Del Monte Fresh Produce Company

July 30, 2012

Mr. Michael C. Gregoire
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road, Unit 98
Riverdale, MD 20737

Mr. Gregoire:

Del Monte Fresh Produce Company, Inc. (hereinafter "Del Monte Fresh") has genetically engineered pineapple (*Ananas comosus* var. *comosus*) and wishes to import whole, fresh fruits and processed products into the United States for commercial distribution and sale. We request confirmation from the United States Department of Agriculture Animal and Plant Health Inspection Service that whole, fresh fruits and processed products of genetically engineered pineapple described herein may be legally imported into the United States in conformance with 7 CFR Part 340. In support of our request, the company provides the following information.

I. Del Monte Rosé Pineapple

Pineapple varieties currently in widest commercial production exhibit internal flesh color that ranges from pale yellow to deep golden yellow. To differentiate its products in the marketplace, Del Monte Fresh has genetically engineered pineapple to increase lycopene levels in the edible tissues to produce a novel rose color. Del Monte Fresh has also genetically engineered pineapple to suppress ethylene biosynthesis to prevent precocious flowering thereby promoting more uniform growth and development of the pineapple plant to aid fruit production, harvest and quality. Del Monte Fresh has designated its pineapple with increased lycopene and controlled flowering phenotype by the variety name, Del Monte Rosé pineapple.

To achieve its novel fruit color, Del Monte Fresh has altered expression of genes involved in lycopene biosynthesis to increase levels in edible tissues of pineapple fruit. The genes of interest are derived from edible plant species, pineapple (*Ananas comosus*) and tangerine (*Citrus reticulata*). Specifically, Del Monte Fresh has overexpressed a tangerine phytoene synthase gene (*Psy*) and suppressed endogenous lycopene β-cyclase (*bLyc*) and lycopene ε-cyclase (*eLyc*) gene expression using RNA

interference (RNAi) technology in order to increase accumulation of lycopene in edible fruit tissues.

To achieve flowering control, Del Monte Fresh has altered expression of pineapple genes involved in ethylene biosynthesis. Ethylene is a plant hormone that plays an important role in every phase of plant development, including seed germination, fruit ripening, leaf and flower senescence, and abscission. Unlike most other crops, pineapple flower initiation is induced by ethylene and, therefore, in order to reduce precocious flowering, Del Monte Fresh has altered endogenous ethylene biosynthesis by suppressing endogenous meristem-specific ACC synthase gene expression using RNAi technology.

Pineapple (*Ananas comosus var. comosus*) variety Del Monte Gold® Extra Sweet Pineapple was transformed by *Agrobacterium tumefaciens*-mediated transformation. To achieve both phenotypes, *Agrobacterium* strains containing transformation plasmids for increased lycopene or decreased ethylene biosynthesis were co-cultivated with pineapple tissues. Transformed cells were selected on growth media containing chlorsulfuron and subsequently screened for the presence of effect genes by polymerase chain reaction (PCR). A summary of genetic elements used in Del Monte Fresh Rosé pineapple is enclosed.

II. United States Pineapple Market

Pineapples, grown by Del Monte Fresh for the United States market, are produced in Costa Rica and sold into four channels: fresh (60%), fresh cut (15%), juice (20%), and frozen (5%). Quality is the main factor that determines the channel into which fruit is sent. Class 1 fruit goes to the fresh market. Class 2 fruit, which may contain cosmetic defects to the crown or shell, is diverted to fresh cut. Class 3 fruit has high shell color due to over-maturity, sunburn, or bruises and is directed to juice or frozen depending on fruit size.

Fresh market fruit is sold in two modes: spot sales and contract sales. Approximately 50% of the fresh fruit volume is sold at contracted prices to large retailers, food service operators, and wholesalers. Most contracted fruit is sold in truckload size lots. The balance of the fresh, whole fruit is sold at prices that are negotiated daily (spot sales) and may go to large retailers, food service operators, or Del Monte Fresh distribution centers (DCs). The DCs function like wholesalers and resell the fruit by the pallet or individual boxes. Fruit that is packed for Del Monte Fresh Cut Operations (FCOs) have the crowns removed during the packing process. FCOs cut the pineapples into cylinders, spears or chunks and sell it alone or in combination with other fruit. FCO customers include retailers, wholesalers and food service operators. Juice and frozen product are prepared in San Jose, Costa Rica. Juice is sold to bottlers and frozen product is sold in bulk to distributors who sometimes repackage the product before selling it to end users (such as bakeries), retail customers or food service operators.

