NY - 101	11	784-35	GEN-03	GS	PPO Suppression	2005
NY - 101	12	773-9	GEN-03	GD	PPO Suppression	2005
NY - 101	13	1000-37	Control	GD	Control	2005
NY - 101	14	714-1	GEN-03	GD	PPO Suppression	2005
NY - 101	15	831-7	GEN-03	GD	PPO Suppression	2005
NY - 101	16	845-5	GEN-03	GD	PPO Suppression	2005
NY - 101	17	702-43	GEN-03	GD	PPO Suppression	2005
NY - 101	18	634-1	GEN-02	GS	PPO Suppression	2005
NY - 101	19	703-56	GEN-03	GD	PPO Suppression	2005
NY - 101	20	707-41	GEN-03	GD	PPO Suppression	2005
NY - 101	21	1002-8	Control	NF	Control	2005
NY - 101	22	1000-36	Control	GD	Control	2005
NY - 101	23	1003-2	Control	PG	Control	2005
NY - 101	24	426-1	GEN-02	NF	PPO Suppression	2005
NY - 101	25	702-44	GEN-03	GD	PPO Suppression	2005
NY - 101	26	872-2	GEN-03	NF	PPO Suppression	2005
NY - 101	27	872-4	GEN-03	NF	PPO Suppression	2005
NY - 101	28	1001-39	Control	GS	Control	2005
NY - 101	29	845-9	GEN-03	GD	PPO Suppression	2005
NY - 101	30	427-1	GEN-02	NF	PPO Suppression	2005
NY - 101	31	273-1	GEN-02	GD	PPO Suppression	2005
NY - 101	32	811-24	GEN-03	GD	PPO Suppression	2005
NY - 101	33	784-30	GEN-03	GS	PPO Suppression	2005
NY - 101	34	294-2	GEN-02	NF	PPO Suppression	2005
NY - 101	35	872-1	GEN-03	NF	PPO Suppression	2005
NY - 101	36	331-2	GEN-02	GS	PPO Suppression	2005
NY - 101	37	1001-44	Control	GS	Control	2005
NY - 101	38	1001-31	Control	GS	Control	2005
NY - 101	39	743-85	GEN-03	GD	PPO Suppression	2005
NY - 101	40	811-8	GEN-03	GD	PPO Suppression	2005

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
NY - 101	41	845-15	GEN-03	GD	PPO Suppression	2005
NY - 101	42	255-1	GEN-02	GD	PPO Suppression	2005
NY - 101	43	707-64	GEN-03	GD	PPO Suppression	2005
NY - 101	44	846-11	GEN-03	GD	PPO Suppression	2005
NY - 101	45	702-45	GEN-03	GD	PPO Suppression	2005
NY - 101	46	846-10	GEN-03	GD	PPO Suppression	2005
NY - 101	47	846-12	GEN-03	GD	PPO Suppression	2005
NY - 101	48	801-34	GEN-03	GD	PPO Suppression	2005
NY - 101	49	784-56	GEN-03	GS	PPO Suppression	2005
NY - 101	50	249-2	GEN-02	GD	PPO Suppression	2005
NY - 101	51	1000-34	Control	GD	Control	2005
NY - 101	52	1002-6	Control	NF	Control	2005
NY - 101	53	520-2	GEN-02	GD	PPO Suppression	2005
NY - 101	54	1003-10	Control	PG	Control	2005
NY - 101	55	705-91	GEN-03	GD	PPO Suppression	2005
NY - 101	56	846-3	GEN-03	GD	PPO Suppression	2005
NY - 101	57	811-1	GEN-03	GD	PPO Suppression	2005
NY - 101	58	784-52	GEN-03	GS	PPO Suppression	2005
NY - 101	59	590-1	GEN-02	GD	PPO Suppression	2005
NY - 101	60	1003-13	Control	PG	Control	2005
NY - 102	1	523-1	GEN-02	GD	PPO Suppression	2005
NY - 102	2	703-58	GEN-03	GD	PPO Suppression	2005
NY - 102	3	743-88[1]	GEN-03	GD	PPO Suppression	2005
NY - 102	4	294-1	GEN-02	NF	PPO Suppression	2005
NY - 102	5	615-3	GEN-02	GD	PPO Suppression	2005
NY - 102	6	260-2	GEN-02	GD	PPO Suppression	2005
NY - 102	7	260-1	GEN-02	GD	PPO Suppression	2005
NY - 102	8	348-4	GEN-02	GD	PPO Suppression	2005
NY - 102	9	255-2	GEN-02	GD	PPO Suppression	2005
NY - 102	10	784-76	GEN-03	GS	PPO Suppression	2005
NY - 102	11	605-1	GEN-02	GD	PPO Suppression	2005
NY - 102	12	348-5	GEN-02	GD	PPO Suppression	2005
NY - 102	13	426-2	GEN-02	NF	PPO Suppression	2005
NY - 102	14	590-2	GEN-02	GD	PPO Suppression	2005
NY - 102	15	1003-12	Control	PG	Control	2005
NY - 102	16	714-2	GEN-03	GD	PPO Suppression	2005
NY - 102	17	520-1	GEN-02	GD	PPO Suppression	2005
NY - 102	18	846-7	GEN-03	GD	PPO Suppression	2005
NY - 102	19	743-87	GEN-03	GD	PPO Suppression	2005
NY - 102	20	811-21	GEN-03	GD	PPO Suppression	2005
NY - 102	21	743-67	GEN-03	GD	PPO Suppression	2005

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
NY - 102	22	534-2	GEN-02	GD	PPO Suppression	2005
NY - 102	23	617-1	GEN-02	GD	PPO Suppression	2005
NY - 102	24	705-90	GEN-03	GD	PPO Suppression	2005
NY - 102	25	331-1	GEN-02	GS	PPO Suppression	2005
NY - 102	26	845-7	GEN-03	GD	PPO Suppression	2005
NY - 102	27	831-5	GEN-03	GD	PPO Suppression	2005
NY - 102	28	702-27	GEN-03	GD	PPO Suppression	2005
NY - 102	29	784-34	GEN-03	GS	PPO Suppression	2005
NY - 102	30	831-9	GEN-03	GD	PPO Suppression	2005
NY - 102	31	703-31	GEN-03	GD	PPO Suppression	2005
NY - 102	32	1001-42	Control	GS	Control	2005
NY - 102	33	554-1	GEN-02	GD	PPO Suppression	2005
NY - 102	34	801-36	GEN-03	GD	PPO Suppression	2005
NY - 102	35	831-6	GEN-03	GD	PPO Suppression	2005
NY - 102	36	801-25	GEN-03	GD	PPO Suppression	2005
NY - 102	37	615-1	GEN-02	GD	PPO Suppression	2005
NY - 102	38	604-1	GEN-02	GD	PPO Suppression	2005
NY - 102	39	1000-38	Control	GD	Control	2005
NY - 102	40	705-85	GEN-03	GD	PPO Suppression	2005
NY - 102	41	534-1	GEN-02	GD	PPO Suppression	2005
NY - 102	42	705-95	GEN-03	GD	PPO Suppression	2005
NY - 102	43	466-1	GEN-02	GD	PPO Suppression	2005
NY - 102	44	743-30	GEN-03	GD	PPO Suppression	2005
NY - 102	45	613-1	GEN-02	GD	PPO Suppression	2005
NY - 102	46	604-2	GEN-02	GD	PPO Suppression	2005
NY - 102	47	249-1	GEN-02	GD	PPO Suppression	2005
NY - 102	48	523-2	GEN-02	GD	PPO Suppression	2005
NY - 102	49	1000-39	Control	GD	Control	2005
NY - 102	50	702-41	GEN-03	GD	PPO Suppression	2005
NY - 102	51	601-1	GEN-02	GD	PPO Suppression	2005
NY - 102	52	427-2	GEN-02	NF	PPO Suppression	2005
NY - 102	53	246-1	GEN-02	GD	PPO Suppression	2005
NY - 102	54	605-2	GEN-02	GD	PPO Suppression	2005
NY - 102	55	601-2	GEN-02	GD	PPO Suppression	2005
NY - 102	56	811-19	GEN-03	GD	PPO Suppression	2005
NY - 102	57	273-2	GEN-02	GD	PPO Suppression	2005
NY - 102	58	743-63	GEN-03	GD	PPO Suppression	2005
NY - 102	59	554-2	GEN-02	GD	PPO Suppression	2005

[1] This tree (743-88) was removed prior to the 2006 field season.

[2] The plant name is a unique identifier for each tree, where the number preceding the dash is the transgenic event name and the number following the dash is the tree number (event name 1000 = GD control; event name 1001 = GS control).

Table 51: Map of the Washington Field Trial - 2004 Block

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA - 15	1	703-22	GEN-03	GD	PPO Suppression	2004	
Yes	WA - 15	2	784-29	GEN-03	GS	PPO Suppression	2004	
	WA - 15	3	743-35	GEN-03	GD	PPO Suppression	2004	
Yes	WA - 15	4	703-57	GEN-03	GD	PPO Suppression	2004	
	WA - 15	5	784-26	GEN-03	GS	PPO Suppression	2004	
	WA - 15	6	784-40	GEN-03	GS	PPO Suppression	2004	
	WA-15	7	784-44	GEN-03	GS	PPO Suppression	2004	
	WA-15	8	703-60	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	9	1001-34	Control	GS	Control	2004	Other
	WA-15	10	705-83	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	11	705-62	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	12	707-62	GEN-03	GD	PPO Suppression	2004	
	WA-15	13	705-78	GEN-03	GD	PPO Suppression	2004	
	WA-15	14	842-5	GEN-03	GD	PPO Suppression	2004	
	WA-15	15	784-42	GEN-03	GS	PPO Suppression	2004	
	WA-15	16	784-45	GEN-03	GS	PPO Suppression	2004	
	WA-15	17[1]	705-59	GEN-03	GD	PPO Suppression	2004	
	WA-15	18	831-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	19	784-31	GEN-03	GS	PPO Suppression	2004	
	WA-15	20	1000-42	Control	GD	Control	2004	
	WA-15	21	784-33	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	22	811-11	GEN-03	GD	PPO Suppression	2004	
	WA-15	23	784-50	GEN-03	GS	PPO Suppression	2004	Other
	WA-15	24	845-12	GEN-03	GD	PPO Suppression	2004	
	WA-15	25	784-38	GEN-03	GS	PPO Suppression	2004	
	WA-15	26	743-55	GEN-03	GD	PPO Suppression	2004	
	WA-15	27	743-64	GEN-03	GD	PPO Suppression	2004	
	WA-15	28	743-59	GEN-03	GD	PPO Suppression	2004	
	WA-15	29	811-10	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	30	872-9	GEN-03	NF	PPO Suppression	2004	
	WA-15	31	743-31	GEN-03	GD	PPO Suppression	2004	
	WA-15	32	1003-9	Control	PG	Control	2004	Other
	WA-15	33	842-7	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	34	872-5	GEN-03	NF	PPO Suppression	2004	
	WA-15	35	845-11	GEN-03	GD	PPO Suppression	2004	
	WA-15	36	880-2	GEN-03	PG	PPO Suppression	2004	Other
Yes	WA-15	37	879-3	GEN-03	PG	PPO Suppression	2004	
	WA-15	38	702-46	GEN-03	GD	PPO Suppression	2004	

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA-15	39	1003-7	Control	PG	Control	2004	
Yes	WA-15	40	872-8	GEN-03	NF	PPO Suppression	2004	
Yes	WA-15	41	885-1	GEN-03	PG	PPO Suppression	2004	
	WA-15	42	1003-8	Control	PG	Control	2004	Other
	WA-15	43	842-6	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	44	846-17	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	45	879-1	GEN-03	PG	PPO Suppression	2004	
	WA-15	46	831-14	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	47	1002-5	Control	NF	Control	2004	
	WA-15	48	831-12	GEN-03	GD	PPO Suppression	2004	
	WA-15	49	872-13	GEN-03	NF	PPO Suppression	2004	FB
	WA-15	50	784-28	GEN-03	GS	PPO Suppression	2004	
	WA-15	51	846-15	GEN-03	GD	PPO Suppression	2004	
	WA-15	52	705-61	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	53	743-68	GEN-03	GD	PPO Suppression	2004	
	WA-15	54	705-79	GEN-03	GD	PPO Suppression	2004	
	WA-15	55	784-66	GEN-03	GS	PPO Suppression	2004	
	WA-15	56	842-8	GEN-03	GD	PPO Suppression	2004	
	WA-15	57	784-65	GEN-03	GS	PPO Suppression	2004	
	WA-15	58	931-1	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	59	743-52	GEN-03	GD	PPO Suppression	2004	
	WA-15	60	831-13	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	61	1003-3	Control	PG	Control	2004	
Yes	WA-15	62	846-4	GEN-03	GD	PPO Suppression	2004	
	WA-15	63	845-14	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	64	872-10	GEN-03	NF	PPO Suppression	2004	
	WA-15	65	872-7	GEN-03	NF	PPO Suppression	2004	
	WA-15	66	784-61	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	67	1003-5	Control	PG	Control	2004	
	WA-15	68	784-46	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	69	885-2	GEN-03	PG	PPO Suppression	2004	
	WA-15	70	1003-11	Control	PG	Control	2004	
	WA-15	71	1001-30	Control	GS	Control	2004	
Yes	WA-15	72	846-1	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	73	784-60	GEN-03	GS	PPO Suppression	2004	
	WA-15	74	872-12	GEN-03	NF	PPO Suppression	2004	
	WA-15	75	1001-29	Control	GS	Control	2004	
	WA-15	76	872-11	GEN-03	NF	PPO Suppression	2004	
	WA-15	77	845-13	GEN-03	GD	PPO Suppression	2004	

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA-15	78	831-10	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	79	1002-7	Control	NF	Control	2004	
Yes	WA-15	80	1003-6	Control	PG	Control	2004	
Yes	WA-15	81	880-1	GEN-03	PG	PPO Suppression	2004	
Yes	WA-15	82	784-23	GEN-03	GS	PPO Suppression	2004	
	WA-15	83	784-21	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	84	846-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	85	784-22	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	86	703-52	GEN-03	GD	PPO Suppression	2004	
	WA-15	87	743-46	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	88	743-65	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	89	707-58	GEN-03	GD	PPO Suppression	2004	
	WA-15	90	743-62	GEN-03	GD	PPO Suppression	2004	
	WA-15	91	1000-15	Control	GD	Control	2004	
	WA-15	92	702-42	GEN-03	GD	PPO Suppression	2004	
	WA-15	93	784-41	GEN-03	GS	PPO Suppression	2004	Other
Yes	WA-15	94	743-60	GEN-03	GD	PPO Suppression	2004	
	WA-15	95	1001-35	Control	GS	Control	2004	
	WA-15	96	743-6	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	97	1001-36	Control	GS	Control	2004	Other
	WA-15	98	1001-37	Control	GS	Control	2004	Other
	WA-15	99	702-47	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	100	707-61	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	101	743-40	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	102	811-17	GEN-03	GD	PPO Suppression	2004	
	WA-15	103	784-48	GEN-03	GS	PPO Suppression	2004	
	WA-15	104	703-54	GEN-03	GD	PPO Suppression	2004	
	WA-15	105	811-18	GEN-03	GD	PPO Suppression	2004	
	WA-15	106	703-55	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	107	1001-33	Control	GS	Control	2004	
	WA-15	108	703-45	GEN-03	GD	PPO Suppression	2004	
	WA-15	109	811-16	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	110	1001-38	Control	GS	Control	2004	
Yes	WA-15	111	758-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	112	714-3	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	113	811-25	GEN-03	GD	PPO Suppression	2004	
	WA-15	114	743-26	GEN-03	GD	PPO Suppression	2004	
	WA-15	115	743-33	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	116	707-25	GEN-03	GD	PPO Suppression	2004	Other

