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Application for an Extension of the Determination of Nonregulated Status for Non-Browning Arctic® Apple (10-161-01p):

Transformation Event NF872

OECD Unique Identifier OKA-NBØØ3-1

Submitted by:

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March 1, 2016

RELEASE OF INFORMATION

The information in this petition is being submitted by Okanagan Specialty Fruits Inc. (OSF) for review by USDA as part of the regulatory process. By submitting this information, OSF does not authorize its release to any third party except to the extent it is requested under the Freedom of Information Act (FOIA), 5 U.S.C. Section 552, and 7 CFR 1, covering all or some of this information. Except in accordance with FOIA, OSF does not authorize the release, publication or other distribution of this information without OSF's prior notice and consent.

CERTIFICATION

The undersigned certifies, that to the best knowledge and belief of the undersigned, 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 petitioner which is unfavorable to the petition.

_____

Neal Carter
President

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SUMMARY

Okanagan Specialty Fruits Inc. (OSF) requests a determination from USDA APHIS that non-browning Arctic® apple event NF872 no longer be considered a regulated article under 7 CFR Part 340, and that APHIS consider this document as an extension to petition 10-161-01p. The subjects of petition 10-161-01p Arctic® apple (Event GD743) and Arctic® apple (Event GS784) received a determination of non-regulated status on February 13, 2015.

For clarity, Arctic® apple event NF872, to be marketed under the name Arctic® Fuji will be referred to NF872 for the remainder of the document. Similarly, deregulated Arctic® apple event GD743 currently marketed as Arctic® Golden will be referred to GD743 and deregulated Arctic® apple event GS784 currently marketed as Arctic® Granny will be referred to GS784.

NF872 was produced by *Agrobacterium*-mediated transformation of the apple cultivar Fuji (NF) with the binary vector GEN-03. The GEN-03 vector contains a chimeric PPO suppression transgene consisting of partial coding sequences of four members of the apple PPO gene family (PPO2, GPO3, APO5 and pSR7) in the sense orientation under control of the cauliflower mosaic virus promoter and nopaline synthase terminator. The transgene is designed reduce expression of the entire PPO gene family and to induce a nonbrowning phenotype in apple fruit. The transgene sequences were derived from the donor organism apple. The antecedent organisms, apple events GD743 and GS784 as described in petition 10-161-01p, were also generated by *Agrobacterium*-mediated transformation with the GEN-03 vector.

Whole genome sequencing of NF872 precisely describes three complex insertions in chromosome 3, 13 and 17 with multiple GEN-03 fragments present in each location. There is approximately 1400 bp of vector backbone (13872 – 67) in chromosome 3, which does not include any functional sequences.

Agronomic evaluations of NF872 demonstrated that no differences in morphology, disease or pest susceptibility were observed compared to the untransformed parent cultivar. Compositional analysis of NF872 demonstrated it to be substantially equivalent to its parent cultivar and within the USDA norms for apple, raw with skin (NDB09003).

Based on all analyses performed, NF872 was found to be comparable to the antecedent organisms, events GD743 and GS784.

A comparison of the characteristics of NF872, GD743 and GS784 is summarized in Table 1 and is discussed in the appropriate sections of this petition.

Table 1: Comparison of Events NF872, GD743 and GS784

Characteristic	**NF872**	**GD743**	**GS784**
Crop	Apple	Apple	Apple
Recipient Organism	*Malus x domestica*	*Malus x domestica*	*Malus x domestica*
Cultivar	Fuji	Golden Delicious	Granny Smith
Transformation Method	*Agrobacterium*-mediated	*Agrobacterium*-mediated	*Agrobacterium*-mediated
Binary Vector	GEN-03	GEN-03	GEN-03
Trait	Non-Browning	Non-Browning	Non-Browning

ACRONYMS AND SCIENTIFIC TERMS

APHIS	Animal and Plant Health Inspection Service
bp	base pairs
CaMV 35S	Cauliflower Mosaic Virus 35s
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FB	fireblight
FDA	Food and Drug Administration
g	gram
GAP	Good Agricultural Practices
GD	Golden Delicious
GD743	Arctic® Golden (Event GD743)
GS	Granny Smith
GS784	Arctic® Granny (Event GS784)
IPM	Integrated Pest Management
JB	Japanese beetle
LB	left border
M	mean
mg	milligram
ml	milliliter
N	number of units in a population
n	number of units in a sample
NF	a non-patented strain of Fuji
NF872	Arctic® Fuji (Event NF872)
ng	nanogram
nptII	neomycin phosphotransferase II gene
NptII	neomycin phosphotransferase II protein
NOS	nopaline synthase
OSF	Okanagan Specialty Fruits Inc.
P	probability
PG	a patented strain of Gala
PGAS	a chimeric suppression sequence comprising fragments of four apple PPO genes (**P**PO2, **G**PO3, **A**PO5 and p**S**R7)
PM	Powdery Mildew
PPO	polyphenol oxidase
P70	duplicated-enhancer CaMV 35S promoter
qPCR	quantitative polymerase chain reaction
RB	right border
RNA	ribonucleic acids
S	standard deviation
SOFA	Statistics Open For All
T-DNA	transfer DNA
TLM	tentiform leafminer
USA	United States of America

USC	United States Code
USDA	United States Department of Agriculture
UTR	untranslated region

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1 RATIONALE FOR NONREGULATED STATUS

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) is responsible for the protection of the US agriculture infrastructure against noxious pests and weeds. Under the Plant Protection Act (7 USC Sections 7701-7772) APHIS considers plants altered or produced by genetic engineering as restricted articles under 7 CFR 340 which cannot be released into the environment without appropriate approvals. APHIS provides that petitions may be filed under 7 CFR 340.6 to evaluate data to determine that a particular regulated article does not present a plant pest risk. Should APHIS determine that the submitted article does not present a plant pest risk; the article may be deregulated and released without further restrictions.

This petition serves an application for an Extension of the Petition for Determination of Nonregulated Status for Arctic® apple events GD743 and GS784. The petition for Arctic apple events GD743 and GS784 received a determination of non-regulated status on February 13, 2015. Event NF872 has been transformed using the same binary vector as events GD743 and GS784, yielding the same non-browning phenotype. Therefore, there are no changes in the rationale from petition 10-161-01p entitled “Petition for the Determination of Non-Regulated Status: Arctic® Apple (*Malus x domestica*) Events GD743 and GS784”.

2 THE APPLE FAMILY

The genus *Malus* belongs to the rose family (Rosaceae).