Del Monte Rosé pineapple is a new variety developed as a point of differentiation between Del Monte Fresh Produce Company, Inc. and other branded pineapple growers. Del Monte Rosé pineapple is not intended to be a replacement for Del Monte Gold® Extra Sweet Pineapple, but rather as a niche product that could expand the market for pineapple. Propagation and planting of Del Monte Rosé pineapple will occur on company-owned farms in Costa Rica; it will not be commercially cultivated in the United States. Del Monte Rosé pineapple will be sold into the same channels as Del Monte Gold® pineapple. It will be offered to selected retailers and it will also be distributed via company FCOs and DCs. Third class fruit will be used for juice and frozen product.

The commercial distribution of Del Monte Rosé pineapple will be always be under the control of the company as all of the facilities for growing, packing, shipping, distributing and processing are company-owned. The product will be branded with a trade name and each fruit will be individually labeled. Additional controls of the distribution are in the form of a plant patent (or plant variety protection outside the United States). Del Monte Fresh has applied to the United States Patent and Trademark Office for a plant patent (Application No. 13/507,101, filed June 4, 2012) and is in the process of filing for similar protections in Canada, Central America, European Union, and China. It is a violation of the Patent Act to asexually reproduce, use, offer for sale, or sell the plant so reproduced, or any of its parts, throughout the United States, or to import the plant so reproduced, or any parts thereof, into the United States, unless authorized by the patent holder (35 U.S.C. §161 et seq.). Additionally, utility patents related to the genetic engineering of pineapple for increased lycopene or flowering control have been granted to Del Monte Fresh in the United States, European Union, Australia and elsewhere. Standard food security measures will protect Del Monte Rosé pineapple throughout the distribution chain.

III. Ecology and Reproductive Biology of Pineapple

Ananas comosus is the most economically important plant in the family *Bromeliaceae.* The genus *Ananas* is recognized among *Bromeliaceae* by its characteristic inflorescence, which is fused into a fleshy compound fruit (syncarp), a unique dense rosette of scape-wide leaves, and medium to large fruits. There are approximately 30 cultivars of *A. comosus* that are grown commercially in tropical and subtropical countries around the world at latitudes ranging from 30° South to 30° North. In the United States, Hawaii is the only commercial producer of pineapples, primarily on Oahu and Maui with smaller amounts on Kauai and the island of Hawaii. Pineapple is also reportedly grown commercially in Puerto Rico and for home landscape use in Florida (USDA, NRCS. 2012. The PLANTS Database (http://plants.usda.gov, accessed 13 July 2012).

Pineapple is asexually propagated from various plant parts for commercial production. For production purposes, the parts used are crowns, slips, hapas and suckers, with crowns and slips being the most common. Sexual reproduction in pineapple is possible, but is essentially limited to intensive breeding programs due to strong gametophytic self-

incompatibility. This incompatibility is generally stronger in *A. comosus* var. *comosus* than in other varieties, which results in near complete absence of seed production in commercial pineapples. Furthermore, pineapple growers take measures to discourage pollination as it is both unnecessary for fruit development and significantly detracts from fruit quality due to presence of hard inedible seed. *A. comosus* var. *comosus* is naturally compatible with other varieties (sub-species) and cultivars of *A. comosus* var. *comosus*. However, none of the five other sexually compatible sub-species are reported to exist in the United States. The enclosed biology document more fully describes the ecology and reproductive biology of pineapple (*A. comosus* var. *comosus*).

IV. Imported Fresh, Whole Rosé Pineapple Is Unlikely to Persist in the Environment

There are two theoretical modes for gene dispersal from imported whole, fresh pineapple fruit: (1) formation and dispersal of seed as a result of fertilization with pollen from an external source in the area of cultivation or (2) formation and dispersal of clonal tissues as a result of asexual propagation. The probability of gene dispersal that leads to persistence in the United States environment from the importation of whole, fresh pineapple fruit by either route is highly unlikely.