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA-15	117	1000-11	Control	GD	Control	2004	
	WA-15	118	707-33	GEN-03	GD	PPO Suppression	2004	
	WA-15	119	703-26	GEN-03	GD	PPO Suppression	2004	
	WA-15	120	784-49	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	121	1000-13	Control	GD	Control	2004	
	WA-15	122	703-23	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	123	728-1	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	124	702-11	GEN-03	GD	PPO Suppression	2004	
	WA-15	125	1000-14	Control	GD	Control	2004	
Yes	WA-15	126	752-3	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	127	705-39	GEN-03	GD	PPO Suppression	2004	
	WA-15	128	707-17	GEN-03	GD	PPO Suppression	2004	FB
	WA-15	129	705-54	GEN-03	GD	PPO Suppression	2004	
	WA-15	130	743-34	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	131	705-26	GEN-03	GD	PPO Suppression	2004	
	WA-15	132	743-37	GEN-03	GD	PPO Suppression	2004	
	WA-15	133	1001-20	Control	GS	Control	2004	
Yes	WA-15	134	801-48	GEN-03	GD	PPO Suppression	2004	
	WA-15	135	784-20	GEN-03	GS	PPO Suppression	2004	
	WA-15	136	1001-13	Control	GS	Control	2004	Other
Yes	WA-15	137	702-13	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	138	1000-12	Control	GD	Control	2004	
	WA-15	139	743-48	GEN-03	GD	PPO Suppression	2004	
	WA-15	140	707-21	GEN-03	GD	PPO Suppression	2004	
	WA-15	141	705-50	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	142	705-52	GEN-03	GD	PPO Suppression	2004	
	WA-15	143	1001-18	Control	GS	Control	2004	
Yes	WA-15	144	707-38	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	145	702-14	GEN-03	GD	PPO Suppression	2004	
	WA-15	146	707-34	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	147	784-25	GEN-03	GS	PPO Suppression	2004	
Yes	WA-15	148	1001-15	Control	GS	Control	2004	
	WA-15	149	707-57	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	150	707-39	GEN-03	GD	PPO Suppression	2004	
	WA-15	151	707-47	GEN-03	GD	PPO Suppression	2004	
	WA-15	152	703-21	GEN-03	GD	PPO Suppression	2004	
	WA-15	153	773-12	GEN-03	GD	PPO Suppression	2004	
	WA-15	154	743-36	GEN-03	GD	PPO Suppression	2004	
	WA-15	155	705-70	GEN-03	GD	PPO Suppression	2004	

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA-15	156	703-20	GEN-03	GD	PPO Suppression	2004	
Yes	WA-15	157	801-43	GEN-03	GD	PPO Suppression	2004	
	WA-15	158	707-37	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	159	743-58	GEN-03	GD	PPO Suppression	2004	
	WA-15	160	743-42	GEN-03	GD	PPO Suppression	2004	
	WA-15	161	743-22	GEN-03	GD	PPO Suppression	2004	Other
	WA-15	162	709-3	GEN-03	GD	PPO Suppression	2004	Other
Yes	WA-15	163	1000-26	Control	GD	Control	2004	
	WA-15	164	703-44	GEN-03	GD	PPO Suppression	2004	
	WA-15	165	743-25	GEN-03	GD	PPO Suppression	2004	
	WA-15	166	1000-27	Control	GD	Control	2004	
Yes	WA-15	167	811-9	GEN-03	GD	PPO Suppression	2004	
	WA-15	168	702-20	GEN-03	GD	PPO Suppression	2004	
	WA-15	169	705-64	GEN-03	GD	PPO Suppression	2004	
	WA-15	170	702-21	GEN-03	GD	PPO Suppression	2004	
	WA-15	171	1000-30	Control	GD	Control	2004	Other
Yes	WA-15	172	702-18	GEN-03	GD	PPO Suppression	2004	
	WA-15	173	702-22	GEN-03	GD	PPO Suppression	2004	
	WA-15	174	702-23	GEN-03	GD	PPO Suppression	2004	
	WA-15	175	811-7	GEN-03	GD	PPO Suppression	2004	
	WA-15	176	709-16	GEN-03	GD	PPO Suppression	2004	
	WA-15	177	702-24	GEN-03	GD	PPO Suppression	2004	
	WA-15	178	811-4	GEN-03	GD	PPO Suppression	2004	
	WA-15	179	1000-28	Control	GD	Control	2004	
	WA-15	180	702-19	GEN-03	GD	PPO Suppression	2004	
	WA-15	181	743-57	GEN-03	GD	PPO Suppression	2004	
	WA-15	182	811-14	GEN-03	GD	PPO Suppression	2004	
	WA-15	183	811-3	GEN-03	GD	PPO Suppression	2004	
	WA-15	184	707-44	GEN-03	GD	PPO Suppression	2004	
	WA-15	185	811-15	GEN-03	GD	PPO Suppression	2004	
	WA-15	186	811-12	GEN-03	GD	PPO Suppression	2004	
	WA-15	187	1001-7	Control	GS	Control	2004	
	WA-15	188	811-13	GEN-03	GD	PPO Suppression	2004	
	WA-15	189	703-32	GEN-03	GD	PPO Suppression	2004	
	WA-15	190	705-57	GEN-03	GD	PPO Suppression	2004	
	WA-15	191	703-30	GEN-03	GD	PPO Suppression	2004	
	WA-15	192	709-8	GEN-03	GD	PPO Suppression	2004	
	WA-15	193	703-49	GEN-03	GD	PPO Suppression	2004	
	WA-15	194	702-35	GEN-03	GD	PPO Suppression	2004	FB

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA-15	195	702-9	GEN-03	GD	PPO Suppression	2004	
	WA-15	196	703-35	GEN-03	GD	PPO Suppression	2004	
	WA-15	197	709-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	198	703-50	GEN-03	GD	PPO Suppression	2004	
	WA-15	199	1001-14	Control	GS	Control	2004	
	WA-15	200	743-47	GEN-03	GD	PPO Suppression	2004	
	WA-15	201	705-73	GEN-03	GD	PPO Suppression	2004	
	WA-15	202	748-1	GEN-03	GD	PPO Suppression	2004	
	WA-15	203	707-36	GEN-03	GD	PPO Suppression	2004	
	WA-15	204	763-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	205	784-36	GEN-03	GS	PPO Suppression	2004	
	WA-15	206	705-27	GEN-03	GD	PPO Suppression	2004	
	WA-15	207	801-10	GEN-03	GD	PPO Suppression	2004	
	WA-15	208	1000-21	Control	GD	Control	2004	
	WA-15	209	703-27	GEN-03	GD	PPO Suppression	2004	
	WA-15	210	743-49	GEN-03	GD	PPO Suppression	2004	
	WA-15	211	1000-24	Control	GD	Control	2004	
	WA-15	212	705-71	GEN-03	GD	PPO Suppression	2004	
	WA-15	213	702-33	GEN-03	GD	PPO Suppression	2004	
	WA-15	214	801-9	GEN-03	GD	PPO Suppression	2004	
	WA-15	215	1000-33	Control	GD	Control	2004	
	WA-15	216	705-72	GEN-03	GD	PPO Suppression	2004	FB
	WA-15	217	704-1	GEN-03	GD	PPO Suppression	2004	
	WA-15	218	702-30	GEN-03	GD	PPO Suppression	2004	
	WA-15	219	801-11	GEN-03	GD	PPO Suppression	2004	
	WA-15	220	707-53	GEN-03	GD	PPO Suppression	2004	
	WA-15	221	1000-22	Control	GD	Control	2004	
	WA-15	222	702-26	GEN-03	GD	PPO Suppression	2004	
	WA-15	223	707-51	GEN-03	GD	PPO Suppression	2004	
	WA-15	224	705-56	GEN-03	GD	PPO Suppression	2004	
	WA-15	225	719-1	GEN-03	GD	PPO Suppression	2004	
	WA-15	226	753-1	GEN-03	GD	PPO Suppression	2004	
	WA-15	227	705-67	GEN-03	GD	PPO Suppression	2004	
	WA-15	228	703-46	GEN-03	GD	PPO Suppression	2004	
	WA-15	229	1000-19	Control	GD	Control	2004	
	WA-15	230	743-54	GEN-03	GD	PPO Suppression	2004	
	WA-15	231	703-48	GEN-03	GD	PPO Suppression	2004	FB
	WA-15	232	730-2	GEN-03	GD	PPO Suppression	2004	
	WA-15	233	702-28	GEN-03	GD	PPO Suppression	2004	

Washington (2004 Block)[2, 4]								
Flagged	**Trial - Row**	**Position**	**Plant Name[3]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA-15	234	707-55	GEN-03	GD	PPO Suppression	2004	
	WA-15	235	707-50	GEN-03	GD	PPO Suppression	2004	
	WA-15	236	702-29	GEN-03	GD	PPO Suppression	2004	
	WA-15	237	811-5	GEN-03	GD	PPO Suppression	2004	
	WA-15	238	707-20	GEN-03	GD	PPO Suppression	2004	
	WA-15	239	743-51	GEN-03	GD	PPO Suppression	2004	
	WA-15	240	743-56	GEN-03	GD	PPO Suppression	2004	
	WA-15	241	709-11	GEN-03	GD	PPO Suppression	2004	
	WA-15	242	743-53	GEN-03	GD	PPO Suppression	2004	

[1] FB: Removed due to fireblight on September 25, 2007; Other: Trees are removed during the growing season for a variety of reasons, mostly commonly transplant mortality, rodent or mechanical damage. Trees noted here as 'other' were removed during the 2009 field season.

[2] Trees in Washington – 15, Positions 76 – 92 are the Control Block of trees for Pest and Disease monitoring (See Section 6.1.3). These trees are monitored on a weekly basis. If pest or disease is detected within this Control Block it triggers the collection of pest and disease data from all flagged trees in the Washington trial (WA2004, WA2008).

[3] The plant name is a unique identifier for each tree, where the number preceding the dash is the transgenic event name and the number following the dash is the tree number (event name 1000 = GD control; event name 1001 = GS control).

[4] Removed: Block of trees removed from position 179 to 244.

Table 52: Map of the Washington Field Trial - 2008 Block

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	2	801-7	GEN-03	GD	PPO Suppression	2004	
	WA - 14	3	142-93-1	OSF-01	PG	PPO Suppression	2008	
	WA - 14	4	OSF.FJ.1-21	OSF-01	NF	PPO Suppression	2008	
	WA - 14	5	GS 18-2-2	OBI-04	GS	PPO Suppression	2008	Other
	WA - 14	6	OSF.FJ.1-1	OSF-01	NF	PPO Suppression	2008	
	WA - 14	7	OSF.FJ.1-4	OSF-01	NF	PPO Suppression	2008	
	WA - 14	8	OSF.FJ.1-27	OSF-01	NF	PPO Suppression	2008	
	WA - 14	9	OSF.FJ.1-3	OSF-01	NF	PPO Suppression	2008	
	WA - 14	10	GS 18-2-1	OBI-04	GS	PPO Suppression	2008	
	WA - 14	11	165-22-2	OSF-01	PG	PPO Suppression	2008	
	WA - 14	12	OSF.FJ.1-2	OSF-01	NF	PPO Suppression	2008	
	WA - 14	13	165-22-5	OSF-01	PG	PPO Suppression	2008	
	WA - 14	14	OSF.FJ.1-28	OSF-01	NF	PPO Suppression	2008	
	WA - 14	15	OSF.FJ.1-14	OSF-01	NF	PPO Suppression	2008	
	WA - 14	16	OSF.FJ.2-3	OSF-01	NF	PPO Suppression	2008	
	WA - 14	17	OSF.PG.3-2	OSF-01	PG	PPO Suppression	2008	
	WA - 14	18	OSF.FJ.1-15	OSF-01	NF	PPO Suppression	2008	
	WA - 14	19	OSF.FJ.1-13	OSF-01	NF	PPO Suppression	2008	
	WA - 14	20	OSF.FJ.1-19	OSF-01	NF	PPO Suppression	2008	
	WA - 14	21	OSF.FJ.1-18	OSF-01	NF	PPO Suppression	2008	
	WA - 14	22	OSF.FJ.1-29	OSF-01	NF	PPO Suppression	2008	
	WA - 14	23	No Tree					
	WA - 14	24	OSF.FJ.1-20	OSF-01	NF	PPO Suppression	2008	
	WA - 14	25	OSF.PG.3-1	OSF-01	PG	PPO Suppression	2008	
	WA - 14	26	OSF.FJ.2-1	OSF-01	NF	PPO Suppression	2008	
	WA - 14	27	OSF.FJ.1-30	OSF-01	NF	PPO Suppression	2008	
	WA - 14	28	OSF.FJ.1-16	OSF-01	NF	PPO Suppression	2008	
	WA - 14	29	OSF.FJ.2-4	OSF-01	NF	PPO Suppression	2008	
	WA - 14	30	OSF.FJ.1-25	OSF-01	NF	PPO Suppression	2008	
	WA - 14	31	OSF.FJ.1-8	OSF-01	NF	PPO Suppression	2008	
	WA - 14	32	OSF.FJ.1-26	OSF-01	NF	PPO Suppression	2008	
	WA - 14	33	OSF.FJ.1-22	OSF-01	NF	PPO Suppression	2008	
	WA - 14	34	OSF.FJ.1-17	OSF-01	NF	PPO Suppression	2008	
	WA - 14	35	OSF.FJ.1-10	OSF-01	NF	PPO Suppression	2008	
	WA - 14	36	OSF.FJ.1-11	OSF-01	NF	PPO Suppression	2008	
	WA - 14	37	OSF.FJ.1-7	OSF-01	NF	PPO Suppression	2008	
	WA - 14	38	142-93-2	OSF-01	PG	PPO Suppression	2008	
	WA - 14	39	No Tree					
	WA - 14	40	801-26	GEN-03	GD	PPO Suppression	2004	
	WA - 14	41	801-39	GEN-03	GD	PPO Suppression	2004	