The CFIA biology document on apple (CFIA 2014) provides information pertaining to the following aspects of apple biology:

- Identity
- Geographical Distribution
- Biology
- Related Species of *Malus domestica*
- Potential Interactions of *Malus domestica* with Other Life Forms

3 DEVELOPMENT OF EVENT NF872

The NF872 event was obtained using the same transformation system (*Agrobacterium*-mediated) as was used to obtain the antecedent organisms GD743 and GS784. The parental cultivar used for the development of NF872 was the virus-free apple cultivar Fuji (NF). A schematic of the development process of event NF872 is shown in Figure 1.

3.1 The Transformation System

The events NF872, GD743 and GS784 were all generated through *Agrobacterium*-mediated gene transfer of the T-DNA from GEN-03.

Agrobacterium-mediated gene transfer of a vector results in the transfer of the DNA fragment between the T-DNA border repeats to the plant genome. The left and right border repeats of *A. tumefaciens* are also inserted in the individual events.

3.2 The Parental Cultivar

The parental cultivar, Fuji (NF), is a commercially grown cultivar of apple.

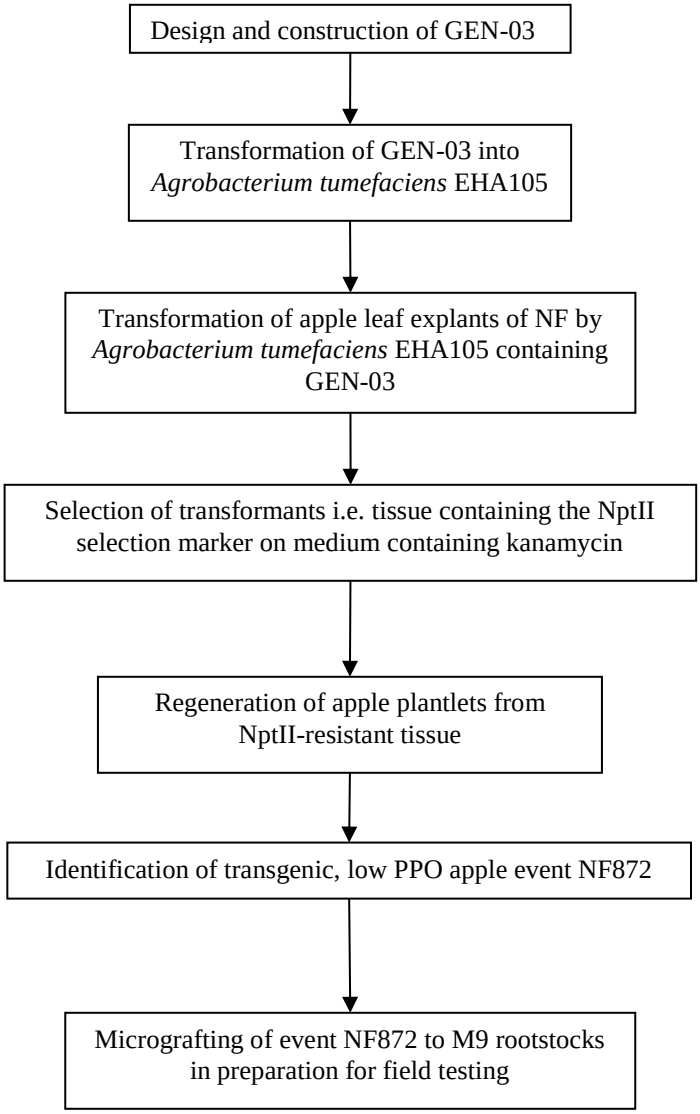

Figure 1: Steps in the Development of NF872

4 DONOR GENES AND REGULATORY SEQUENCES

Event NF872 was 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) is transferred into the apple genome by *Agrobacterium tumefaciens* during the transformation process. The portion of the vector transferred to the plant genome begins near the right border (RB), extends through the PPO suppression transgene and NptII selection marker, and ends near the left border (LB).

The components of the T-DNA used to develop NF872 are provided (Table 2). The sequences of the components of the T-DNA used to develop NF872 are available.

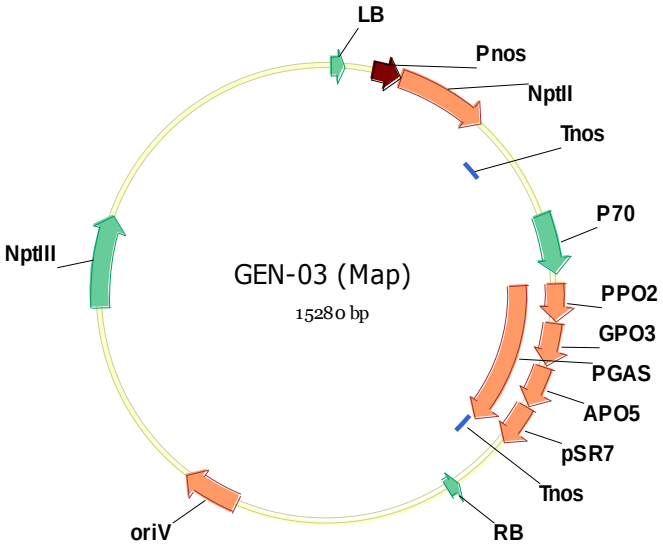

Figure 2: Map of the GEN-03 Vector

Table 2: Components of the T-DNA Used to Develop NF872

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 (M. W. Bevan et al. 1983).
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) (M. Bevan et al. 1983).
P70	0.65	The duplicated-enhancer CaMV 35S promoter with untranslated leader sequence from alfalfa mosaic virus RNA4 (Datla et al. 1992) that directs transcription of the PGAS chimeric suppression sequence.
PGAS	1.82	A chimeric suppression sequence comprising fragments of four apple PPO genes (PPO2, GPO3, APO5 and pSR7), designed to suppress the entire 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) (M. Bevan et al. 1983).
RB	0.14	A right border sequence derived from *Agrobacterium tumefaciens* pTIT37 (Depicker et al. 1982) .

5 GENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION

Whole genome sequencing of NF872 demonstrated that there are three copies of the T-DNA present. There is approximately 1400 bp of vector backbone associated with one of the T-DNA insertions, although no functional elements of the vector backbone were transferred to the plant.

5.1 Whole Genome Sequencing

The intent of OSF's genetic modification was to insert T-DNA from the binary vector GEN-03, including the PPO suppression transgene and NptII selection marker into the genome of the apple cultivar Fuji (NF). A rapid and economical method for precise identification of T-DNA insertion sites using low coverage (20X) Illumina sequencing technology combined with a novel bioinformatics approach was used to describe three complex T-DNA insertions mapped to chromosome 3, 13 and 17 in NF872.