1. Pineapple fruits develop parthenocarpically by the complete fusion of berry-like fruitlets. Commercial pineapple plantations are grown as a monoculture, which essentially precludes seed production due to strong gametophytic self-incompatibility of pineapple. At the company's Costa Rica plantations, no external pollen sources from sexually compatible species are present, which essentially precludes seed formation. In the remote possibility that flower pollination leads to seed formation, seeds remain embedded within the fleshy fruit and are not dispersed. Pineapple seeds exhibit significant dormancy due to an impermeable seed coat and require scarification to facilitate germination. Furthermore, pineapple seedlings are very delicate and do not establish in the environment without significant human intervention.
2. Pineapple plants can regenerate from crowns excised from whole, fresh fruit, but are unlikely to persist in the environment without human intervention. Plant crowns can be intentionally nurtured into whole plants, but when discarded as waste, do not readily establish in the environment. Pineapple exhibits few if any characteristics of weedy species and is not considered a weed in any environment where cultivated. Imported whole, fresh pineapple fruit cannot pollinate any plant species in the United States since no viable pollen remains on mature fruit.
3. Pineapple cultivation favors a temperature range of 18°C to 32°C. Areas subject to mean temperatures below 15°C or above 32°C do not support pineapple cultivation. Plants do not tolerate frost and prolonged cold exposure (0°C) of established plantings will destroy the plant canopy, delay maturity, and adversely affect fruit quality. Accordingly, pineapple can be grown outdoors only in the southern-most United States and Hawaii.
4. Current United States phytosanitary regulations prohibit entry of whole, fresh pineapple fruit into Hawaii.

In light of the above reasons, the probability that importation of whole, fresh fruit and processed products could lead to persistence of Del Monte Fresh Rosé pineapple in the United States environment is essentially nil.

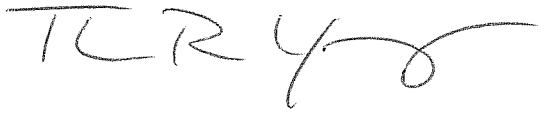
V. Finding that Whole, Fresh Rosé Pineapple and Processed Products May be Imported is Consistent With Previous APHIS Determinations

APHIS has previously determined that certain genetically engineered plants and plant products may be imported into the United States in conformance with 7 CFR 340. These include, among others:

> A genetically engineered Baby's Breath (*Gypsophila paniculata*) produced by inserting a plant gene whose expression results in altered flower color and a kanamycin resistance gene from *Escherichia coli* K-12. APHIS determined that commercial distribution of imported cut Baby's Breath flowers poses little, if any risk of gene dispersal and persistence in the environment. APHIS confirmed that genetically engineered cut Baby's Breath does not require an importation or interstate movement permit under 7 CFR part 340.

We are currently collecting data and information to complete the food safety consultation for Del Monte Rosé pineapple with the U.S. Food and Drug Administration and to support Costa Rican government approvals. We respectfully request that APHIS confirm that whole, fresh fruit and processed products described above may be legally imported into the United States in conformance with 7 CFR Part 340, so that we can advance this exciting new product introduction with confidence. Thank you for your assistance in this matter.

Very truly yours,



Thomas Young, PhD
Senior Vice President
R&D and Agricultural Services
241 Sevilla Avenue
Coral Gables, FL 33134
305-520-8089
tyoung@freshdelmonte.com