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	42	OSF.FJ.1-2	OSF-01	NF	PPO Suppression	2008	
	WA - 14	43	OSF.PG.3-4	OSF-01	PG	PPO Suppression	2008	
	WA - 14	44	OSF.FJ.1-9	OSF-01	NF	PPO Suppression	2008	
	WA - 14	45	OSF.FJ.1-6	OSF-01	NF	PPO Suppression	2008	
	WA - 14	46	OSF.FJ.1-23	OSF-01	NF	PPO Suppression	2008	
	WA - 14	47	OSF.FJ.1-5	OSF-01	NF	PPO Suppression	2008	
	WA - 14	48	OSF.PG.3-3	OSF-01	PG	PPO Suppression	2008	
	WA - 14	49	GS 18-2-4	OBI-04	GS	PPO Suppression	2008	Other
	WA - 14	50	142-93-3	OSF-01	PG	PPO Suppression	2008	
	WA - 14	51	GS 18-2-3	OBI-04	GS	PPO Suppression	2008	
	WA - 14	52	No Tree					
	WA - 14	53	142-93-4	OSF-01	PG	PPO Suppression	2008	
	WA - 14	54	No Tree					
	WA - 14	55	707-73	GEN-03	GD	PPO Suppression	2008	
	WA - 14	56	707-74	GEN-03	GD	PPO Suppression	2008	
	WA - 14	57	707-75	GEN-03	GD	PPO Suppression	2008	
	WA - 14	58	707-76	GEN-03	GD	PPO Suppression	2008	
	WA - 14	59	No Tree					
	WA - 14	60	707-77	GEN-03	GD	PPO Suppression	2008	
	WA - 14	61	707-78	GEN-03	GD	PPO Suppression	2008	
	WA - 14	62	707-79	GEN-03	GD	PPO Suppression	2008	
	WA - 14	63	801-47	GEN-03	GD	PPO Suppression	2004	
	WA - 14	64	707-80	GEN-03	GD	PPO Suppression	2008	
	WA - 14	65	707-81	GEN-03	GD	PPO Suppression	2008	
	WA - 14	66	707-82	GEN-03	GD	PPO Suppression	2008	
	WA - 14	67	707-83	GEN-03	GD	PPO Suppression	2008	
	WA - 14	68	707-84	GEN-03	GD	PPO Suppression	2008	
	WA - 14	69	707-85	GEN-03	GD	PPO Suppression	2008	
	WA - 14	70	707-86	GEN-03	GD	PPO Suppression	2008	
	WA - 14	71	707-87	GEN-03	GD	PPO Suppression	2008	
	WA - 14	72	707-89	GEN-03	GD	PPO Suppression	2008	
	WA - 14	73	707-88	GEN-03	GD	PPO Suppression	2008	
	WA - 14	74	707-90	GEN-03	GD	PPO Suppression	2008	
	WA - 14	75	707-91	GEN-03	GD	PPO Suppression	2008	
	WA - 14	76	707-92	GEN-03	GD	PPO Suppression	2008	
	WA - 14	77	707-93	GEN-03	GD	PPO Suppression	2008	
	WA - 14	78	707-94	GEN-03	GD	PPO Suppression	2008	
	WA - 14	79	707-95	GEN-03	GD	PPO Suppression	2008	
	WA - 14	80	707-96	GEN-03	GD	PPO Suppression	2008	
	WA - 14	81	707-98	GEN-03	GD	PPO Suppression	2008	
	WA - 14	82	707-97	GEN-03	GD	PPO Suppression	2008	

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	83	705-98	GEN-03	GD	PPO Suppression	2008	
	WA - 14	84	705-99	GEN-03	GD	PPO Suppression	2008	
	WA - 14	85	705-100	GEN-03	GD	PPO Suppression	2008	
	WA - 14	86	705-101	GEN-03	GD	PPO Suppression	2008	
	WA - 14	87	705-102	GEN-03	GD	PPO Suppression	2008	
	WA - 14	88	705-103	GEN-03	GD	PPO Suppression	2008	
	WA - 14	89	705-104	GEN-03	GD	PPO Suppression	2008	
	WA - 14	90	801-35	GEN-03	GD	PPO Suppression	2004	
	WA - 14	91	801-41	GEN-03	GD	PPO Suppression	2004	
	WA - 14	92	705-105	GEN-03	GD	PPO Suppression	2008	
	WA - 14	93	705-106	GEN-03	GD	PPO Suppression	2008	
	WA - 14	94	705-107	GEN-03	GD	PPO Suppression	2008	
	WA - 14	95	705-108	GEN-03	GD	PPO Suppression	2008	
	WA - 14	96	705-109	GEN-03	GD	PPO Suppression	2008	
	WA - 14	97	705-110	GEN-03	GD	PPO Suppression	2008	
	WA - 14	98	705-111	GEN-03	GD	PPO Suppression	2008	
	WA - 14	99	705-112	GEN-03	GD	PPO Suppression	2008	
	WA - 14	100	705-113	GEN-03	GD	PPO Suppression	2008	
	WA - 14	101	705-114	GEN-03	GD	PPO Suppression	2008	
	WA - 14	102	705-115	GEN-03	GD	PPO Suppression	2008	
	WA - 14	103	No Tree					
	WA - 14	104	801-38	GEN-03	GD	PPO Suppression	2004	
	WA - 14	105	No Tree					
	WA - 14	106	801-33	GEN-03	GD	PPO Suppression	2004	
	WA - 14	107	No Tree					
	WA - 14	108	705-116	GEN-03	GD	PPO Suppression	2008	
	WA - 14	109	No Tree					
	WA - 14	110	705-117	GEN-03	GD	PPO Suppression	2008	
	WA - 14	111	792-1	GEN-03	GD	PPO Suppression	2004	
	WA - 14	112	705-118	GEN-03	GD	PPO Suppression	2008	
	WA - 14	113	705-119	GEN-03	GD	PPO Suppression	2008	
	WA - 14	114	705-120	GEN-03	GD	PPO Suppression	2008	
	WA - 14	115	705-121	GEN-03	GD	PPO Suppression	2008	
	WA - 14	116	705-122	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	117	784-116	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	118	784-117	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	119	784-118	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	120	784-119	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	121	784-120	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	122	784-121	GEN-03	GS	PPO Suppression	2008	
	WA - 14	123	792-2	GEN-03	GD	PPO Suppression	2004	

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
Yes	WA - 14	124	784-122	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	125	784-123	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	126	784-124	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	127	784-125	GEN-03	GS	PPO Suppression	2008	
	WA - 14	128	784-126	GEN-03	GS	PPO Suppression	2008	
	WA - 14	129	784-127	GEN-03	GS	PPO Suppression	2008	
	WA - 14	130	784-128	GEN-03	GS	PPO Suppression	2008	
	WA - 14	131	784-129	GEN-03	GS	PPO Suppression	2008	
	WA - 14	132	784-130	GEN-03	GS	PPO Suppression	2008	
	WA - 14	133	No Tree					
	WA - 14	134	784-131	GEN-03	GS	PPO Suppression	2008	
	WA - 14	135	784-132	GEN-03	GS	PPO Suppression	2008	
	WA - 14	136	784-133	GEN-03	GS	PPO Suppression	2008	
	WA - 14	137	784-134	GEN-03	GS	PPO Suppression	2008	
	WA - 14	138	784-135	GEN-03	GS	PPO Suppression	2008	
	WA - 14	139	784-136	GEN-03	GS	PPO Suppression	2008	
	WA - 14	140	784-137	GEN-03	GS	PPO Suppression	2008	
	WA - 14	141	784-138	GEN-03	GS	PPO Suppression	2008	
	WA - 14	142	784-139	GEN-03	GS	PPO Suppression	2008	
	WA - 14	143	784-140	GEN-03	GS	PPO Suppression	2008	
	WA - 14	144	784-142	GEN-03	GS	PPO Suppression	2008	
	WA - 14	145	784-143	GEN-03	GS	PPO Suppression	2008	
	WA - 14	146	784-144	GEN-03	GS	PPO Suppression	2008	
Yes	WA - 14	147	743-104	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	148	743-107	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	149	743-108	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	150	743-109	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	151	743-110	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	152	743-111	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	153	743-112	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	154	743-113	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	155	743-114	GEN-03	GD	PPO Suppression	2008	
Yes	WA - 14	156	743-115	GEN-03	GD	PPO Suppression	2008	
	WA - 14	157	743-116	GEN-03	GD	PPO Suppression	2008	
	WA - 14	158	743-117	GEN-03	GD	PPO Suppression	2008	
	WA - 14	159	743-118	GEN-03	GD	PPO Suppression	2008	
	WA - 14	160	743-119	GEN-03	GD	PPO Suppression	2008	
	WA - 14	161	743-120	GEN-03	GD	PPO Suppression	2008	
	WA - 14	162	743-122	GEN-03	GD	PPO Suppression	2008	
	WA - 14	163	743-123	GEN-03	GD	PPO Suppression	2008	
	WA - 14	164	743-124	GEN-03	GD	PPO Suppression	2008	

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	165	743-125	GEN-03	GD	PPO Suppression	2008	
	WA - 14	166	743-126	GEN-03	GD	PPO Suppression	2008	
	WA - 14	167	743-127	GEN-03	GD	PPO Suppression	2008	
	WA - 14	168	743-128	GEN-03	GD	PPO Suppression	2008	
	WA - 14	169	743-129	GEN-03	GD	PPO Suppression	2008	
	WA - 14	170	743-130	GEN-03	GD	PPO Suppression	2008	
	WA - 14	171	743-106	GEN-03	GD	PPO Suppression	2004	
Yes	WA - 14	172	1001-114	Control	GS	Control	2008	
Yes	WA - 14	173	1001-115	Control	GS	Control	2008	
Yes	WA - 14	174	1001-116	Control	GS	Control	2008	
Yes	WA - 14	175	1001-117	Control	GS	Control	2008	
Yes	WA - 14	176	1001-119	Control	GS	Control	2008	
Yes	WA - 14	177	1001-120	Control	GS	Control	2008	
Yes	WA - 14	178	1001-122	Control	GS	Control	2008	
Yes	WA - 14	179	1001-123	Control	GS	Control	2008	
Yes	WA - 14	180	1001-124	Control	GS	Control	2008	
Yes	WA - 14	181	1001-126	Control	GS	Control	2008	
	WA - 14	182	1001-128	Control	GS	Control	2008	Other
	WA - 14	183	1001-130	Control	GS	Control	2008	
	WA - 14	184	1001-132	Control	GS	Control	2008	
	WA - 14	185	1001-133	Control	GS	Control	2008	
	WA - 14	186	1001-134	Control	GS	Control	2008	
	WA - 14	187	1001-135	Control	GS	Control	2008	
	WA - 14	188	1001-137	Control	GS	Control	2008	
	WA - 14	189	1001-138	Control	GS	Control	2008	
	WA - 14	190	1001-122	Control	GS	Control	2004	
	WA - 14	191	1000-106	Control	GD	Control	2008	
	WA - 14	192	1000-118	Control	GD	Control	2008	
Yes	WA - 14	193	1000-167	Control	GD	Control	2008	
Yes	WA - 14	194	1000-168	Control	GD	Control	2008	
Yes	WA - 14	195	1000-169	Control	GD	Control	2008	
	WA - 14	196	792-6	GEN-03	GD	PPO Suppression	2004	
Yes	WA - 14	197	1000-170	Control	GD	Control	2008	
Yes	WA - 14	198	1000-171	Control	GD	Control	2008	
Yes	WA - 14	199	1000-172	Control	GD	Control	2008	
Yes	WA - 14	200	1000-173	Control	GD	Control	2008	
Yes	WA - 14	201	1000-174	Control	GD	Control	2008	
Yes	WA - 14	202	1000-175	Control	GD	Control	2008	
	WA - 14	203	792-5	GEN-03	GD	PPO Suppression	2004	
Yes	WA - 14	204	1000-176	Control	GD	Control	2008	
	WA - 14	205	792-8	GEN-03	GD	PPO Suppression	2004	

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	206	792-7	GEN-03	GD	PPO Suppression	2004	
	WA - 14	207	1000-177	Control	GD	Control	2008	
	WA - 14	208	792-9	GEN-03	GD	PPO Suppression	2004	
	WA - 14	209	1000-178	Control	GD	Control	2008	
	WA - 14	210	1000-179	Control	GD	Control	2008	
	WA - 14	211	1000-180	Control	GD	Control	2008	
	WA - 14	212	1000-181	Control	GD	Control	2008	
	WA - 14	213	1000-182	Control	GD	Control	2008	
	WA - 14	214	1000-183	Control	GD	Control	2008	
	WA - 14	215	1000-184	Control	GD	Control	2008	
	WA - 14	216	1000-185	Control	GD	Control	2008	
	WA - 14	217	1000-186	Control	GD	Control	2008	
	WA - 14	218	1000-187	Control	GD	Control	2008	
	WA - 14	219	281-11-1	OSF-02	PG	PPO Suppression	2009	
	WA - 14	220	277-55-1	OSF-02	PG	PPO Suppression	2009	
	WA - 14	221	277-19-4	OSF-02	PG	PPO Suppression	2009	
	WA - 14	222	277-19-3	OSF-02	PG	PPO Suppression	2009	
	WA - 14	223	281-11-8	OSF-02	PG	PPO Suppression	2009	
	WA - 14	224	277-19-1	OSF-02	PG	PPO Suppression	2009	
	WA - 14	225	277-19-7	OSF-02	PG	PPO Suppression	2009	
	WA - 14	226	277-19-2	OSF-02	PG	PPO Suppression	2009	
	WA - 14	227	GD Control-5	Control	GD	Control	2009	
	WA - 14	228	277-55-6	OSF-02	PG	PPO Suppression	2009	
	WA - 14	229	277-94-5	OSF-02	PG	PPO Suppression	2009	
	WA - 14	230	277-19-6	OSF-02	PG	PPO Suppression	2009	
	WA - 14	231	277-94-7	OSF-02	PG	PPO Suppression	2009	
	WA - 14	232	277-55-4	OSF-02	PG	PPO Suppression	2009	
	WA - 14	233	277-19-8	OSF-02	PG	PPO Suppression	2009	
	WA - 14	234	277-19-5	OSF-02	PG	PPO Suppression	2009	
	WA - 14	235	222-1-6	OSF-01	GS	PPO Suppression	2009	
	WA - 14	236	221-7-1	OSF-01	PG	PPO Suppression	2009	
	WA - 14	237	277-55-8	OSF-02	PG	PPO Suppression	2009	
	WA - 14	238	283-1-2	OSF-02	GD	PPO Suppression	2009	
	WA - 14	239	281-11-9	OSF-02	PG	PPO Suppression	2009	Cold
	WA - 14	240	277-55-5	OSF-02	PG	PPO Suppression	2009	
	WA - 14	241	GS Control-2	Control	GS	Control	2009	Cold
	WA - 14	242	277-130-2	OSF-02	PG	PPO Suppression	2009	
	WA - 14	243	277-94-6	OSF-02	PG	PPO Suppression	2009	
	WA - 14	244	277-101-1	OSF-02	PG	PPO Suppression	2009	
	WA - 14	245	277-69-3	OSF-02	PG	PPO Suppression	2009	
	WA - 14	246	No Tree					

Washington (2008 Block)								
Flagged	**Trial - Row**	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**	**Removed[1]**
	WA - 14	247	No Tree					
	WA - 14	248	No Tree					
	WA - 14	249	No Tree					
	WA - 14	250	No Tree					
	WA - 14	250	739-9	GEN-03	GS	PPO Suppression	2008	
	WA - 14	251	739-2	GEN-03	GS	PPO Suppression	2008	
[1] Cold: Removed throughout the 2011 growing season due to damage caused by extreme cold. Other: Trees are removed during the growing season for a variety of reasons, mostly commonly transplant mortality, rodent or mechanical damage. Trees noted here as 'other' were removed at the beginning of the 2011 field season. [2] The plant name is a unique identifier for each tree, where the number preceding the dash is the transgenic event name and the number following the dash is the tree number (event name 1000 = GD control; event name 1001 = GS control).								