Whole Genome Sequencing of NF872

Genomic DNA was extracted from leaf samples of NF872 and its parent NF control with a DNeasy Plant Mini Kit (Qiagen). The concentration of the DNA was determined using the Qubit dsDNA BR Assay Kit (Invitrogen/Molecular Probes) and the quality was evaluated by 1% agarose gel electrophoresis. 200 ng DNA was used to prepare each library using the TruSeq Nano DNA LT Sample Prep Kit (Illumina) as per the manufacturer's instructions for the 550 bp insert size. The following adapters were used for ligation: Adapter AD006 for NF control and AD012 for NF872. The quality of each library was then checked on a DNA 1000 chip on the 2100 Bioanalyzer (Agilent Technologies Inc.) and the concentration was determined by qPCR using the KAPA SYBR FAST ABI Prism qPCR Kit (Kapa Biosystems) and the StepOnePlus Real-Time PCR System (Applied Biosystems). Equimolar concentrations of the libraries were then pooled and a concentration of 12 pM was used for clustering in one lane of a flowcell on the cBOT (Illumina). The samples were then sequenced (2 x 150 cycles, paired-end reads) on the HiSeq2500 (Illumina) using the TruSeq Rapid SBS Kit-HS Kit (Illumina). Low quality reads were filtered out using custom PERL scripts with the threshold of Q20.

<u>Identification of T-DNA/Apple Junctions</u>

Employing bbDuk[1] open source software, the NF872 reads were searched and those containing 21 bp with 100% identity to the GEN-03 binary vector placed in a separate file. Prior to use in this fetch operation, all apple PPO sequences were deleted from the GEN-03 binary vector sequence. A total of 6,069 GEN-03-coding reads (458 kb total sequence[2]) were identified.

The subset of the GEN-03 reads containing both vector and apple sequences were identified employing a local Multiple BLAST search against the downloaded apple genome (Velasco et al. 2010) followed by assessment using in-house R-script software to characterize T-DNA/apple junction sequences.

[1] BBMap (Brian Bushnell bbushnell@lbl.gov) is available from (http://sourceforge.net/projects/bbmap/).
[2] The identification of 458,000 bp of sequence that maps to the GEN-03 vector (not the PPO part) at 20x coverage, predicts 22,900 of inserted T-DNA sequence, exclusive of PPO sequence. In fact, there are 24,658 bp of inserted sequence, exclusive of the PPO region, inserted into NF872.

A total of 16 reads (2,416 bp) in which the complete read were defined by domains with 100% identity to T-DNA and apple genomic sequences were identified. Additional reads containing these T-DNA/apple junctions were identified by a simple search of the original 6,069 GEN-03 specific reads employing 40 bp of the junction (20 bp T-DNA and 20 bp apple).

A total of six T-DNA/apple junctions were identified, indicating three T-DNA insertions. Individual locations of these insertions in the apple genome were evaluated by using the Phytozome v9.1 BLAST server[3] of the v1.0 contigs of *Malus domestica* cultivar 'Golden Delicious' (GDR[4]). These insertions mapped to three genomic locations on four separate contigs (Table 3). The Chromosome 17 insertion occurred between two mapped contigs, with each end of the insertion mapping to a separate database entry.

Table 3: Chromosomal Locations of T-DNA Insertions

Chromosome	Contig Size (Mb)	Contig Name
3	24.01	MDC009179.475
13	31.60	MDC006811.297
17	5.76	MDC019498.104 / MDC016794.218

Identification of Internal T-DNA Junctions.

As indicated below, all three of the T-DNA insertions in the NF872 apple event are complex, with multiple GEN-03 fragments present. Each of these insertion events will generate specific internal T-DNA junction reads with part of a read originating from different GEN-03 locations. Individual reads crossing these junctions were identified by simple matrix analysis of the original 6,069 GEN-03 specific reads versus the GEN-03 binary vector. The 150 bp reads with only partial identity to a given GEN-03 region, and not included in the T-DNA/apple junctions described above, were identified. In these cases, the section of the read without identity to the first GEN-03 location was found to have identity to a second GEN-03 location. These reads, and their associated pairs, allowed assembly of the individual insertion events described below.

Note that no internal junctions were found for the Chromosome 3 insertion event[5]. Given that the data employed in the internal junction search did not contain the apple PPO reads, an internal site involving recombination between the apple PPO sequences on the two GEN-03 T-DNA fragments is indicated. However, the border junctions for this insertion event are clearly defined.

[3] Phytozome v9.1 BLAST server (http://www.phytozome.net/apple.php).
[4] GDR: Genome Database for Rosaceae (http://www.rosaceae.org/node/1).
[5] One of the two internal recombination sites in the inverted repeat transgenes has been defined (within the RB sequences) while the other recombination site has not been defined and is presumed to be located within the PPO sequences. This undefined internal recombination site could be assigned to the inverted repeat insertion present in either CHR3 or CHR13.

Overview of Insertions in NF872

CHR3 Insertion:

The insertion in CHR3 is comprised of three GEN-03 T-DNA fragments (Figure 3). The arrangement includes an inverted repeat structure with the PPO suppression transgenes pointing towards each other. The recombination site between the two inverted transgenes was not found amongst the sequencing reads, indicating that the recombination site is within the PPO sequences (i.e. the NOS terminators and RB sequences are missing)[6]. The insertion includes 1409 bp of vector backbone (13872 – 67), which does not include any functional sequences.

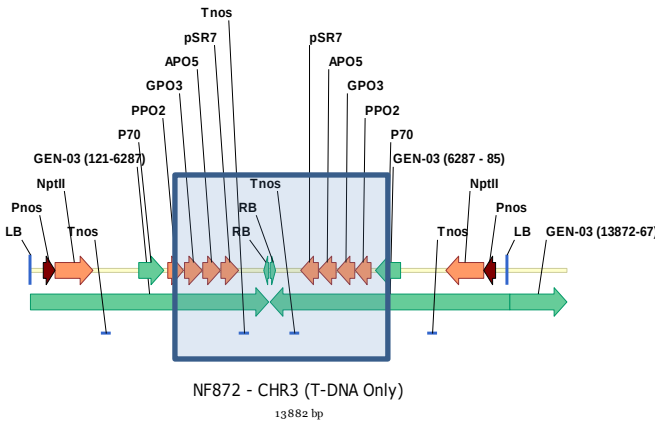

Figure 3: Chromosome 3 Insertion

CHR13 Insertion:

The insertion in CHR13 is comprised of two GEN-03 T-DNA fragments (Figure 4). The arrangement includes an inverted repeat structure with the PPO suppression transgenes pointing towards each other. The recombination site between the two inverted transgenes is within RB sequences. The CHR13 insertion does not include any binary vector backbone sequence.