Enclosure

Genetic Elements for Increased Lycopene

Genetic Element	Function
BRI promoter	A promoter derived from the bromelaine inhibitor (BRI) gene from *Ananas comosus* that drives fruit-enhanced expression of the target gene.
Psy	A phytoene synthase (Psy) gene from *Citrus reticulata*, which encodes an enzyme in lycopene biosynthesis.
Ubi terminator	A terminator derived from the polyadenylation sequence of the ubiquitin (Ubi) gene from *Ananas comosus* terminates transcription of the target gene.
BRI promoter	A promoter derived from the promoter sequence from the bromelaine inhibitor (BRI) gene from *Ananas comosus* that drives fruit-enhanced expression of the RNAi target gene.
eLyc sense fragment	A fragment of the lycopene ε-cyclase gene from *Ananas comosus* in sense orientation, which is used in an RNAi expression system to down-regulate endogenous lycopene ε-cyclase, an enzyme in lycopene biosynthesis.
ST-LS1 intron	An intron of the light-inducible tissue-specific ST-LS1 gene from *Solanum tuberosum* that functions as a spacer between sense and antisense gene fragments enhancing vector stability.
eLyc antisense fragment	A fragment of the lycopene ε-cyclase gene from *Ananas comosus* in antisense orientation, which is used in an RNAi expression system to down-regulate endogenous lycopene ε-cyclase, an enzyme in lycopene biosynthesis.
Ubi terminator	A terminator derived from the polyadenylation sequence of the ubiquitin (Ubi) gene from *Ananas comosus* terminates transcription of the RNAi target gene.
BRI promoter	A promoter derived from the promoter sequence from a bromelaine inhibitor (BRI) gene from *Ananas comosus* that drives fruit-enhanced expression of the RNAi target gene.
bLyc sense fragment	A fragment of the lycopene β-cyclase gene from *Ananas comosus* in sense orientation, which is used in an RNAi expression system to down-regulated endogenous lycopene β-cyclase, an enzyme in lycopene biosynthesis.
ST-LS1 intron	An intron of the light-inducible tissue-specific ST-LS1 gene from *Solanum tuberosum* that functions as a spacer between sense and antisense gene fragments enhancing vector stability.
bLyc antisense fragment	A fragment of the lycopene β-cyclase gene from *Ananas comosus* in antisense orientation, which is used in an RNAi expression system to down-regulated endogenous lycopene β-cyclase, an enzyme in lycopene biosynthesis.
Ubi terminator	A terminator derived from the polyadenylation sequence of the ubiquitin (Ubi) gene from *Ananas comosus* terminates transcription of the RNAi target gene.
Selectable Marker	
EHS-Ubi promoter	A promoter derived from the epoxide hydrolase (EHS) gene fused to the ubiquitin (Ubi) gene promoter and the native intron from *Ananas comosus* that drives constitutive expression of the selectable marker gene.
ALS	A modified acetolactate synthase gene from *Nicotiana tabacum* that confers resistance to sulfonylurea and allows selection of transformed plant cells.

ALS 3'	A terminator derived from the untranslated polyadenylation signal from the acetolactate synthase (ALS) gene from *Nicotiana tabacum*.

Genetic Elements for Flowering Control

Genetic Element	Function
Ubi promoter	A promoter derived from the ubiquitin (Ubi) gene from *Ananas comosus* and the native intron that drives constitutive expression of the target gene(s).
ACC3' sense fragment	A 3' sequence of the meristem-specific aminocyclopropane carboxylic acid synthase (ACC) gene from *Ananas comosus* in sense orientation, which is used in an RNAi expression system to down-regulate endogenous ACC synthase, a key enzyme in ethylene biosynthesis.
ST-LS1 intron	An intron of the light-inducible tissue-specific ST-LS1 gene from *Solanum tuberosum* that functions as a spacer between sense and antisense gene fragments enhancing vector stability.
ACC3' antisense fragment	A 3' sequence of the meristem-specific aminocyclopropane carboxylic acid synthase (ACC) gene from *Ananas comosus* in antisense orientation, which is used in an RNAi expression system to down-regulate endogenous ACC synthase, a key enzyme in ethylene biosynthesis.
Ubi terminator	A terminator derived from the polyadenylation sequence of the ubiquitin (Ubi) gene from *Ananas comosus* terminates transcription of the RNAi target gene.
Selectable Marker	
EHS-Ubi promoter	A promoter derived from the epoxide hydrolase (EHS) gene fused to the promoter from the ubiquitin (Ubi) gene promoter and the native intron from *Ananas comosus* that drives constitutive expression of the selectable marker gene.
ALS	A modified acetolactate synthase gene from *Nicotiana tabacum* that confers resistance to sulfonylurea and allows selection of transformed plant cells.
ALS 3'	A terminator derived from the untranslated polyadenylation signal from the acetolactate synthase (ALS) gene from *Nicotiana tabacum*.