Appendix 3: Pest and Disease Data – Field Reports

Table 53: Field Report - Powdery Mildew - WA2008 - April 19, 2010

Field Trial:	Washington GEN-03 (2008)	Date of Incident:	April 19, 2010
Pest / Disease:	**Powdery Mildew (PM)**		
Data Collected:	The control block of trees was inspected for the incidence of Powdery Mildew (PM) by a top to bottom visual scan of each tree. A PM incident would be defined as a single or multiple spore lesions on the leaf surface. If a PM incident was discovered it triggered the inspection of all of the flagged trees within the Washington field trial. Ten actively growing shoots from each tree were inspected for the incidence of PM. If single or multiple lesions were detected the shoot was counted as infected. Total = number of shoots.		
Incident Summary:	PM appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (7.5%) than Granny Smith (GS + GS784) (3.5%). However, Chi-square test revealed that the percentage of PM affected shoots did not significantly differ by cultivar (X^2(1, N=400) =3.08, P>0.05). PM infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GD743 and GD (X^2(1, N=200) =0.65, P>0.3). Similarly, Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GS784 and GS (X^2(1, N=200) = 0.59, P>0.3).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	15	185	200
GS + GS784	7	193	200
Total	22	378	400
Chi Square =	3.08	P > 0.05	
df =	1		
Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	9	91	100
GD Control	6	94	100
Total	15	185	200
Chi Square =	0.65	P > 0.3	
df =	1		
Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	5	95	100
GS Control	2	98	100
Total	7	193	200
Chi Square (Yates) =	0.59	P > 0.3	
df =	1		

Table 54: Field Report - Powdery Mildew - NY2005 - July 21, 2008

Field Trial:	New York GEN-03 (2005)	Date of Incident:	July 21, 2008
Pest / Disease:	**Powdery Mildew (PM)**		
Data Collected:	PM infection was assayed according to a rating scale where 1 < 10%, 2 = 10 – 50%, and 3 > 50%. The percentages refer to the percent (%) of shoot tips colonized by PM (data not shown). Since the rate of infection was similar (<10%) in the PM infected trees, the number of trees infected is reported here. Total = number of trees.		
Incident Summary:	PM was very light affecting only GS events. However, Chi-square (Yates) test revealed that the percentage of PM affected trees did not differ significantly cultivar (X^2(1, N=22) =0.37, P>0.5). PM infection was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly unaffected by PM. Similarly, Chi-square (Yates) test revealed that the percentage of PM infected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0.6, P>0.3).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	0	10	10
GS + GS784	2	10	12
Total	2	20	22
Chi Square (Yates) =	0.37	P > 0.5	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	0	5	5
GD Control	0	5	5
Total	0	10	10
Chi Square =		P not calculated	
df =			

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	1	5	6
GS Control	1	5	6
Total	2	10	12
Chi Square (Yates) =	0.6	P > 0.3	
df =	1		

Table 55: Field Report - Powdery Mildew - WA2008 - August 16, 2010

Field Trial:	Washington GEN-03 (2008)	Date of Incident:	August 16, 2010
Pest / Disease:	**Powdery Mildew (PM)**		
Data Collected:	The control block of trees was inspected for the incidence of Powdery Mildew (PM) by a top to bottom visual scan of each tree. A PM incident would be defined as a single or multiple spore lesions on the leaf surface. If a PM incident was discovered it triggered the inspection of all of the flagged trees within the Washington field trial. Ten actively growing shoots from each tree were inspected for the incidence of PM. If single or multiple lesions were detected the shoot was counted as infected. Total = number of shoots.		
Incident Summary:	PM does not appear to be cultivar specific with the rate of PM incidence in Golden Delicious (GD + GD743) (11.7%) than Granny Smith (GS + GS784) (13.6%) being very similar. Chi-square test revealed that the percentage of PM affected shoots did not significantly differ by cultivar (X^2(1, N=400) =0.23, P>0.5). PM infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GD743 and GD (X^2(1, N=200) =2.61, P>0.1). Similarly, Chi-square test revealed that the percentage of PM infected shoots did not significantly differ between GS784 and GS (X^2(1, N=200) =3.03, P>0.05).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	21	179	200
GS + GS784	24	176	200
Total	45	355	400
Chi Square =	0.23	P > 0.5	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	7	93	100
GD Control	14	86	100
Total	21	179	200
Chi Square =	2.61	P > 0.1	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	8	92	100
GS Control	16	84	100
Total	24	176	200
Chi Square =	3.03	P > 0.05	
df =	1		

Table 56: Field Report - Fireblight

Field Trial:	Washington GEN-03 (2003, 2004, 2008)	Date of Incident:	2005, 2006, 2007
Pest / Disease:	**Fireblight (FB)**		
Data Collected:	Field blocks WA2003, WA2004 and WA2008 have been routinely checked for the presence of FB. The presence (+) or absence (-) of Fireblight is assessed. The number of FB affected trees is reported here. In response to the detection of FB, trees may be pruned to remove infected wood (dormant pruning) or the tree itself may be removed. A common practice is to remove the fireblight affected tree plus one tree on either side to prevent spread of the disease. The removal of these additional trees is not reported in this data set as they are not confirmed as FB affected. Total = number of trees.		
Incident Summary:	FB was detected in at a higher rate in Golden Delicious (GD + GD743) (4% of trees) than in Granny Smith (GS + GS784) (0% of trees). However, Chi-square (Yates) test revealed that the percentage of FB affected trees did not significantly differ by cultivar (X^2(1, N=327) =1.61, P>0.2). FB was not influenced by incorporation of the GEN-03. Chi-square (Yates) test revealed that the percentage of FB affected trees did not significantly differ between GEN-03 events (GD702, GD703, GD705, GD707 and GD743) and the untransformed control GD (X^2(1, N=258) =0.34 P>0.5). Both GS874 and GS were similarly unaffected by FB. FB has never been detected in the WA2008 block.		

By Cultivar			
Event Name[1]	Infected	Not Infected	Total[2]
GD + GD743 (plus)	10	248	258
GS + GS784	0	69	69
Total	10	317	327
Chi Square (Yates) =	1.61	P > 0.2	
df =	1		
Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743 (plus)	8	225	233
GD Control	2	23	25
Total	10	248	258
Chi Square (Yates) =	0.34	P > 0.5	
df =	1		
Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	44	44
GS Control	0	25	25
Total	0	69	69
Chi Square =		P not calculated	
df =			

[1] GD743 (plus) = GD743 plus additional PPO suppressed GEN-03 events GD702, GD703, GD705, and GD707.
[2] FB was only ever detected in the WA20003 and WA2004. The FB affected trees reported here and summarized in Table 26 are all from the WA2003 and WA2004 blocks. Therefore the totals reported are for the total number of trees for the selected Event Name in the WA2003 and WA2004 blocks.

Table 57: Field Report - Aphids - NY2005 - November 19, 2009

Field Trial:	New York GEN-03 (2005)	Date of Incident:	November 19, 2009
Pest / Disease:	**Aphids**		
Data Collected:	Aphids are assayed as 1 < 10%, 2 = 10 – 50%, 3 > 50% (data not shown). The percentages refer to the percent of shoot tips colonized by aphids. Aphid infection was rated at > 50% in 21/22 trees assessed (Arctic™ Apple and Control). Therefore, the number of trees infected is reported here. Total = number of trees.		
Incident Summary:	Aphids were present throughout the NY field trial in the Fall of 2009. The rate of aphid infection did not appear to be influenced by affected by cultivar or PPO suppression.		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	10	0	10
GS + GS784	12	0	12
Total	22	0	22
Chi Square =		P not calculated	
df =			

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	5	0	5
GD Control	5	0	5
Total	10	0	10
Chi Square =		P not calculated	
df =			

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	6	0	6
GS Control	6	0	6
Total	12	0	12
Chi Square =		P not calculated	
df =			

Table 58: Field Report - Green Apple Aphid - WA2004 - August 9, 2010

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	August 9, 2010
Pest / Disease:	**Green Apple Aphid (GAA)**		
Data Collected:	The control block of trees was inspected for the presence of any live Green Apple Aphid (GAA) on actively growing shoots by a top to bottom visual scan of each tree. If GAA were present in the control trees it triggered inspection of all the flagged trees within the Washington field trial. Ten actively growing shoots per tree were observed for the presence of GAA. If a shoot had one or more GAA present it was counted as infected. Total = number of shoots.		
Incident Summary:	GAA appeared to be cultivar specific with a higher rate of GAA incidence in Golden Delicious (GD + GD743) (22.5%) than in Granny Smith (GS + GS784) (14.3%). However, Chi-square test revealed that the percentage of GAA affected shoots did not significantly differ by cultivar ($X^2(1, N=150) =2.14, P>0.1$). GAA infection was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of GAA infected shoots did not significantly differ between GD743 and GD ($X^2(1, N=80) =0.62, P>0.3$). Similarly, Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GS784 and GS ($X^2(1, N=70) =0.70, P>0.3$).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	19	61	80
GS + GS784	10	60	70
Total	29	121	150
Chi Square =	2.14	P > 0.1	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	11	29	40
GD Control	8	32	40
Total	19	61	80
Chi Square =	0.62	P > 0.3	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	4	36	40
GS Control	6	24	30
Total	10	60	70
Chi Square (Yates) =	0.70	P > 0.3	
df =	1		

Table 59: Field Report - Green Apple Aphid - WA2004 - August 16, 2010

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	August 16, 2010
Pest / Disease:	**Green Apple Aphid (GAA)**		
Data Collected:	The control block of trees was inspected for the presence of any live Green Apple Aphid (GAA) on actively growing shoots by a top to bottom visual scan of each tree. If GAA were present in the control trees it triggered inspection of all the flagged trees within the Washington field trial. Ten actively growing shoots per tree were observed for the presence of GAA. If a shoot had one or more GAA present it was counted as infected. Total = number of shoots.		
Incident Summary:	GAA was not cultivar specific with a similar rate of GAA incidence in Golden Delicious (GD + GD743) (6.3%) and Granny Smith (GS + GS784) (4.3%). Chi-square (Yates) test revealed that the percentage of GAA affected shoots did not significantly differ by cultivar (X^2(1, N=150) =0.03, P>0.8). GAA infection was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GD743 and GD (X^2(1, N=80) =0, P>0.95). Similarly, Chi-square (Yates) test revealed that the percentage of GAA infected shoots did not significantly differ between GS784 and GS (X^2(1, N=70) =0.88, P>0.3).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	5	75	80
GS + GS784	3	67	70
Total	8	142	150
Chi Square (Yates) =	0.03	P > 0.8	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	2	38	40
GD Control	3	37	40
Total	5	75	80
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	3	37	40
GS Control	0	30	30
Total	3	67	70
Chi Square (Yates) =	0.88	P > 0.3	
df =	1		

Table 60: Field Report - Woolly Aphids - WA2004 - July 12, 2010

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	July 12, 2010
Pest / Disease:	**Woolly Aphids**		
Data Collected:	The control block of trees was inspected for the presence of any live Woolly Aphids on actively growing shoots by a top to bottom visual scan of each tree. If Woolly Aphids were present in the control trees it triggered inspection of all the flagged trees within the Washington field trial. Ten actively growing shoots per tree were observed for the presence of Woolly Aphids. If a shoot had one or more Woolly Aphids present it was counted as infected. Total = number of shoots.		
Incident Summary:	Woolly Aphid appears to be cultivar specific with a higher rate of incidence in Golden Delicious (GD + GD743) (11.3%) than in Granny Smith (GS + GS784) (1.4%). Chi-square (Yates) test revealed that the percentage of Woolly Aphid affected shoots differed significantly by cultivar (X^2(1, N=150) =4.32, P<0.05). Woolly Aphid infection was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Woolly Aphids infected shoots did not significantly differ between GD743 and GD (X^2(1, N=80) =0, P>0.95). Similarly, Chi-square (Yates) test revealed that the percentage of Woolly Aphid infected shoots did not significantly differ between GS784 and GS (X^2(1, N=70) =0.02, P>0.8).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	9	71	80
GS + GS784	1	69	70
Total	10	140	150
Chi Square (Yates) =	4.32	P < 0.05	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	5	35	40
GD Control	4	36	40
Total	9	71	80
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	30	30
GS Control	1	39	40
Total	1	69	70
Chi Square (Yates) =	0.02	P > 0.8	
df =	1		

Table 61: Field Report - Japanese Beetle - NY2005 - July 21, 2008

Field Trial:	New York GEN-03 (2005)	Date of Incident:	July 21, 2008
Pest / Disease:	**Japanese Beetle (JB)**		
Data Collected:	Japanese Beetle infection is assessed as 1 = light, 2 = moderate, 3 = severe, where: Scale 1: 1 – 5 Japanese Beetles; 1 – 5 % LS Scale 2: 6 – 10 Japanese Beetles; 6 – 10 % LS Scale 3: > 10 Japanese Beetles; > 10 % LS LS = Leaf Skeletonization The rate of infection was similar (light) in the infected trees. Therefore, the number of trees infected is reported. Total = number of trees.		
Incident Summary:	JB was not cultivar specific with a similar level of incidence in Golden Delicious (GD + GD743) (30%) and Granny Smith (GS + GS784) (25%). Chi-square (Yates) test revealed that the percentage of JB affected trees did not significantly differ by cultivar ($X^2(1, N=22) =0.05$, $P>0.8$). JB was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GD743 and GD ($X^2(1, N=10) =0$, $P>0.95$). Similarly, Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GS784 and GS ($X^2(1, N=12) =0$, $P>0.95$).		