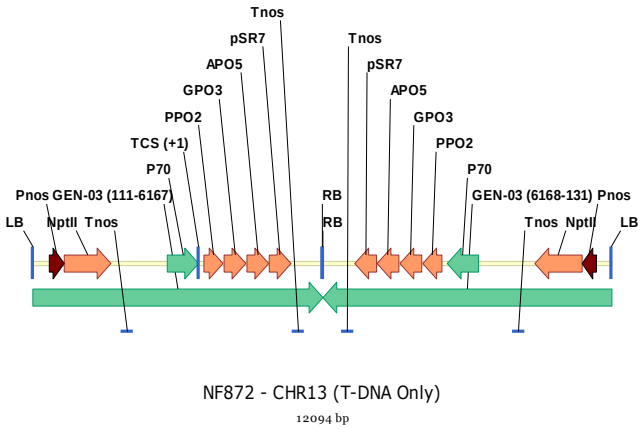

Figure 4: Chromosome 13 Insertion

[6] If the recombination site between the inverted repeat was within the non-PPO regions of the vector, it would have been identified. Therefore, it is presumed that recombination occurred within the PPO regions of the transgenes indicated by the box in Figure 3.

CHR17 Insertion:
The insertion in CHR17 is comprised of two GEN-03 T-DNA fragments (Figure 5). The arrangement includes one partial T-DNA fragment linked to one complete PPO suppression transgene. The insertion leaves a partial CaMV 35S promoter (P70) pointing out into the apple genomic sequence. In this partial CaMV 35S insertion, 127 bp of the CaMV 35S core promoter are missing, including the entire proximal and medial regions and 3' end of the distal region. The CHR17 insertion does not include any binary vector backbone sequence.

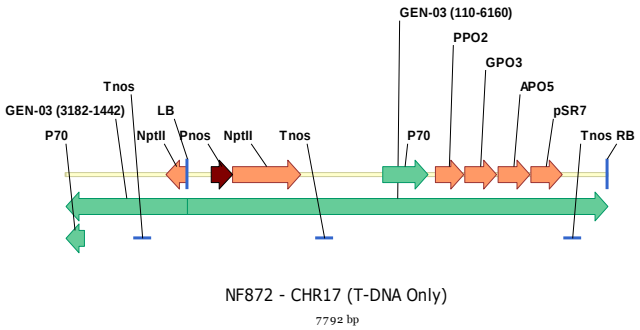

Figure 5: Chromosome 17 Insertion

Sequences of insertion junctions, internal junctions and sequence reads covering these junction sites can be made available on request.

No Potential for Unintended Consequences

Low coverage (20X) sequencing was combined with a novel method for mapping T-DNA insertion sites in NF872. The insertion maps generated are submitted to regulatory agencies in place of Southern Analysis for molecular characterization of NF872 as part of the safety assessment this enhanced apple cultivar. Our assessment is consistent with a minimum risk for unintended consequences associated with the T-DNA insertions that generate NF872 from its parent cultivar NF.

The mapping approach has allowed the precise mapping of the three transgene insertion sites in NF872, providing both the chromosomal location of the insertions and the precise limits of the T-DNA that was inserted at each location. In all but one case, the internal structure of the transgene insertion was resolved. In the case of the CHR3 insertion, the internal recombination site occurred within the PPO sequences, so the bioinformatics approach used here could not resolve the precise location of the crossover.

For insertions in CHR3 and CHR13, the structure of the transgenes in such that there is no potential for expression of unknown endogenous sequences by orphaned promoter sequences. The fragment of GEN-03 binary vector in the CHR13 insertion does not contain any functional sequences and is not a safety concern.

The insertion in CHR17 includes a partial CaMV 35S promoter that is no longer attached to our PPO suppression transgene and which is oriented such that it is pointing into the apple genome. An examination of the sequence shows that most of the functional elements of the promoter fragment are deleted, including the entire proximal and medial regions, the entire distal region of the first enhancer, and the 3' end of the distal region of the second enhancer which will render the promoter fragment non-functional. There is no potential for this partial CaMV 35S insertion to drive expression of unknown endogenous sequences and therefore does represent a safety issue.

5.2 Basis for Resistance to Enzymatic Browning in NF872

The intended consequence of genetic modification in NF872 is to reduce the expression of the PPO gene family. Reduced gene expression should lead to lower total PPO activity and result in a nonbrowning phenotype. To confirm this result, we measure PPO activity (see Section 5.2.1), and bruise response (Section 5.2.2) in NF872 relative to its untransformed control cultivar (NF).

5.2.1 PPO Enzyme Activity

PPO activity is measured is tissue culture leaves, field leaves, immature fruit and mature fruit in NF872 relative to its control cultivar NF according to (Broothaerts et al. 2000).

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, immature 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

Tissue culture plants of NF872 and NF control were sub-cultured ca. every 6 weeks. On two successive sub-cultures, leaf samples (4–5 leaves) of NF872 and the NF control were collected, 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 NF872 was first identified as PPO suppressed on January 29, 2004. 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 >20 highly PPO suppressed events.

PPO activity was reduced 76% in NF872 relative to its control (Table 4). Statistical analysis of the results was not performed.

Table 4: PPO Activity in NF872 - Tissue Culture Leaves

Cultivar	Mean SpActivity[1]	n[2]	PPO Suppression[3]
NF872	1225	2	76 %
NF	5130	2	

[1] SpActivity = Specific Activity of PPO
[2] n = number of leaf samples per cultivar
[3] PPO Suppression = ((Mean SpActivity of NF – Mean SpActivity of NF872) / Mean SpActivity of NF)*100

Field Leaves

Shoots of NF872 were grafted onto M9 rootstocks (Lane 1992) and grown under greenhouse conditions before being sent to field trials. Mature leaves of field-grown plants were collected from the field trial in the summer of 2015 (June 23, 2015). Two trees of NF872 and two trees of NF control were sampled. Since apples are clonally propagated, only a subset of the plants that went to the field was tested. For each tree, a sample comprising 4–5 leaves was collected, snap frozen in liquid nitrogen and stored at -80^{o}C until processing. In preparation for the PPO assay each sample of 4–5 leaves was ground under liquid nitrogen into a powder and was subsampled three times.