By Cultivar

Event Name	Infected	Not Infected	Total
GD + GD743	3	7	10
GS + GS784	3	9	12
Total	6	16	22
Chi Square (Yates) =	0.05	P > 0.8	
df =	1		

Golden Delicious

Event Name	Infected	Not Infected	Total
GD743	1	4	5
GD Control	2	3	5
Total	3	7	10
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Granny Smith

Event Name	Infected	Not Infected	Total
GS784	1	5	6
GS Control	2	4	6
Total	3	9	12
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Table 62: Field Report - Japanese Beetle - NY2005 - July 21, 2010

Field Trial:	New York GEN-03 (2005)	Date of Incident:	July 21, 2010
Pest / Disease:	Japanese Beetle		
Data Collected:	Japanese Beetle infection is assessed as 1 = light, 2 = moderate, 3 = severe, where: Scale 1: 1 – 5 Number of Japanese Beetles: 1 – 5 % LS Scale 2: 6 – 10 Number of Japanese Beetles: 6 – 10 % LS Scale 3: > 10 Number of Japanese Beetles: > 10 % LS LS = Leaf Skeletonization The rate of infection was similar (light) in the infected trees. Therefore, the number of trees infected is reported. Total = number of trees.		
Incident Summary:	JB appeared to be cultivar specific with lower level of incidence in Golden Delicious (GD + GD743) (0%) than in Granny Smith (GS + GS784) (17%). However, Chi-square (Yates) test revealed that the percentage of JB affected trees did not significantly differ by cultivar (X^2(1, N=22) =0.37, P>0.5). JB was not influenced by incorporation of the GEN-03 transgene. JB was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly unaffected by JB. Chi-square test revealed that the percentage of JB infected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0.6, P>0.3).		

By Cultivar

Event Name	Infected	Not Infected	Total
GD + GD743	0	10	10
GS + GS784	2	10	12
Total	2	20	22
Chi Square (Yates) =	0.37	P > 0.5	
df =	1		

Golden Delicious

Event Name	Infected	Not Infected	Total
GD743	0	5	5
GD Control	0	5	5
Total	0	10	10
Chi Square =		P not calculated	
df =			

Granny Smith

Event Name	Infected	Not Infected	Total
GS784	1	5	6
GS Control	1	5	6
Total	2	10	12
Chi Square (Yates) =	0.6	P > 0.3	
df =	1		

Table 63: Field Report - Tentiform Leaf Miner - WA2004 - August 23, 2005

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	August 23, 2005
Pest / Disease:	**Tentiform Leaf Miner (TLM)**		
Data Collected:	The control block of trees was inspected for the presence of Tentiform Leaf Miner (TLM) by a top to bottom visual scan of each tree. If a TLM egg or mine was discovered it triggered a full inspection of all the flagged[1] trees in the Washington field trial. An inspection would consist of a random 25 leaf sample per tree. Each leaf would be viewed under a hand lens for the presence or absence of TLM. Any leaf that had one or more TLM present would be counted as infected. Total = number of leaves.		
Incident Summary:	TLM was not cultivar specific. Chi-square test revealed that the percentage of TLM affected trees did not significantly differ by cultivar ($X^2(1, N=250) =0.70, P>0.3$). TLM was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of TLM infected leaves did not significantly differ between GD743 and GD ($X^2(1, N=100) =0.03, P>0.8$). Conversely, for GS, Chi-square test revealed that the percentage of infected leaves differed significantly between GS784 and GS ($X^2(1, N=150) =5.33, P<0.05$), with a higher incidence of TLM in GS784 (21%) than in GS (8%).		

By Cultivar

Event Name	Infected	Not Infected	Total
GD + GD743	11	89	100
GS + GS784	22	128	150
Total	33	217	250
Chi Square =	0.70	P > 0.3	
df =	1		

Golden Delicious

Event Name	Infected	Not Infected	Total
GD743	8	67	75
GD Control	3	22	25
Total	11	89	100
Chi Square (Yates) =	0.03	P > 0.8	
df =	1		

Granny Smith

Event Name	Infected	Not Infected	Total
GS784	16	59	75
GS Control	6	69	75
Total	22	128	150
Chi Square =	5.33	P < 0.05	
df =	1		

[1] Trees tagged in 2005 were limited to: GD743 (3 trees), GD (1 tree), GS784 (3 trees) and GS (3 trees). WA 2004 trees were reflagged in 2007 to provide a larger dataset or future data collections.

Table 64: Field Report - Burr Knot - NY2005 - August 24, 2006

Field Trial:	New York GEN-03 (2005)	Date of Incident:	August 24, 2006
Pest / Disease:	**Burr Knot**		
Data Collected:	The presence (+) or absence (-) of Burr Knot is assayed. The number of Burr Knot affected trees is reported here. Total = number of trees.		
Incident Summary:	Burr Knot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (70%) than Granny Smith (GS + GS784) (25%). However, Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ by cultivar (X^2(1, N=22) =2.83, P>0.05). Burr Knot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ between GD743 and GD (X^2(1, N=10) =1.91, P>0.1). Similarly, Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0, P>0.95).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	7	3	10
GS + GS784	3	9	12
Total	10	12	22
Chi Square (Yates) =	2.83	P > 0.05	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	2	3	5
GD Control	5	0	5
Total	7	3	10
Chi Square (Yates) =	1.91	P > 0.1	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	1	5	6
GS Control	2	4	6
Total	3	9	12
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Table 65: Field Report - Leaf Spot - NY2005 - August 24, 2006

Field Trial:	New York GEN-03 (2005)	Date of Incident:	August 24, 2006
Pest / Disease:	**Leaf Spot**		
Data Collected:	The presence (+) or absence (-) of Leaf Spot is assessed. The number of Leaf Spot affected trees is reported here. Total = number of trees.		
Incident Summary:	Leaf Spot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (100%) than Granny Smith (GS + GS784) (17%). Chi-square test revealed that the percentage of Leaf Spot affected trees significantly differed by cultivar (X^2(1, N=22) =12.10, P<0.001). Leaf Spot was not influenced by incorporation of the GEN-03 transgene. Both GD743 and GD were similarly affected by Leaf Spot (100% in both). Similarly, Chi-square (Yates) test revealed that the percentage of Leaf Spot affected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0.60, P>0.3).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	10	0	10
GS + GS784	2	10	12
Total	12	10	22
Chi Square (Yates) =	12.10	P < 0.001	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	5	0	5
GD Control	5	0	5
Total	10	0	10
Chi Square =		P not calculated	
df =			

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	1	5	6
GS Control	1	5	6
Total	2	10	12
Chi Square (Yates) =	0.60	P > 0.3	
df =	1		

Table 66: Field Report - Russet - NY 2005 - November 19, 2009

Field Trial:	New York GEN-03 (2005)	Date of Incident:	November 19, 2009
Pest / Disease:	Russet		
Data Collected:	When russet is assayed at the NY field trial, it is done so on a tree by tree basis. The percentage of fruit on each tree affected by russet is observed and the tree is assigned a rating with 1 < 10%, 2 = 10 – 50%, and 3 > 50%. The %'s refer to the % of total fruits with russet. The level of russet present on each tree was generally similar. Therefore, the number of russet-affected trees is reported here. Total = number of trees.		
Incident Summary:	Russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (80%) than Granny Smith (GS + GS784) (8%). Chi-square (Yates) test revealed that the percentage of russet affected trees significantly differed by cultivar (X^2(1, N=22) =8.81, P<0.01). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GD743 and GD (X^2(1, N=10) =0.63, P>0.3). Similarly, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0, P>0.95).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD7443	8	2	10
GS + GS784	1	11	12
Total	9	13	22
Chi Square (Yates) =	8.81	P < 0.01	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	4	1	5
GD Control	4	1	5
Total	8	2	10
Chi Square (Yates) =	0.63	P > 0.3	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	6	6
GS Control	1	5	6
Total	1	11	12
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Table 67: Field Report - Russet - WA2004 - June 21, 2010

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	June 21, 2010
Pest / Disease:	**Russet**		
Data Collected:	Russet has appeared on the fruit as it has started to size. Damage occurred sometime between bloom and first cover. Sampled ten pieces of fruit between the second and fourth wire along the main trunk. If no fruit is present along the trunk fruit on the first available scaffold limb is checked. Each apple is handled and rotated 360 degrees in order to visualize any fruit russeting. On the GEN-03 (2008) Grannies all fruit was sample due to poor set. The number of fruit sampled is reported. Total = number of fruit.		
Incident Summary:	Russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (91%) than Granny Smith (GS + GS784) (23%). Chi-square test revealed that the percentage of russet affected fruit significantly differed by cultivar (X^2(1, N=150) =72.4, P<0.001). Russet was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected fruit differed significantly between GD743 and GD (X^2(1, N=80) =5.64, P<0.05) with a higher incidence of russet in GD (100%) than GD743 (82.5%) fruit. Conversely, Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GS784 and GS (X^2(1, N=70) =1.14, P>0.2).		

By Cultivar

Event Name	Infected	Not Infected	Total
GD + GD743	73	7	80
GS + GS784	16	54	70
Total	89	61	150
Chi Square =	72.37	P < 0.001	
df =	1		

Golden Delicious

Event Name	Infected	Not Infected	Total
GD743	33	7	40
GD Control	40	0	40
Total	73	7	80
Chi Square (Yates)=	5.64	P < 0.05	
df =	1		

Granny Smith

Event Name	Infected	Not Infected	Total
GS784	11	29	40
GS Control	5	25	30
Total	16	54	70
Chi Square =	1.14	P > 0.2	
df =	1		

Table 68: Field Report - Russet - WA2008 - June 21, 2010

Field Trial:	Washington GEN-03 (2008)	Date of Incident:	June 21, 2010
Pest / Disease:	**Russet**		
Data Collected:	Russet has appeared on the fruit as it has started to size. Damage occurred sometime between bloom and first cover. Sampled ten pieces of fruit between the second and fourth wire along the main trunk. If no fruit is present along the trunk fruit on the first available scaffold limb is checked. Each apple is handled and rotated 360 degrees in order to visualize any fruit russeting. On the GEN-03 (2008) Grannies all fruit was sampled due to poor set. The number of fruit sampled is reported. Total = number of fruit.		
Incident Summary:	Russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (86%) than Granny Smith (GS + GS784) (20%). Chi-square test revealed that the percentage of russet affected fruit significantly differed by cultivar (X^2(1, N=323) =139.3, P<0.001). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GD743 and GD (X^2(1, N=197) =0.10 P>0.7). Similarly, Chi-square test revealed that the percentage of russet affected fruit did not significantly differ between GS784 and GS (X^2(1, N=126) =3.05, P>0.05).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	169	28	197
GS + GS784	25	101	126
Total	194	129	323
Chi Square =	139.3	P < 0.001	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	85	15	100
GD Control	84	13	97
Total	169	28	197
Chi Square =	0.10	P > 0.7	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	8	52	60
GS Control	17	49	66
Total	25	101	126
Chi Square =	3.05	P > 0.05	
df =	1		

Table 69: Field Report - Russet - NY2005 - July 21, 2010

Field Trial:	New York GEN-03 (2005)	Date of Incident:	July 21, 2010
Pest / Disease:	**Russet**		
Data Collected:	When russet is assayed at the NY field trial, it is done so on a tree by tree basis. The percentage of fruit on each tree affected by russet is observed and the tree is assigned a rating with1 < 10%, 2 = 10 – 50%, and 3 > 50%. The %'s refer to the % of total fruits with russet. The level of russet present on each tree was generally similar. Therefore, the number of russet-affected trees is reported here. Total = number of trees.		
Incident Summary:	Russet appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (70%) than Granny Smith (GS + GS784) (8%). Chi-square (Yates) test revealed that the percentage of russet affected trees significantly differed by cultivar (X^2(1, N=22) =6.50, P<0.05). Russet was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GD743 and GD (X^2(1, N=10) =0, P>0.95). Similarly, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between GS784 and GS (X^2(1, N=12) =0, P>0.95).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	7	3	10
GS + GS784	1	11	12
Total	8	14	22
Chi Square (Yates) =	6.50	P < 0.05	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	4	1	5
GD Control	3	2	5
Total	7	3	10
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	6	6
GS Control	1	5	6
Total	1	11	12
Chi Square (Yates) =	0	P > 0.95	
df =	1		

Table 70: Field Report - Campylomma - WA2004 - May 24, 2010

Field Trial:	Washington GEN-03 (2004)	Date of Incident:	May 24, 2010
Pest / Disease:	**Campylomma Bug**		
Data Collected:	The control block of trees was inspected for the incidence of Campylomma stings by a top to bottom visual scan. If Campylomma damage was present, an inspection of all the flagged trees within the Washington field trial was triggered. Ten pieces of fruit from each tree were selected between the second and fourth wires. Sampling started with the fruit on the leader, working upward from the second to the fourth wire. If no fruit was available on the leader the first fruit on a scaffold limb was selected. Fruit was taken from the east side of the tree in order for the sun to be at the back of the individual inspector. Each apple was held and rotated 360 degrees to look for defects. Any apple that had one or more Campylomma stings was counted as infected. Total = number of fruit.		
Incident Summary:	Campylomma Bug appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (16%) than Granny Smith (GS + GS784) (4%). Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit significantly differed by cultivar ($X^2(1, N=150) =4.42, P<0.05$). Campylomma Bug was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit differed significantly between GD743 and GD ($X^2(1, N=80) =5.88, P<0.05$) with a higher incidence of Campylomma in GD (38%) than GD743 (5%). Conversely, Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit did not significantly differ between GS784 and GS ($X^2(1, N=70) =2.10, P>0.1$), although there was a higher incidence of Campylomma in GS (10%) than GS784 (0%).		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	13	67	80
GS + GS784	3	67	70
Total	16	134	150
Chi Square (Yates) =	4.42	P < 0.05	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	2	38	40
GD Control	11	29	40
Total	13	67	80
Chi Square (Yates) =	5.88	P < 0.05	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	40	40
GS Control	3	27	30
Total	3	67	70
Chi Square (Yates) =	2.10	P > 0.1	
df =	1		

Table 71: Field Report - Campylomma - WA2008 - May 24, 2010

Field Trial:	Washington GEN-03 (2008)	Date of Incident:	May 24, 2010
Pest / Disease:	**Campylomma Bug**		
Data Collected:	The control block of trees was inspected for the incidence of Campylomma stings by a top to bottom visual scan. If Campylomma damage was present, an inspection of all the flagged trees within the Washington field trial was triggered. Ten pieces of fruit from each tree were selected between the second and fourth wires. Sampling started with the fruit on the leader, working upward from the second to the fourth wire. If no fruit was available on the leader the first fruit on a scaffold limb was selected. Fruit was taken from the east side of the tree in order for the sun to be at the back of the individual inspector. Each apple was held and rotated 360 degrees to look for defects. Any apple that had one or more Campylomma stings it was counted as infected. Total = number of fruit.		
Incident Summary:	Campylomma Bug appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (2.5%) than Granny Smith (GS + GS784) (0%). However, Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit did not significantly differ by cultivar ($X^2(1, N=400) =3.24, P>0.05$). Campylomma Bug was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Campylomma Bug affected fruit did not significantly differ between GD743 and GD ($X^2(1, N=200) =3.28, P>0.05$). Both GS784 and GS were similarly unaffected by Campylomma.		

By Cultivar			
Event Name	Infected	Not Infected	Total
GD + GD743	5	195	200
GS + GS784	0	200	200
Total	5	395	400
Chi Square (Yates) =	3.24	P > 0.05	
df =	1		

Golden Delicious			
Event Name	Infected	Not Infected	Total
GD743	5	95	100
GD Control	0	100	100
Total	5	195	200
Chi Square (Yates) =	3.28	P > 0.05	
df =	1		

Granny Smith			
Event Name	Infected	Not Infected	Total
GS784	0	100	100
GS Control	0	100	100
Total	0	200	200
Chi Square =		P not calculated	
df =			

Table 72: Field Report - 2008 Fruit Rot After Storage - NY2005 - June 30, 2009

Field Trial:	New York GEN-03 (2005)	Date of Incident:	June 30, 2009
Pest / Disease:	2008 Fruit Rot (After Storage)		
Data Collected:	Fruit Rot after storage was reported as the number of fruit showing tan or black rot and the total number of fruit examined. Black rot is caused by *Botryosphaeria obtuse*. Tan rot is probably mostly caused by *Colletotrichum* spp. However, we didn't culture from any of these fruits, so we can't be 100% certain what caused each rot. Total = number of fruit.		
Incident Summary:	Tan Rot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (20%) than Granny Smith (GS + GS784) (2%). Chi-square test revealed that the percentage of Tan Rot affected fruit significantly differed by cultivar (X^2(1, N=469) =46.19, P<0.001). Tan Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GD743 and GD (X^2(1, N=156) =0.13, P>0.7). Similarly, Chi-square test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GS784 and GS (X^2(1, N=313) =3.44, P>0.05). Black Rot appears to be cultivar specific with a higher overall rate of incidence in Golden Delicious (GD + GD743) (9%) than Granny Smith (GS + GS784) (1%). Chi-square test revealed that the percentage of Black Rot affected fruit significantly differed by cultivar (X^2(1, N=469) =19.50, P<0.001). Black Rot was not uniformly influenced by incorporation of the GEN-03 transgene. Chi-square test revealed that the percentage of Black Rot affected fruit differed significantly between GD743 and GD (X^2(1, N=156) =7.51, P<0.01) with GD having a higher incidence of Black Rot (15%) than GD743 (1%). Conversely, Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not significantly differ between GS784 and GS (X^2(1, N=313) =0.35, P>0.5).		

By Cultivar – Tan Rot			
Event Name	Infected	Not Infected	Total
GD + GD743	31	125	156
GS + GS784	6	307	313
Total	37	432	469
Chi Square =	46.19	P < 0.001	
df =	1		

Golden Delicious – Tan Rot			
Event Name	Infected	Not Infected	Total
GD743	15	56	71
GD Control	16	69	85
Total	31	125	156
Chi Square =	0.13	P > 0.7	
df =	1		

Granny Smith – Tan Rot			
Event Name	Infected	Not Infected	Total
GS784	0	143	143
GS Control	6	164	170
Total	6	307	313
Chi Square (Yates) =	3.44	P > 0.05	
df =	1		

By Cultivar – Black Rot			
Event Name	Infected	Not Infected	Total
GD + GD743	14	142	156
GS + GS784	2	311	313
Total	16	453	469
Chi Square (Yates) =	19.50	P < 0.001	
df =	1		
Golden Delicious – Black Rot			
Event Name	Infected	Not Infected	Total
GD743	1	70	71
GD Control	13	72	85
Total	14	142	156
Chi Square (Yates) =	7.51	P < 0.01	
df =	1		
Granny Smith – Black Rot			
Event Name	Infected	Not Infected	Total
GS784	1	142	143
GS Control	1	169	170
Total	2	311	313
Chi Square (Yates) =	0.35	P > 0.5	
df =	1		

Table 73: Field Report - 2009 Fruit Rot After Storage - NY2005 - December 30, 2010

Field Trial:	New York GEN-03 (2005)	Date of Incident:	December 30, 2010
Pest / Disease:	**2009 Fruit Rot (After Storage)**		
Data Collected:	Fruit Rot after storage was reported as the number of fruit showing tan or black rot and the total number of fruit examined. Black rot is caused by *Botryosphaeria obtuse*. Tan rot is probably mostly caused by *Colletotrichum* spp. However, we didn't culture from any of these fruits, so we can't be 100% certain what caused each rot. Total = number of fruit.		
Incident Summary:	Tan Rot did not appear to be cultivar specific with a similar rate of incidence in Golden Delicious (GD + GD743) (3%) and Granny Smith (GS + GS784) (3%). Chi-square test revealed that the percentage of Tan Rot affected fruit did not significantly differ by cultivar (X^2(1, N=372) =0.04, P>0.8). Tan Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Tan Rot affected fruit did not significantly differ between GD743 and GD (X^2(1, N=176) =0.17, P>0.5). Similarly, Chi-square (Yates) test revealed that the percentage of Tan Rot affected fruit did not differ significantly between GS784 and GS (X^2(1, N=196) =3.35, P>0.05). Black Rot appears to be cultivar specific with a higher rate of incidence in Golden Delicious (GD + GD743) (2.3%) than Granny Smith (GS + GS784) (0%). However, Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not differ significantly by cultivar (X^2(1, N=372) =2.62, P>0.1). Black Rot was not influenced by incorporation of the GEN-03 transgene. Chi-square (Yates) test revealed that the percentage of Black Rot affected fruit did not significantly differ between GD743 and GD (X^2(1, N=176) =0.26, P>0.5). Both GS and GS784 were similarly unaffected by Black Rot in 2009 fruit after storage.		

By Cultivar – Tan Rot			
Event Name	Infected	Not Infected	Total
GD + GD743	6	170	176
GS + GS784	6	190	196
Total	12	360	372
Chi Square =	0.04	P > 0.8	
df =	1		

Golden Delicious – Tan Rot			
Event Name	Infected	Not Infected	Total
GD743	4	84	88
GD Control	2	86	88
Total	6	170	176
Chi Square (Yates) =	0.17	P > 0.5	
df =	1		

Granny Smith – Tan Rot			
Event Name	Infected	Not Infected	Total
GS784	0	88	88
GS Control	6	102	108
Total	6	190	196
Chi Square (Yates) =	3.35	P > 0.05	
df =	1		

By Cultivar – Black Rot			
Event Name	Infected	Not Infected	Total
GD + GD743	4	172	176
GS + GS784	0	196	196
Total	4	368	372
Chi Square (Yates) =	2.62	P > 0.1	
df =	1		
Golden Delicious – Black Rot			
Event Name	Infected	Not Infected	Total
GD743	3	85	88
GD Control	1	87	88
Total	4	172	176
Chi Square (Yates) =	0.26	P > 0.5	
df =	1		
Granny Smith – Black Rot			
Event Name	Infected	Not Infected	Total
GS784	0	88	88
GS Control	0	108	108
Total	0	196	196
Chi Square =		P not calculated	
df =			

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Biology of Cultivated Apple

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Submitted October 25, 2011

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Nomenclature

Throughout this document *Malus x domestica* is referred to by its common name, cultivated apple or simply apple. The table below summarizes the synonyms for apple.

Malus x domestica	
Taxonomy ID: 3750[1]	
synonym:	*Malus pumila var. domestica (Borkh.) C. K.Schneid.*
synonym:	*Malus sylvestris var. domestica (Borkh.) Mansf.*
synonym:	*Malus sylvestris var. domestica*
synonym:	*Malus domestica Borkh.*
synonym:	*Malus pumila var. domestica*
synonym:	*Malus pumila auct.*
synonym:	*Malus domestica*
common name:	cultivated apple
common name:	apple tree
common name:	apple
[1] Reference: NCBI Taxonomy Browser (*Malus x domestica*) (NCBI, 2011)	

1. OVERVIEW

In this document the biology of cultivated apple and plants related to apple are considered along with potential routes of gene escape. Since the mechanism by which genes are moved from one flowering plant to another is through cross pollination of sexually compatible plants, the plants with which apple can cross-pollinate are also described. Below is an analysis of the biology of cultivated apple. This review focuses solely on apple in the United States.

This document is a companion to the United States Department of Agriculture (USDA) regulatory submission "Petition for the Determination of Nonregulated Status: Arctic™ Apple (*Malus domestica*) Events GD743 and GS784," submitted by Okanagan Specialty Fruits Inc. (OSF). It is intended to provide background information on the biology of cultivated apple, its centers of origin, its related species and the potential for gene introgression from cultivated apple into relatives, and details of the life forms with which it may interact.

Such species-specific information will serve as a guide for addressing some of the information requirements of the regulatory guidelines. Specifically, it will be used to determine whether there are significantly different or altered interactions with other life forms resulting from the transgenic plant event, which could potentially cause it to become a weed of agriculture, become invasive of natural habitats or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of cultivated apple relate only to conventional plants of this species, and not to transgenic ones.

2. THE BIOLOGY OF CULTIVATED APPLE

2.1 General Description, Use as a Crop Plant and Origin of Species

The cultivated apple, *Malus* x *domestica*, is a member of the rose family Rosaceae, which is made up in general of herbs, shrubs or trees that are deciduous or evergreen and often thorny. Fruit produced by members of the family can be a follicle, achene, drupe, hip or pomme. Flowers are usually perfect.

Rosaceae consists of about 100 genera with more than 2,000 species distributed throughout the world, but is most common in temperate regions. It is sometimes divided into tribes or subfamilies based on fruit characteristics, namely: Spiraeaceae, Pomaceae (Malaceae) and Amygdalaceae (Drupaceae) (Rehder, 1958). Rosaceous species of agronomic importance because of their fruit include apple, pear, quince, peach, cherry, apricot, plum, strawberry, raspberry and blackberry. Those of ornamental importance include: crab apple, pyrocantha, spirea, mountain ash, rose and others.

The genus *Malus,* to which cultivated apple and crab apple belong, includes about 25 species worldwide. The center of diversity of cultivated apple is central Asia, but native or naturalized *Malus* occur in central and northern Europe and a number of *Malus* species are native to temperate Asia and western China (Way *et al.*, 1990).

Cultivated apple in the United States of America (USA) includes cultivars used for fresh consumption, as well as, processing into juice, sweet (non-alcoholic) cider, pie filling, sauce, hard (alcoholic) cider, juice concentrate, fruit leather, dehydrated fruit bars and other products (Downing, 1989). The pomace left over from juice production and other byproducts of manufacturing may be fed to livestock or wild animals, or be used as an ingredient in foods such as, baked goods, for extraction of ester flavors, etc.

Fresh apples are the primary apple product consumed. Processed apples generally account for less than 50 percent of the U.S. crop, the actual proportion varying from year to year and from location to location (Outlook, 2007). Apples compete with processed snack foods and confectionaries, as well as with fruits and fresh vegetables. Apple is notable for its positive nutritional qualities. It is a natural snack food with low fat content, sugar content of 11-16 percent, is a good source of potassium, is a good source of dietary fiber including soluble pectin, and phenol antioxidants (Vinson *et al.*, 2001) including vitamin C. Children and infants consume a disproportionate amount of fresh and processed products of apple (Dennison, 1966).

Cultivated apple is the most important temperate fruit crop. Countries with significant apple production are: China, Europe, USA, India, Turkey, Russia, Iran, Japan, Chile, New Zealand, Canada, Australia, South Africa, Argentina and Brazil (O'Rourke, 1994). Per-capita annual consumption in the USA is on the order of 16 pounds of fresh apples and 29 pounds of processed apple products (O'Rourke, 2010). The farm-gate value of apples in the USA is about $1.6 billion (Perez and Pollack, 2008).

2.2 Brief Outlook of Agronomic Practices for Cultivated Apple

Cultivated apple is grown as a tree in orchards throughout temperate regions of the world (Westwood, 1993). Fruit cultivars with desirable quality and production characteristics are propagated by grafting a scion onto a rootstock, since apple does not root easily from cuttings (Rom and Carlson, 1987). Rootstock cultivars – also usually *Malus* x *domestica* – are propagated in layer beds and affect the scion cultivar by inducing traits such as dwarf growth habit, precocity and resistance to root diseases or cold temperatures, or have other characteristics useful for efficient apple production (Kester *et al.*, 2011). Seedlings of hardy cultivars such as Wealthy and crab apples are also sometimes used as rootstock.

Trees in modern orchards are planted at a density of 200-2,000+ trees per acre; trees are seldom higher than 15 feet tall at maturity. Production averages about 16 tons per acre, varying considerably depending on orchard age, cultivar, management practices and weather. An apple orchard in full production produces about 80,000 apples per acre (Gallardo *et al.*, 2010).

Orchards are often located on slopes to avoid the colder temperatures and frost pockets found in low-lying elevations that can kill flowers, and to avoid waterlogged soil conditions that can result in tree death. Climatic areas with a hardiness zone rating of 5a (McKenney *et al.*, 2001) or warmer, but cool enough to result in the 1,200 or more chilling hours, that are most advantageous for commercial production. Apple production is centered in, but not exclusive to, areas with low to moderate rainfall, to promote good fruit quality and to avoid diseases such as apple scab and canker that are problematic in damper climates. In a modern orchard setting, trees are replaced after about 20 years as new and improved cultivars become available or land use changes. Specimen apple trees growing in favorable locations can be very long lived, probably as long as a pear trees that has been documented to be more than 400 years old (Wikipedia(1), 2011).

Apple trees in commercial plantings reach maturity at an age of 4-5 years; they flower in the early spring, producing white or pink flowers about two inches in diameter that develop on fruiting spurs in groups of six flowers. Cultivated apple often develops a biennially-bearing habit, tending to produce abundant flowers in one year but fewer in the next; this is a significant production hindrance that can be ameliorated by thinning fruit soon after bloom. Fruits can be uniformly red, green, yellow, or bicolored, such as striped or blushed red on yellow or green

background (Wikipedia(2), 2011). The most desirable weight of commercial apple ranges from 6 to 9 ounces (oz) per piece. Apples are picked from mid-August to November in north temperate zones, and can be stored for up to a year. There are about 12 significant commercial apple cultivars in international trade with many of these grown in all major production areas. Widely grown cultivars are: Golden Delicious, Delicious, Granny Smith, Gala, McIntosh and Fuji. Minor international cultivars include: Jonagold, Cox's Orange Pippen, Braeburn, Mutsu and Pink Lady.

Most cultivars are diploid with a chromosome number of 34 (n=17); a few are triploid (Darlington and Wylie, 1955). Natural or induced mutations of many cultivars, especially red-skinned ones, have been selected by horticulturalists and are commercially important. They are known by the synonymous terms: clonal selections, strains or sports of the parent cultivar. The most common commercially-important characteristics of these are enhanced fruit color, altered tree growth habit and earlier fruit maturity time. The genetic differences between the strain and its parent are usually small, and have not yet been described even with molecular genetic techniques.