PPO activity was reduced 76% in NF872 relative to its control (Table 5).

Table 5: PPO Activity in NF872 - Field Leaves

Cultivar	Mean SpActivity[1]	*S*[2]	n[3]	PPO Suppression[4]
NF872	466	78	6	76 %
NF	1971	213	6	

[1] SpActivity = Specific Activity of PPO
[2] *S* = standard deviation
[3] n = number of samples per cultivar; in this case pooled leaf samples from two trees per cultivar with each pooled leaf sample subsampled three times
[4] PPO Suppression = ((Mean SpActivity of NF – Mean SpActivity of NF872) / Mean SpActivity of NF)*100

Independent sample t-test of average PPO specific activity in NF872 versus NF control was performed using SOFA Statistics v1.4.5. The nominal alpha criterion level is = 0.01. 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, $t(10) = 16.27$, $p = < 0.001$, with NF872 having lower PPO specific activity than NF.

Immature Fruit

Immature fruit, approximately 15 mm in length, were collected from field trial in the summer of 2015 (June 23, 2015). Six apples of NF872 and six apples of NF were randomly harvested and stored at 4^{o}C until processing. Skin samples were taken from each fruit, snap frozen in liquid

nitrogen and stored at -80°C until processing. When sampling for PPO activity, each skin sample was ground under liquid nitrogen into a powder and sampled (50 mg) for the PPO assay.

PPO activity was reduced 96% in NF872 relative to its control (Table 6).

Table 6: PPO Activity in NF872 - Immature Fruit

Cultivar	Mean SpActivity[1]	S^2	n[3]	PPO Suppression[4]
NF872	229	46	6	96 %
NF	6174	3135	6	

[1] SpActivity = Specific Activity of PPO
[2] *S* = standard deviation
[3] n = number of fruit per cultivar
[4] PPO Suppression = ((Mean SpActivity of NF – Mean SpActivity of NF872) / Mean SpActivity of NF)*100

Independent sample t-test of average PPO Specific Activity in NF872 versus NF control was performed using SOFA Statistics v1.4.5. The nominal alpha criterion level is = 0.01. 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, $t(10) = 4.644$, $p = <0.001$, with NF872 having lower PPO specific activity than NF.

Mature Fruit

Mature apple fruit were harvested from field trial in 2015. Six apples of NF872 and six apples of NF were randomly harvested and stored at 4°C until processing. Skin samples were taken from each fruit, snap frozen in liquid nitrogen and stored at -80°C until processing. For each apple, eight samples (5o mg) were taken and measured for PPO activity.

PPO activity was reduced 98% in NF872 relative to its control (Table 7).

Table 7: PPO Activity in NF872 - Mature Fruit

Cultivar	Mean SpActivity[1]	S^2	n[3]	PPO Suppression[4]
NF872	7	6	48	98 %
NF	904	390	48	

[1] SpActivity = Specific Activity of PPO
[2] *S* = standard deviation
[3] n = number of samples per cultivar; in this case six fruit per cultivar with each fruit sampled eight times
[4] PPO Suppression = ((Mean SpActivity of NF – Mean SpActivity of NF872) / Mean SpActivity of NF)*100

Independent sample t-tests of average PPO Specific Activity in NF872 versus NF control were performed using SOFA Statistics v1.4.5. The nominal alpha criterion level is = 0.01. 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, $t(94) = -15.93$, $p < 0.001$, with NF872 having lower PPO specific activity than NF.

5.2.2 Controlled Bruising of Apple

Enzymatic browning that occurs in response to mechanical bruising is measured in mature fruit of NF872 relative to its control cultivar NF using Minolta Chroma Meter CR-400.

Mature apple fruit were harvested from field trial in 2015. Six apples of NF872 and six apples of NF were randomly harvested and were stored at 4^{o}C until processing. Each apple was bruised three times in three different locations on the mid-section of the apple (n = 18). The apples were left to sit for 2 hours at room temperature to allow bruise development. An apple peeler was used to expose an area that was larger than the bruised area. The peeled area was measured using a colorimeter 'off' and 'on' the bruise. The color of the apple flesh is compared on and off the bruise location. Bruising is reported as Total Change in Color (ΔE*).

The average ΔE* for NF (20.89) produced visible brown bruising of the apple flesh, while the average ΔE* for NF872 (6.72) did not produce visible bruising of the apple flesh (Table 8).

Table 8: Controlled Bruising of NF872 – Mature Fruit

Cultivar	ΔE*	*S*[1]	n[2]	Temp[3]
NF872	6.73	2.49	18	18
NF	20.89	4.51	18	18

[1] *S* = standard deviation
[2] n = number of bruises per cultivar; in this case six fruit per cultivar with each fruit bruised three times
[3] Bruising done while apple flesh temperature was at room temperature.

Independent sample t-tests of average ΔE* in NF872 versus NF control was performed using SOFA Statistics v1.4.5. The nominal alpha criterion level is = 0.01. 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, $t(34) = 11.65$, $p = <0.001$, with NF872 having lower ΔE* than NF.

Images and Other Data

NF872 showed markedly reduced browning response relative to its untransformed parent cultivar, further demonstrating success of the genetic transformation and that a nonbrowning phenotype had in fact resulted. Images of the bruise response are included in the photographic record provided in Appendix 1 (Figure 6).

Other forms of mechanical damage, such as slicing or juicing, have consistently yielded the same dramatic nonbrowning phenotype for NF872.

Method for Measuring Bruising Response in Apples

An Impact Device is used to deliver a controlled bruise to the apple with minimal destruction to the tissue. Apples are bruised in a consistent manner using the Impact Device. Bruise response is reported as Total Change in Color (ΔE) between bruised and non-bruised tissue as measured using a Minolta Chroma Meter CR-400.*

The Impact Device

The Impact Device consists of a 35 mm glass marble and a 28 cm cardboard tube, plus a foam support used to stabilize and protect the apple during bruise. The bruise is made by dropping the marble down the tube which is placed on the surface of the apple. The foam support, 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 Impact Device is designed to bruise the apple, but with minimum physical damage to the apple tissue. The impact will produce an observable bruise 2 hours after impact, but does not produce a significant bruise at time zero.

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 3 times and allowed to sit at room temperature for2 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). Total Change in Color (ΔE) are calculated as:*

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

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

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

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

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.*

6 AGRONOMIC EVALUATION

6.1 Description of the Field Trial

6.1.1 Site Selection

OSF's field trial site in the USA is covered by USDA APHIS Notifications and Permits detailed in Table 9. This trial is located in New York and represents a major apple growing region.

Table 9: 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	Annual report submitted
14-027-102r	March 19, 2014	March 31, 2017	Annual report submitted

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 three NF872 trees and two NF control trees. The balance of the trial consists of control trees and other events (see Appendix 2, Table 30: Map of the New York Field Trial – 2005 Block).

Agronomic and pest and disease data reported in this petition are from the New York field trial.

6.1.2 Horticultural Management

OSF operates the NY field trial with the assistance of a local collaborator who provides the day-to-day onsite horticulture management.

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 and fruit quality. For this reason, little pest and disease pressure is tolerated or observed in commercial apple orchards.

6.1.3 Data Collection

In New York State, OSF's collaborator provided the independent horticultural support and data. This collaborator has considerable experience in running tree fruit field trials and is capable of handling this task.

6.2 Agronomic Performance

Agronomic performance data was collected at the New York field trial. A sampling of data from 2009 from field trial planted in 2005 (5th leaf) is presented (Table 10). 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. This is particularly true over the first few years or until the trees reach their expected height (approximately 10ft) and they begin to get heading cuts. Other factors in the field, such as the quality of the graft union, tree location etc., result in considerable variability in the tree growth and performance. Apple orchards are managed for fruit quality, and we have established this in other sections.

Table 10: Agronomic Performance - New York

Sample Description		TCA[2] (cm2)			Scaffolds			Fruit Number at Harvest		
Cultivar	**Leaf**	**Average**	***S*[2]**	**n[3]**	**Average**	***S***	**n**	**Average**	***S***	**n**
All Trees	5th	764	14	118	14	5	118	58	42	118
NF872	5th	1394	423	3	15	3	3	27	6	3
NF	5th	346	52	2	10	4	2	45	7	2

[1] TCA = trunk cross-sectional area measured at the end of each season

[2] Scaffolds = the side limbs that form as a tree grows and where typically most of the fruit buds are located.

[2] *S* = standard deviation

[3] n = number of trees

6.3 Pest and Disease Characteristics

OSF evaluated how NF872 performed in the field with respect its untransformed parent cultivar. Pest and disease characteristics were monitored, and data was collected that would help to analyze if NF872 was less, equal or more susceptible to pest and diseases than the NF control. As described in section 6.1, OSF engaged a horticulturalist to monitor field trials and to report incidents of pest and disease and the related data associated with these incidents. The following sections provide data specific to relevant pest and disease incidents as these occurred in the field trial over the multiple years of evaluation and are used to establish if there was a significant variance between the NF872 and the control cultivar.

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 = 0.01. An effect is considered to be significant if the actual alpha value (P) is not greater than the nominal alpha criterion level.

6.3.1 Scab (*Venturia inaequalis)*

Scab is a fungal disease infects both apple fruit and leaves. Scab is of serious concern to commercial apple growers particularly in wet and humid areas. In 2006, scab lesions in the fruit were reported in the NY field trial, but there were very few trees or fruit infected (4/119 trees).

NF872 and NF control trees were similarly not infected by scab (Table 11).

Table 11: Scab - NY2005 - August 24, 2006

Cultivar	Infected	Not Infected	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square (Yates) =		P not calculated	
df =			

6.3.2 Mildew (*Podosphara leucotricha)*

Mildew is a fungal disease that 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. Mildew is a common disease of concern to commercial growers in both humid and drier growing areas. Mildew was identified on the NY field trial in 2006 infecting only 2/119 trees in the field trial. The number of mildew infected trees is reported.

NF872 and NF control trees were similarly not infected by mildew (Table 12).

Table 12: Powdery Mildew - NY2005 - July 21, 2008

Cultivar	Infected	Not Infected	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square (Yates) =		P not calculated	
df =			

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. Fireblight was reported in 2009 and 2011 (Table 13), but the incidences did not involve NF872 or NF trees.

NF872 and NF control trees were similarly not infected by fireblight (Table 13).

Table 13: Incidences of Fireblight

Trial – Row	Position	Plant Name	Vector	Cultivar	Year Reported
NY – 102	26	845-7	GEN-03	GD	2011
NY – 102	59	554-2	GEN-02	GD	2009

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. Aphid infestation 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. In 2008, aphids were identified in 5 of 119 trees in the field trial with each tree similarly infested at less than 50%. In 2009, aphids infested all trees in the field trial with each tree similarly infested at >50%. Therefore, the number of aphid infested trees is reported.

In 2008, NF872 and NF control trees were similarly not infested by aphids (Table 14).

Table 14: Aphids - NY2005 - July 21, 2008

Cultivar	Infested	Not Infested	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square (Yates) =		P not calculated	
df =			

In 2009, NF872 and NF control trees were similarly infested by aphids (Table 15).

Table 15: Aphids - NY2005 - November 19, 2009

Cultivar	Infested	Not Infested	Total
NF872	3	0	3
NF control	2	0	2
Total	5	0	5
Chi Square =		P not calculated	
df =			

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. Mite infestation 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 mites. In 2009, mites were identified in 6 of 119 trees in the field trial, with each tree similarly infested at < 10%. Therefore, the number of mite infested trees is reported.

NF872 and NF control trees were similarly not infested by Mites (Table 16).

Table 16: Mites - NY2005 - November 19, 2009

Cultivar	Infested	Not Infested	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square =		P not calculated	
df =			

6.3.6 Japanese Beetle *(Popillia japonica)*

This newly-emerging apple insect pest was monitored in the New York field trial site which reported incidences of Japanese Beetle (JB) in 2008 affecting 24 of 119 trees, 2010 affecting 5 of 119 trees and 2011 affecting 30 of 119 trees. JB Monitoring involves an inspection of each leaf looking for the presence of JB and Leaf Skeletonization (LS). LS is assessed as 1 = light (1-5%) , 2 = moderate (6-10%), and 3 = severe (>10%). The number of trees JB infested is reported.

In 2008, Chi-square (Yates) revealed that the percentage of JB infested trees did not significantly differ between NF872 and NF (Table 17).

Table 17: Japanese Beetle (JB) - NY2005 - July 21, 2008

Cultivar	Infested	Not Infested	Total
NF872	0	3	3
NF	1	1	2
Total	1	4	5
Chi Square (Yates) =	0.05	P = 0.82	
df =	1		

In 2010, NF872 and NF control trees were similarly not affected by JB (Table 18).

Table 18: Japanese Beetle - NY2005 - July 21, 2010

Cultivar	Infested	Not Infested	Total
NF872	0	3	3
NF control	0	2	0
Total	0	5	5
Chi Square (Yates) =		P not Calculated	
df =			

In 2011, Chi-square (Yates) test revealed that the percentage of JB infested trees did not significantly differ between NF872 and NF (Table 19).