Apple is a labor intensive, highly-managed crop requiring about 200 hours of labor per acre per year. Major crop management operations include: fruit harvesting, tree pruning, fruit thinning, irrigation, and pest and disease control. Mature commercial orchards generate cash flows of about $4,000 per acre per year (Gallardo *et al.*, 2010). Apple scab and powdery mildew are the major fungal diseases; fire blight (*Erwinia amylovora)* is the major bacterial disease; and codling moth the key insect pest in most production areas; while leaf roller, aphids, mites, and other minor pests are of concern in most areas.

Virus diseases infect apple, with most viruses spread during propagation through use of contaminated grafting wood (WSU, 2010). Virus diseases have become a limited concern to apple growers because of the success of certified budwood schemes that distribute virus-free wood for propagation. Apple can be infected by a number of quarantinable diseases, and certification standards are in place for the import and export of fruit, propagating wood and trees.

2.3 The Reproductive Biology of Cultivated Apple

Apple is propagated by grafting a scion bud to a rootstock. Most nursery trees are produced in specialized commercial fruit-tree nurseries. These companies have access to favorable growing locations, to specialized technology, to certified propagating wood of the most popular cultivars and to information useful to growers deciding on planting decisions. Some growers produce their own nursery trees. The sequence of operations in apple propagation is: rootstock liners are planted in a nursery in the spring; they are budded in August; the stock is cut back to the bud the following March; the scion bud develops into a nursery tree in the following growing season. Nursery trees are dug up in the fall, graded and stored over the winter; and then are distributed to growers for planting the following spring (OVTFA, 1993). Flowering occurs in year 2, following planting with full fruit production in year 4 or 5.

About 10 percent of apple flowers develop to mature fruit; the others fail due to lack of pollination, competition between fruits, or cultural practices to remove some fruit to promote the size and quality of the remaining fruit. That proportion is higher for crab apple. Nearly all apple cultivars require cross-pollination for consistent cropping, with about 3-5 percent of the orchard area devoted to pollinizer cultivars of either another fruit cultivar or a specialized crab-apple pollinizer cultivar. Genes conferring incompatibility have been described and are sufficient in

number that the diversity between cultivars results in nearly all cultivars being cross compatible. Some cultivars are at least partially self-fertile due to environmentally-induced breakdown of that incompatibility system when flowers age or temperatures are high (Warmund, 1996).

Pollination is carried out by insects, primarily cultivated or wild honeybees or other insect pollinators such as bumblebees, *osmia* bees and other species (Dupree *et al.*, 1987). The period of flowering during which viable pollen is produced by an apple plant varies, depending on weather conditions, from about 7-30 days. Wind pollination is inconsequential for apple; emasculated flowers seldom, if ever, set fruit even though flowers on nearby branches produce abundant pollen.

Rootstock cultivars seldom flower since this component of the tree consists of only the root and a short stem; any rootstock-derived shoots that might develop are removed to prevent competition with the scion. Rootstock layer beds are maintained in vigorous vegetative growth and are selected for juvenility to promote rooting. Ungrafted rootstocks, should they be managed in the same way as fruiting cultivars, develop much the same way producing fruit after 2-3 years. The fruit of most rootstock cultivars is unattractive, small and insipid.

Pollen fertility of most apple cultivars is close to 100 percent, but is reduced in some cultivars such as McIntosh by unknown factors and in others such as Jonagold by their triploidy. An apple flower commonly has 10-15 and as many as 20 ovules, some of which fail to develop into seeds. Cultivars vary in average seed number per fruit from about 3-12, with pollination conditions influencing this average. A small percentage of fruit may develop by parthenocarpy (the production of fruit without fertilization) (Olien, 1987). Apomixix – development of an embryo without the occurrence of fertilization – has been documented in some *Malus* species. Seeds require conditioning by stratification at an optimum temperature of about 35°F for several months to germinate (Bradford and Nonogaki, 2007).

Seedling trees have a juvenile growth phase of 5-10 years – shortened by grafting onto rootstock – before producing flowers; they are characterized by lanky shoot growth and thorny spurs, and are adapted to growing in communal thickets. Damaged trees can regenerate shoots from roots, which contributes to the dense structure of wild crab apple communities and raises the question of what proportion of tree stems in these communities originate directly from seed compared to those that regenerated from roots of established plants.

A large, mature cultivated apple tree can produce in the order of 2,000 fruits per year, potentially yielding 10,000 seeds. They may live for 50 years or longer, theoretically producing 500,000 seeds in its lifetime. For a naturalized apple-tree population to be at equilibrium, each individual apple tree need reproduce just one other to replace itself. This suggests that the probability of an individual apple seed developing into a mature tree is small in comparison to annual plants that produce fewer seeds per plant and require, on average, a replacement plant each year to maintain a stable population.

2.4 The Centers of Origin of the Species

The domesticated and cultivated apple originates from mountainous regions in Central Asia, notably the western foothills of the Tian Shan Mountains of Kazakhstan and nearby areas with similar ecology. Cultivars, seedlings and hybrids derived from these populations were distributed to temperate areas of Europe and Asia by ancient travelers, resulting in naturalized populations in the Caucasus Mountains and in Persia, Russia and Europe and were later brought to America

by pioneers in the 17th and 18th century. The cultivated domestic apple is thought to be derived primarily from *Malus sieversii;* for some old cultivars, interspecific hybridization is a possibility, although such history remains to be clearly described.

Crab apple species introduced to North America for use as ornamentals are native to southwestern China and the middle latitudes of Asia in general. Five species of crab apples within the section *Chloromeles* are native to North America; one, Pacific Crab (*Malus fusca*), occurs on the west coast. The other three species are similar to each other and closely related to *Malus fusca*, and occur in the east (Hosie, 1979); they are: *M. angustifolia, M. coronaria* and *M. ioensis. Malus glabrata* (Biltmore crab apple) occurs from northwest Carolina to Alabama. The introduced ornamental species *Malus baccata* and *Malus prunifolia*, originating in Asia, have escaped from cultivation in the northeastern USA but are not naturalized (Little, 1979). All crabs have small, insipid fruit, *M. fusca* about 0.20 inches in diameter and 0.10 oz. and *M. coronaria,* about 1.38 inches and 0.52 oz.. *M. fusca* is adapted to wet, often disturbed habitats such as the edge of ponds or creek banks in West Coast rain forest habitat. New cultivars of crab apple, frequently interspecific hybrids of two crab apple cultivars with disease resistance or enhanced ornamental traits, have been introduced by breeders and nurseries (Draper and Chatfield, 1996).

Cultivated apple cultivars or seedlings were valuable on mixed farms in pioneer times because their fruit could be stored for use as fresh produce in the winter or processed to canned and dried apple, juice and cider. Rudimentary breeding efforts, or selection and propagation of exceptional chance seedlings, in Canada and the USA resulted in cultivars such as McIntosh, Delicious and Golden Delicious.

Later, government-sponsored breeding programs established in the 20th century resulted in introduction of cultivars with improved fruit quality, storage and production characteristics and improved rootstock cultivars. Many significant introductions of apple cultivars were clonal improvements originating from spontaneous mutants occurring in trees in commercial orchards. Over 180 clones of Delicious originating in this way have been named and introduced (Higgins, 2005) (Wikipedia(3), 2011). Clonal improvements of the cultivars McIntosh, Gala, Fuji, Pink Lady and Braeburn are also commercially important.

The 1971 National Apple Registrar of the United Kingdom describes over 8,000 historic and modern named cultivars (Smith, 1971).

2.5 Cultivated Apple as a Volunteer Weed

Cultivated apple is not regarded as a weedy species, although has been reported to be a successional species in abandoned pasture but not abandoned cultivated field (Stover and Mark, 1998). Animals such as bears can carry fruit containing seed away from cultivated areas, and occasionally establishing feral trees in undisturbed habitat.

Cultivated apple tree seedlings can be persistent; the species has escaped cultivation and naturalized in southern Canada, in the eastern USA, and from British Columbia south to California (Little, 1979). Four species of crab apples within the section *Chloromeles* are native to North America (see above). The introduced ornamental species *Malus baccata* and *Malus prunifolia* have escaped from cultivation in the northeastern USA, but are not naturalized (Little, 1979). Research using molecular techniques found no introgression of cultivated apple genes to native *Malus* species of North America (Dickson *et al.*, 1991). Nonetheless hybridization

between cultivated apples and at least native and introduced crab apple species of the group *Chloromeles* can occur from time to time when the two species are grown together.

Domesticated apple has been grown in North America for several centuries, allowing more than sufficient time for hybrids between cultivated apple and native or introduced *Malus* species to naturalize, should they occur in sufficient numbers and successfully compete. Hybrids involving cultivated apple have been reported and are expected to be recognizable by their intermediate fruit characteristics. Plants of the hybrids *malus* x *platycarpa* (*platycarpa* is sometimes united taxonomically with *coronaria;* the common name of the hybrid is big fruit crab) and *malus* x *soulardii* and *malus* x *ioensis* appear to be local and not very common. Should they occur, hybrids between cultivated apple and crab apple are expected to be easily recognized because fruit size of the hybrid is intermediate between the domestic apple and crab.

Volunteer plants originating from seed in apple orchards are very rare due to the perennial nature of the crop, and associated orchard management practices such as herbicide treatment of tree rows and mowing of the alley between rows. Local authorities have enacted legislation to enforce removal of abandoned orchards and seedling trees occurring near orchards. Apples are poorly suited to the ecology of higher elevation areas dominated by conifer species. Many commercial cultivars are damaged by temperatures below -13°F, their response being tempered by preconditioning temperature and genetic composition. Hardy cultivars have been developed to withstand colder temperatures and these are grown to some extent in colder regions. Apples supercool to a minimum of about -40°F, at which temperature ice crystals form in the cells, killing the fruit. This results in even very hardy cultivars of apple being uncommon in areas where temperatures are regularly below -40°F. Individual trees may survive if protected by snow or growing in some other special microenvironment.

Nearly all apple trees in orchards are grafted on rootstock, usually specialized rootstock cultivars or less commonly seedlings grown for this purpose, thus shoots originating from roots are the rootstock cultivar. Removal of feral or abandoned orchard trees by cutting at ground level is often not effective unless measures were taken to kill the root by removing the stump or treating it with herbicide.

2.6 Summary of Ecology of Cultivated Apple

Cultivated apple – an introduced domesticated and cultivated species naturalized in Europe – is derived from *Malus sieversii,* native to the foothills of central Asia. It is related to both native North American *Malus* and introduced crab apple species, and can hybridize with them. Feral cultivated apple is not weedy nor very common, but is successional in abandoned pasture and has naturalized in some areas of North America. Cultivated apple occurs in the same general area as trees of introduced ornamental crab apple, and backyard cultivated apple trees may be near native or naturalized crab apple. Feral cultivated apple trees near commercial plantings are usually removed to prevent them becoming a source of pest infestation and disease inoculum. The occurrence of volunteers in commercial plantings is very rare because of orchard management practices. Shoots can regenerate from the roots or stumps of injured apple trees. Apple is a long-lived tree that depends on bees or other insects for cross-pollination. The ratio of number of seeds produced by a cultivated apple tree in its lifetime (several hundred thousand) to the number of plants needed to maintain a stable population is several orders of magnitude larger than for annual crop species, but apple is an effective colonizer of disturbed land such as abandoned pasture, and trees may persist in such locations for many years.

3. THE CLOSE RELATIVES OF CULTIVATED APPLE

3.1 Interspecific/Genus Hybridization

In considering the potential environmental impact following the unconfined release of genetically modified apple, it is important to understand the possible development of hybrids through interspecific and intergeneric crosses with other cultivars and related species. Development of hybrids could result in the introgression of the novel traits into these related species and resulting in:

- the related species becoming more weedy, and/or
- the introduction of a novel trait with potential for ecosystem disruption into the related species.

For a trait to become incorporated into a species genome, both repeated backcrossing of plants of that species by the hybrid intermediaries, and survival and fertility of the resulting offspring are necessary.

3.2 Potential for Introgression of Genetic Information from Cultivated Apple into Relatives

Several crab apple species are native to North America and hybridization between cultivated apple and native apple and some introduced crab apple species is possible. Domesticated apple has been grown in North America for several centuries, allowing more than sufficient time for hybrids between cultivated apple and native or introduced *Malus* species to naturalize should they occur frequently and successfully compete. Naturalized hybrids involving cultivated apple have not been reported, but should be recognizable by their intermediate fruit characteristics.

Establishment of native crab apple plants from seed is calculated to be a lower-probability event than for annual crops, as the number of seeds produced by an individual apple tree in its lifetime is large yet few trees are needed to replace the parent. Native crab apples grow in thickets, and it is unclear if local populations are maintained primarily through seed or by vegetative regeneration of shoots from roots.

Bee colonies facilitate pollination, transferring pollen from cultivated apple or crab apple pollen source trees to receptor trees and incidental pollen transfer in the opposite direction. Effective transfer of pollen from orchards to native crab apple flowers depends on synchronous flowering of the two species and close proximity. Pollination efficiency decreases rapidly as the distance between pollen source and receptor tree increases; the frequency of transfer is influenced by the size and proximity of competing sources of pollen.

Commercial growers have traditionally planted about 3-5 percent of orchards trees as pollen source cultivars, arranging them so that pollinating bees need travel 50 feet or less to reach receptor trees. Thus, the vast majority of cross-pollination of apple in commercial orchards is between receptive flowers and adjacent pollen sources. When pollinating, individual bees are usually loyal to the location and species and/or cultivar from which they are collecting nectar or pollen.