Table 19: Japanese Beetle - NY2005 - July 13, 2011

Cultivar	Infested	Not Infested	Total
NF872	1	2	3
NF	0	2	2
Total	1	4	5
Chi Square (Yates) =	0.05	P = 0.82	
df =	1		

6.3.7 Codling Moth *(Lapeyresia pomonella)*

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 other 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.

Tentiform Leaf Miner was not detected in the NY field trial.

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 Sutton 2005). Burr Knot was identified throughout the NY field trial in 2006 affecting 68 of 119 trees in the field trial. The presence or absence of burr knot is assessed. The number of burr knot affected trees is reported.

Chi-square (Yates) test revealed that the percentage of Burr Knot affected trees did not significantly differ between NF872 and NF (Table 20).

Table 20: Field Report - Burr Knot - NY2005 - August 24, 2006

Cultivar	Affected	Not Affected	Total
NF872	2	1	3
NF control	1	1	2
Total	3	2	5
Chi Square (Yates) =	0.31	P = 0.58	
df =	1		

6.3.10 Leaf Spot

The majority are probably due to *Alternaria mali* infections, although a small proportion may be due to black rot (*Botryosphaeria obtusa*) infections (Jones & Aldwinckle 1990). Leaf Spot was identified throughout the NY field trial in 2006 affecting 81 of 119 trees in the field trial. 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 presence or absence of leaf spot is assessed.

NF872 and NF control trees were similarly not affected by leaf spot (Table 21).

Table 21: Leaf Spot - NY2005 - August 24, 2006

Cultivar	Affected	Not Affected	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square (Yates) =		P not calculated	
df =			

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). In New York, russet is assessed while the apples are still on the trees. Therefore, the number of russet-affected trees is reported here.

Russet was identified throughout the NY field trial in 2009 affecting 69 of 119 trees, 2010 affecting 65 of 119 trees and 2011 affecting 52 of 119 in the field trial. Data is reported as trees affected or not affected with russet.

In 2009, NF872 and NF control trees were similarly not affected by russet (Table 22).

Table 22: Russet - NY 2005 - November 19, 2009

Cultivar	Affected	Not Affected	Total
NF872	0	3	3
NF control	0	2	2
Total	0	5	5
Chi Square (Yates) =		P not Calculated	
df =			

In 2010, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between NF872 and NF control (Table 23).

Table 23: Russet - NY2005 - July 21, 2010

Cultivar	Affected	Not Affected	Total
NF872	0	3	3
NF control	1	1	2
Total	1	4	5
Chi Square (Yates) =	0.05	P = 0.82	
df =	1		

In 2011, Chi-square (Yates) test revealed that the percentage of russet affected trees did not significantly differ between NF872 and NF (Table 24).

Table 24: Field Report - Russet - NY2005 - July 13, 2011

Cultivar	Affected	Not Affected	Total
NF872	0	3	3
NF	1	1	2
Total	1	4	5
Chi Square (Yates) =	0.05	P = 0.82	
df =	1		

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

Campylomma Bug was not detected in the NY field trial.

6.3.13 Fruit Rot after Storage

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. Fruit rot after storage was monitored in 2009 on fruit harvested in 2008 fruit.

NF872 and NF control apples were similarly not affected by fruit rot (Table 25).

Table 25: 2008 Fruit Rot After Storage - NY2005 - June 30, 2009

Tan Rot			
Cultivar	**Infected**	**Not Infected**	**Total**
NF872	0	11	11
NF control	0	2	2
Total	0	13	13
Chi Square =		P not Calculated	
df =			
Black Rot			
Cultivar	**Infected**	**Not Infected**	**Total**
NF872	0	11	11
NF control	0	2	2
Total	0	13	13
Chi Square =		P not Calculated	
df =			

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 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 the field trial. Notably, management of codling moth and scab was consistent with normal commercial production of apples. Normal management procedures were sufficient to prevent these pests and diseases in NF872 and no additional control measures were required. This indicates that NF872 is 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 2009, aphids infested the entire NY field trial.

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

A summary of pest and disease incidences in the NY field trial is presented in Table 26. Under normal field management conditions, the incidences of pest and disease over the long duration of the field have been minimal and the NF872 has not distinguished itself as significantly more or less susceptible to any particular pest or disease,

Table 26: Effect of Cultivar or Arctic® apple Trait on Pest and Disease Resistance

Bacterial Disease				
Disease[1]	**Date[2]**	**Table**	**Arctic® apple Trait[3]**	
			Effect Present	**Type of Effect**
FB	2009, 2011	Table 13	No	
Fungal Disease				
Disease	**Date**	**Table**	**Arctic® apple Trait**	
			Effect Present	**Type of Effect**
Scab	August 24, 2006	Table 11	No	
PM	July 21, 2008	Table 12	No	
Leaf Spot	August 24, 2006	Table 21	No	
Tan Rot	June 30, 2009	Table 25	No	
Black Rot	June 30, 2009	Table 25	No	
Insect Pest				
Pest	**Date**	**Table**	**Arctic® apple Trait**	
			Effect Present	**Type of Effect**
Aphids	July 21, 2008	Table 14	No	
	November 19, 2009	Table 15	No	
Mites	November 19, 2009	Table 16	No	
JB	July 21, 2008	Table 17	No	
JB	July 21, 2010	Table 18	No	
JB	July 13, 2011	Table 19	No	
CM				
TLM				
CB				
Other Conditions				
Condition	**Date**	**Table**	**Arctic® apple Trait**	
			Effect Present	**Type of Effect**
Burr Knot	August 26, 2006	Table 20	No	
Russet	November 19, 2009	Table 22	No	
	July 21, 2010	Table 23	No	
	July 13, 2011	Table 24	No	

[1] FB = Fire Blight; PM = Powdery Mildew; JB = Japanese Beatle; CM = Codling Moth; TLM = Tentiform Leafminer; CB = Campylomma Bug.

[2] Date = Date on which data was collected.

[3] If a significant effect of the Arctic apple trait were present (Effect Present), it would be indicated if the pest, disease or condition were greater in NF872 or the NF control (Type of Effect).

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 event NF872 and the NF control were subjected to nutritional and proximate analysis, and measured for total phenolics. 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 29). 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 2015. Six apples of NF872 and six apples of NF were randomly harvested for each testing facility. The apples were shipped to Exova for the proximate analysis and Brunswick Laboratories for the total phenolics analysis.