3.3 Occurrence of Cultivated Apple in the United States

Cultivated apple occurs in commercial orchard plantings, as fruit trees in gardens or pastures, in nurseries, or as escaped or naturalized trees that are long lived. With proper care and attention it can be grown in nearly all areas

4. POTENTIAL INTERACTIONS OF CULTIVATED APPLE WITH OTHER LIFE FORMS

4.1 Examples of Potential Interactions of Cultivated Apple With Other Life Forms During its Lifecycle

For a description of diseases of apple and the organisms causing bacterial, fungal, virus and other diseases: see (Jones and Aldwinckle, 1990)

For a description of insect and related pests of apple: see (MacNay and Creelman, 1958)

For a description of insect-observed pollinating apple in British Columbia: see (Dupree *et al.*, 1987)

Table 1: Life Forms Potentially Interacting with Cultivated Apple

Organism Class	Reference/Description
Bacterial Diseases	Table 2
Fungal Diseases	Table 3
Parasitic Nematodes	Table 4
Viral Diseases	Table 5
Viroid Diseases	Table 6
Suspected Viral and Viroid-Like Diseases	Table 7
Phytoplasmal	Table 8
Insect Pests	Table 9
Mite Pests	Table 10
Pollinators	Table 11
Wildlife	Table 12
Soil Microbes	Table 13
other *Malus x domestica*	orchards, gardens, nurseries, feral trees, abandoned orchards
other	introduced ornamental crab apple species and breeding derived hybrids, native and naturalized crab apple species or crab apple pollinizer cultivars derived from introduced ornamental species

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Appendix 1. Life Forms Potentially Interacting with Cultivated Apple

Table 2: Bacterial Diseases of Cultivated Apple

Blister spot	*Pseudomonas syringae* pv. *papulans* (Rose 1917) Dhanvantari 1977
Crown gall	*Agrobacterium tumefaciens* (Smith and Townsend 1907) Conn 1942
Fire blight (FB)	*Erwinia amylovora* (Burrill 1882) Winslow *et al*. 1920
Hairy root	*Agrobacterium rhizogenes* (Riker et al. 1930) Conn 1942
Source: The American Phytopathological Society	

Table 3: Fungal Diseases of Cultivated Apple

FUNGAL DISEASES	
Alternaria blotch	*Alternaria mali* Roberts = *A. alternata* (Fr.:Fr) Keissl, apple pathotype
Alternaria rot	*Alternaria alternata* (Fr.:Fr.) Keissl
American brown rot	*Monilinia fructicola* (G. Wint.) Honey
Anthracnose canker and bull's-eye rot	*Pezicula malicorticis* (H. Jacks.) Nannf *Cryptosporiopsis curvispora* (Peck) Gremmen in Boerema & Gremmen [anamorph]
Apple scab	*Venturia inaequalis* (Cooke) G. Wint. *Spilocaea pomi* Fr.:Fr. [anamorph]
Apple ring rot and canker	*Botryosphaeria berengeriana* De Not. (Japan, China) = *Physalospora piricola* Nose
Armillaria root rot = shoestring root rot	*Armillaria mellea* (Vahl:Fr.) P. Kumm.
Bitter rot	*Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. [anamorph] *C. acutatum* J. H. Simmons
Black pox	*Helminthosporium papulosum* Berg.
Black root rot	*Xylaria mali* Fromme *X. polymorpha* (Pers.:Fr.) Grev.
Black rot, frogeye leafspot and canker	*Botryosphaeria obtusa* (Schwein.) Shoemaker *Sphaeropsis malorum* Berk. [anamorph]
Blister canker = nailhead canker	*Biscogniauxia marginata* (Fr.) Pouzar = *Nummularia discreta* (Schwein.) Tul. & C. Tul.
Blue mold	*Penicillium* spp. *P. expansum* Link
Brooks fruit spot	*Mycosphaerella pomi* (Pass.) Lindau *Cylindrosporium pomi* C. Brooks [anamorph]
Brown rot blossom blight and spur infection*	*Monilinia laxa* (Aderhold & Ruhland) Honey
Calyx-end rot	*Sclerotinia sclerotiorum* (Lib.) de Bary
Clitocybe root rot	*Armillaria tabescens* (Scop.) Dennis *et al.* = *Clitocybe tabescens* (Scop.) Bres.
Diaporthe canker*	*Diaporthe tanakae* Kobayashi & Sakuma (Japan) *Phomopsis tanakae* Kobayashi & Sakuma[anamorph]
Diplodia canker	*Botryosphaeria stevensii* Shoemaker = *Physalospora malorum* Shear *et al.* *Diplodia mutila* (Fr.: Fr.) Mont. [anamorph]
European brown rot	*Monilinia fructigena* Honey in Whetzel *Monilia fructigena* Pers.:Fr. [anamorph] *Monilinia laxa* (Aderhold & Ruhland) Honey
Fisheye rot	*Butlerelfia eustacei* Weresub & Illman = *Corticium centrifugum* (Lév.) Bres.
Flyspeck	*Schizothyrium pomi* (Mont.:Fr.) Arx *Zygophiala jamaicensis* E. Mason [anamorph]

FUNGAL DISEASES	
Fruit blotch, leaf spot and twig canker	*Phyllosticta solitaria* Ellis & Everh.
Glomerella leaf spot	*Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. [anamorph]
Gray mold rot = dry eye rot, blossom-end rot	*Botrytis cinerea* Pers.Fr. *Botryotinia fuckeliana* (de Bary) Whetzel [teleomorph]
Leptosphaeria canker and fruit rot	*Diapleella coniothyrium* (Fuckel) Barr = *Leptosphaeria coniothyrium* (Fuckel) Sacc. *Coniothyrium fuckelii* Sacc. [anamorph]
Leucostoma canker and dieback	*Leucostoma cincta* (Fr.:Fr.) Hohn. *Cytospora cincta* Sacc. [anamorph] *Valsa auerswaldii* Nitschke = *Leucostoma auerswaldii* (Nitschke) Hohn. *Cytospora personata* Fr. [anamorph]
Marssonina blotch*	*Diplocarpon mali* Harada & Sawamura (Japan, Canada, India, Korea, Rumania) *Marssonina coronaria* (Ellis & J. J. Davis) J. J. Davis [anamorph]
Moldy core and core rot	*Alternaria* spp. *Cladosporium* spp. *Coniothyrium* sp. *Epicoccum* spp. *Pleospora herbarum* (Pers.) Rabenh. *Stemphylium* spp. *Ulocladium* spp. wet core rot, mainly *Penicillium* spp.
Monilia leaf blight*	*Monilinia mali* (Takahashi) Whetzel (Japan, China, former Soviet Union) *Monilia* sp. [anamorph]
Monochaetia twig canker	*Seiridium unicorne* (Cooke & Ellis) Sutton = *Monochaetia mali* (Ellis & Everh.) Sacc. *Lepteutypa cupressi* (Nattras *et al*.) H. J. Swart [teleomorph]
Mucor rot	*Mucor* spp. *M. piriformis* E. Fischer
Nectria canker	*Nectria galligena* Bres. in Strass. *Cylindrocarpon heteronemum* (Berk. & Broome) Wollenweb. [anamorph]
Nectria twig blight = coral spot	*Nectria cinnabarina* (Tode:Fr.) Fr. *Tubercularia vulgaris* Tode:Fr. [anamorph]
Peniophora root canker*	*Peniophora sacrata* G. H. Cunn.
Perennial canker	*Neofabrae perennans* Kienholz *Cryptosporiopsis perennans* (Zeller & Childs) Wollenweb. [anamorph]
Phomopsis canker, fruit decay and rough bark	*Phomopsis mali* Roberts *Diaporthe perniciosa* Em. Marchal [teleomorph]
Phymatotrichum root rot = cotton	*Phymatotrichopsis omnivora* (Duggar) Hennebert = *Phymatotrichum*

FUNGAL DISEASES	
root rot	*omnivorum* Duggar
Phytophthora crown, collar and root rot = sprinkler rot	*Phytophthora* spp. *P. cactorum* (Lebert & Cohn) J. Schröt. *P. cambivora* (Petr.) Buisman *P. cryptogea* Pethybr. & Lafferty *P. megasperma* Drechs. *P. syringae* (Kleb.) Kleb.
Phytophthora fruit rot	*Phytophthora cactorum* (Lebert & Cohn) J. Schrot. *P. syringae* (Kleb.) Kleb.
Pink mold rot	*Trichothecium roseum* (Pers.:Fr.) Link = *Cephalothecium roseum* Corda
Powdery mildew	*Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon
Rosellinia root rot = Dematophora root rot	*Rosellinia necatrix* Prill. *Dematophora necatrix* R. Hartig [anamorph]
Rusts	American hawthorne rust *Gymnosporangium globosum* (Farl.) Farl. Cedar apple rust *Gymnosporangium juniperi-virginianae* Schwein Japanese apple rust* *Gymnosporangium yamadae* Miyabe ex Yamada (Asia) Pacific Coast pear rust *Gymnosporangium libocedri* (C. Henn.) F. Kern Quince rust *Gymnosporangium clavipes* (Cooke & Peck) Cooke & Peck in Peck
Side rot	*Phialophora malorum* (M. N. Kidd & A. Beaumont) McColloch
Silver leaf	*Chondrostereum purpureum* (Pers.:Fr.) Pouzar
Sooty blotch complex	*Peltaster fructicola* (Johnson, Sutton, Hodges) *Geastrumia polystigmatis* Batista & M. L. Farr *Leptodontium elatius* (G. Mangenot) De Hoog
Southern blight	*Sclerotium rolfsii* Sacc. *Athelia rolfsii* (Curzi) Tu & Kimbrough [teleomorph]
Thread blight = Hypochnus leaf blight	*Corticium stevensii* Burt = *Pellicularia koleroga* Cooke = *Hypochnus ochroleucus* Noack
Valsa canker*	*Valsa ceratosperma* (Tode:Fr.) Maire (Japan, China, Korea) *Cytospora sacculus* (Schwein.) Gvritischvili [anamorph]
Violet root rot*	*Helicobasidium mompa* Tanaka (Japan, China, Korea)
White root rot	*Scytinostroma galactinum* (Fr.) Donk = *Corticium galactinum* (Fr.) Burt
White rot	*Botryosphaeria dothidea* (Moug.) Ces. & De Not. *Fusicoccum aesculi* Corda [anamorph]
X-spot = Nigrospora spot	*Nigrospora oryzae* (Berk. & Broome) Petch
Zonate leaf spot*	*Cristulariella moricola* (Hino) Redhead (Japan) *Grovesinia pyramidalis* M. Cline *et al*. [teleomorph]
*Not known to occur naturally on apple in the United States of America	

FUNGAL DISEASES
Source: The American Phytopathological Society

Table 4: Nematode Disease of Cultivated Apples

NEMATODES, PARASITIC	
Dagger nematode	*Xiphinema americanum* Cobb *X. rivesi* Dalmasso *X. vuittenezi* Luc *et al*.
Lesion nematode	*Pratylenchus* spp. *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven
Pin nematode	*Paratylenchus* spp.
Ring nematode	*Criconemella* spp.
Root-knot nematode	*Meloidogyne* spp.
Source: The American Phytopathological Society	

Table 5: Viral Diseases of Cultivated Apple

VIRAL DISEASES	
Apple chlorotic leafspot	genus *Trichovirus, Apple chlorotic leafspot virus* (ACLSV
Apple dwarf (*Malus platycarpa*)	Apple stem pitting virus (ASPV)
Apple flat apple	genus *Nepovirus, Cherry rasp leaf virus* (CRLV)
Apple mosaic	genus *Ilarvirus, Apple mosaic virus* (ApMV) genus *Ilarvirus, Tulare apple mosaic virus* (TAMV)
Apple stem grooving = Apple decline of Virginia crab	genus *Capillovirus, Apple stem grooving virus* (ASGV)
Apple stem pitting = apple Spy 227 epinasty and decline	Apple stem pitting virus (ASPV)
Apple union necrosis and decline	genus *Nepovirus, Tomato ringspot virus* (ToRSV)
Source: The American Phytopathological Society	

Table 6: Viroid Disease of Cultivated Apple

VIROID DISEASES	
Apple blister bark (‘Delicious’)	Apple fruit crinkle viroid (AFCVd)
Apple dimple fruit	Apple scar skin viroid (ASSVd)
Apple fruit crinkle*	Apple fruit crinkle viroid (AFCVd) (Japan)
Apple scar skin = apple dapple, apple sabi-ka, apple bumpy fruit	Apple scar skin viroid (ASSVd)
*Not known to occur naturally on apple in the United States of America. Source: The American Phytopathological Society	

Table 7: Suspected Viral- and Viroid-Like Diseases of Cultivated Apple

SUSPECTED VIRAL- AND VIROID-LIKE DISEASES (graft-transmissible pathogens [GTP])	
Dead spur	GTP, unidentified
False sting	GTP, virus suspected
Green crinkle	GTP, virus suspected
Rough skin	GTP, virus suspected
Star crack	GTP, virus suspected
Source: The American Phytopathological Society	

Table 8: Phytoplasmal and Spiroplasmal Diseases of Cultivated Apple

PHYTOPLASMAL and SPIROPLASMAL DISEASES	
Apple chat fruit	Phytoplasma suspected
Apple decline	Phytoplasma suspected
Apple proliferation	Phytoplasma
Rubbery wood	Phytoplasma suspected
Source: The American Phytopathological Society	

Table 9: Insects Interacting with Cultivated Apple

INSECTS	
Codling Moth	*Lapeyresia pomonella*
Fruittree Leafroller	*Archips argyrosplus*
European Leafroller	*Archips rosanus*
Obliquebanded Leafroller	*Choristoneura rosaceana*
Threelined Leafroller	*Pandemis limitata*
Bruce Spanworm	*Operophtera bruceata*
Green Fruitworm	*Lithophane georgii*
Eyespotted Budmoth	*Spilonota ocellana*
Fall Webworm	*Hyphantria cunea*
Apple Aphid	*Aphis pomi*
Wooly Apple Aphid	*Eriosoma lanigerum*
Rosy Apple Aphid	*Dysaphis plantaginea*
Apple Grain Aphid	*Rhopalosiphum fitchii*
San Jose Scale	*Quadraspidiosus perniciosus*
European Fruit Scale	*Quadraspidiosus ostraeformis*
Oystershell Scale	*Lepidosaphes ulmi*
Apple Mealybug	*Phenacoccus aceris*
Onespotted Stinkbug	*Euschistus variolarius*
Mullein Bug	*Campylomma verbasci*
Lygus Bugs	*Lygus* spp.
Western Flower Thrips	*Frankliniella occidentalis*
White Apple Leafhopper	*Typhlocyba pomaria*
Dock Sawfly	*Ametastegia glabrata*
Tentiform Leafminer (TLM)	*Phyllonorycter blancardella*
Apple Leaf Midge	*Dasineura mali*
Japanese Beetle (JB)	*Popillia japonica*
Source: (MacNay and Creelman, 1958)	

Table 10: Mites Interacting with Cultivated Apple

MITES	
McDaniel Spider Mite	*Tetranychus McDanieli*
Twospotted Spider Mite	*Tetranychus urticae*
Apple Rust Mite	*Aculus schlechtendali*
Source: (MacNay and Creelman, 1958)	

Table 11: Pollinators of Cultivated Apple

POLLINATORS	
Honey bees	*Apis mellifera*
Orchard Mason bees	*Osmia* spp.
Bumblebees	Genus *Bombus*
Solitary bees	*Andrena* spp. *Halictus* spp.
Hover flies	*Eristalis cerealis* *Eristalis tenax*
Source: (Dupree *et al.*, 1987)	

Table 12: Wildlife Interacting with Cultivated Apple

WILDLIFE	
Birds	starling, robin, crow, geese, songbirds, raptors
Mammals	small: mice, voles, shrew large: deer, bear, coyote, rabbits, cats, dogs

Table 13: Soil Microbes Interacting with Cultivated Apple

SOIL MICROBES	
Nematodes	Root Lesion Nematode, *Pratylenchus penetrans* Dagger Nematodes, *Xiphinema*