The composition of NF872 and NF control fall within the range of, or closely approximates the published data for apple, raw with skin (NDB09003) provided by the USDA (Table 27).

Table 27: Apple Fruit Compositional Analysis - New York

Description	Units	NF872	NF	Apple (NDB09003)		
				Average	Min	Max
Fat	%	ND	ND	0.17	0.05	0.31
Protein	%	0.33	0.25	0.26	0.17	0.57
Moisture	%	79.5	81.7	85.56	82.4	87.5
Ash	%	0.33	0.28	0.19	0.07	0.48
Carbohydrates	%	19.8	17.7	13.81		
Calories	cal / 100 g	81	72	52		
Sugar Profile	%	12.85	11.14	10.39	8.77	12.0
Dietary Fiber	%	3.9	3.6	2.4	1.4	3.5
Potassium	mg / 100 g	139	97	107	88	136
Vitamin C	mg / 100 g	4.3	ND	4.6	4	5.5
Phenolics	mg GAE/100g	118	54	262	165	396
Number of Composite Samples =		n = 6	n = 6			

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)

Phenolic content was assessed by Brunswick Laboratories (Norton, MA). The acceptable precision is <15% relative standard deviation. The phenolic result is expressed as mg gallic acid equivalency (Slinkard et al. 1977).

Substantially Equivalent

In terms of proximates (fat, protein, moisture, ash, carbohydrates, calories and sugar profile) and dietary fiber, apples of NF872 are nutritionally equivalent to apples of the control NF (Table 28). Apples of NF872 were found to have significantly higher levels of potassium and vitamin C than apples of the control NF. Although it appears that NF872 has higher levels of phenolics than NF the difference is not statistically significant.

Table 28: Apple Fruit Compositional Analysis - New York - Statistical Analysis

Description	Units	NF872	NF	Statistics		
				p	t statistic	df
Fat	%	ND	ND			
Protein	%	0.33	0.25	0.111	-1.746	10
Moisture	%	79.5	81.7	0.028	2.562	10
Ash	%	0.33	0.28	0.092	-1.861	10
Carbohydrates	%	19.8	17.7	0.039	-2.381	10
Calories	cal / 100 g	81	72	0.035	-2.442	10
Sugar Profile	%	12.85	11.14	10.39	8.77	10
Dietary Fiber	%	3.9	3.6	0.387	-0.904	10
Potassium	mg / 100 g	139	97	**<0.001**	-5.484	10
Vitamin C	mg / 100 g	4.3	ND	**0.009**	-3.253	10
Phenolics	mg GAE/100g	118	54	0.066	-2.062	10
	Number of Fruit =	n = 6	n = 6			

Independent sample t-tests of average NF872 versus NF control were performed using SOFA Statistics v1.4.5. The nominal alpha criterion level is = 0.01. 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 with NF872 having higher vitamin C and Potassium than NF.

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

In trying to understand why vitamin C is higher in NF872 the following rational is presented. 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 & Klein 1982). Since there is no vitamin C in the control apples, it indicates to us that there was a delay between the processing of the apple samples (i.e. grinding) and the measurement of the vitamin C.

It is not immediately clear why potassium content is higher in NF872 than in the control NF. However, potassium content in both NF872 and NF control approximates the USDA norms.

Evidence provided here is consistent with the concept that NF872 is nutritionally equivalent with its parent cultivar, prior to processing. After processing, NF872 is nutritionally stable, whereas NF loses vitamin C and some phenolic compounds, through the action of PPO.

6.5 Conclusions

The following statements support the conclusion that event NF872 is equivalent to its parent cultivar under commercial cultivation and does not pose a plant pest risk:

(a) A field trial maintained and observed by an independent horticultural consultant has been established in a major apple growing area in the USA;

(b) Assessment of agronomic performance shows that NF872 is equivalent to its parent cultivar. 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, NF872 was not systematically more or less susceptible to plant pests and disease;

(d) NF872 is nutritionally equivalent to the published norms for apple and may have improved phenolic, vitamin C and potassium stability;

(e) NF872 did not demonstrate any level of increased weediness that the control trees;

7 ENVIRONMENTAL CONSEQUENCES OF INTRODUCTION OF THE TRANSFORMED CULTIVAR

Importance of Apple, Impact of Enzymatic Browning in Fresh and Processed Apple Products, Expected Economic Impact of Arctic® apple, Gene Flow and Stewardship of Arctic® apple Gene Flow were discuss previously.

Fuji is the fifth most popular cultivar in the United States (Table 29). It is anticipated Arctic® Fuji (Event NF872) covered under this petition, will be a direct replacement for Fuji.

Table 29: 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: U.S. Apple Association official, e-mail message to Commission staff, August 24, 2008. Available in (Lynch 2010).						

8 ADVERSE CONSEQUENCES OF INTRODUCTION

Given that NF872 was developed using the same binary vector that was used to develop GD743 and GS784 and that there is no evidence held within this petition that would distinguish NF872 from its commercial parent cultivar NF, there should be no adverse consequence of the introduction of NF872 beyond what was previously discussed for GD743 and GS784.

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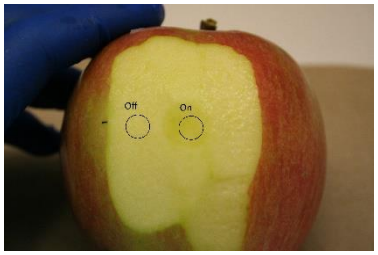
Appendix 1: Photographs

NF872 

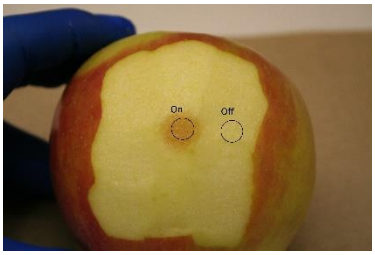
NF 

Figure 6: Bruise Response in NF872

These images were taken during controlled bruising tests. NF872 and its untransformed control were subjected to 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 circle labeled 'On' shows where the bruise was made. Note that for NF872, no bruising is visible.

Appendix 2: Field Map

The following table (Table 30) is a map of our New York field trial planted in 2005. The NF872 and NF controls trees are identified in bold at positions 21, 26, 27, 35 and 52 in Row 101.

Table 30: 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**

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
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
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

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
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
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

New York (2005 Block)						
Trial - Row	**Position**	**Plant Name[2]**	**Vector**	**Cultivar**	**Vector Purpose**	**Year Planted**
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. Since apple trees are clonally propagated trees with the same event name are genetically identical (i.e. 872-2 and 872-4 are clones).