USDA/APHIS Draft Environmental Assessment

In response to University of Florida Petition 04-337-01P seeking a Determination of Nonregulated Status for X17-2 Papaya Resistant to Papaya Ringspot Virus

OECD Unique Identifier UFL-X17CP-6

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

TABLE OF CONTENTS

I. Summary

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-337-01p) from the University of Florida, Institute of Food and Agricultural Sciences (UFL-IFAS). The petition requests a determination of non-regulated status for genetically engineered (transformed) UFL-X17CP-6 papaya (*Carica papaya* L.) derived from their transformation event X17-2 (referred to hereafter as X17-2 papaya). The genetically engineered X17-2 papaya (*C. papaya* L.) was developed to resist infection by papaya ringspot virus (PRSV). This X17-2 papaya is currently a regulated article under USDA regulations at 7 CFR part 340, and as such, field tests of X17-2 papaya have been conducted under notifications issued by APHIS (#'s 99-251-02n, 03-160-02n, 04-309-09n, and 06-044-01n). UFL-IFAS petitioned APHIS requesting a determination that X17-2 papaya does not present a plant pest risk and that X17-2 papaya and progeny derived from crosses with other non-regulated papaya should no longer be considered regulated articles under these APHIS regulations.

This EA describes the biology of papaya and papaya ringspot virus and the use of pathogen-derived resistance as a mechanism for developing new plant varieties. A number of potential environmental impacts are also addressed. These include the following: gene introgression, weediness, effects on non-target organisms, effects on threatened and endangered species, biodiversity, viral interactions, commercial use, agricultural practices, conventional and organic farming, and potential cumulative impacts resulting from adoption of X17-2 papaya. Various Executive Orders and international standards and treaties are also considered and addressed.

II. Introduction

Papaya (*Carica papaya* L.) is described as an almost herbaceous, typically unbranched small (2-10 meters tall) tree cultivated worldwide in tropical and subtropical climates (OECD 2005). Papaya is in the family Caricaceae and is generally considered the only member of the genus *Carica* L. within the family comprised of 5 other genera.

Papaya ringspot virus (PRSV) is reported to cause major crop losses in papaya in many growing areas (OECD 2005). PRSV, a potyvirus, is spread by mechanical means and by aphids (OECD 2005). Treatment of growing areas with insecticides to control disease-carrying insect vectors has generally been ineffective in controlling spread of PRSV.

X17-2 papaya was developed using genetic engineering techniques to introduce the PRSV coat protein (*cp*[1]) gene into papaya trees. The PRSV-*cp* gene was introduced into X17-2 papaya via *Agrobacterium*-mediated transformation (Petition, Section III, page 5) and enables X17-2 papaya to resist infection by PRSV. The PRSV-*cp* gene was introduced into the papaya along with one plant-expressed selectable marker gene, *nptII* (Petition, Section V.A., Insertion Analysis, pp.8-11). This marker gene is commonly used

[1] By convention, notations to genes are made lower case letters and are italicized.

and enables researchers to select those plant tissues that have been successfully transformed with the gene of interest. The *nptII* gene is under the control of a nopaline synthase (*nos)* promoter from *Agrobacterium tumefaciens*, a common soil bacterium. PRSV-*cp* gene expression is controlled by a cauliflower mosaic virus (CaMV) 35S promoter. X17-2 plants and their progeny do produce both NPT II[2] and PRSV-CP proteins.

The DNA regulatory sequences derived from the plant pathogens *Agrobacterium tumefaciens* and CaMV cannot cause plant disease by themselves or in conjunction with the genes that they regulate in the X17-2 papaya.

Analysis of X17-2 papaya shows that it is resistant to PRSV infection in areas where it has been grown (Petition, Section V.D., pp. 13-15). X17-2 and its progeny have been grown in the field since 1999 at the University of Florida Center in Homestead, FL. These trials have provided evidence that X17-2 is resistant to infection by PRSV in this location and that this trait is stable under field conditions.

In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR part 372), this EA has been prepared for X17-2 papaya in order to specifically address the potential for impact to the human environment[3] through the unconfined cultivation and use in agriculture of the regulated article.

III. PURPOSE and NEED

The developer of the X17-2 papaya trees, the University of Florida, submitted a petition to USDA-APHIS requesting that APHIS make a determination that these papaya trees shall no longer be considered regulated articles under 7 CFR part 340. From a commercial perspective, current methods for control of PRSV are mostly ineffective and growers who choose to grow X17-2 or its progeny would have less concern for loss of trees and fruit due to PRSV infection. Under regulations in 7 CFR part 340, APHIS is required to give a determination on the petition for nonregulated status. APHIS has prepared this EA before making a determination on the status of X17-2 papaya as regulated articles under APHIS regulations.

This EA has been prepared to comply with the provisions of the National Environmental Policy Act of 1969 (NEPA) (42 United States Code (U.S.C.) 4321 *et seq*.) as prescribed in implementing regulations adopted by the Council on Environmental Quality (40 Code of Federal Regulations (CFR) §§ 1500–1508), USDA's NEPA regulations (7 CFR 1b), and APHIS' NEPA implementing procedures (7 CFR 372).

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[2] By convention, notations to proteins are capitalized.

[3] Under NEPA regulations, the "human environment" includes the natural and physical environment and the relationship of people with that environment" (40 CFR § 1508.14).

A. USDA regulatory authority

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulations and is also a plant pest, or if there is reason to believe that it is a plant pest. These papaya trees have been considered regulated articles because they were genetically engineered with regulatory sequences and a viral coat protein gene derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted data and 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 is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

B. U.S. Environmental Protection Agency and Food and Drug Administration Regulatory Authorities

In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating biotechnology in the United States: the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA), and the U.S. Department of Health and Human Services' Food and Drug Administration (FDA). Products are regulated according to their intended use, and some products are regulated by more than one agency. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment. USDA, EPA, and FDA apply regulations to biotechnology that are based on the specific nature of each genetically engineered (GE) organism.

Under the Coordinated Framework, the U.S. Environmental Protection Agency (EPA) is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. In order to be registered as a pesticide under FIFRA, it must be demonstrated that when used with common practices, a pesticide will not cause unreasonable adverse effects in the environment. Because the use of Plant Incorporated Protectants, such as viral coat proteins, is considered pesticidal, the University of Florida has submitted a registration package to EPA for X17-2 papaya.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the U.S. Food and Drug Administration (FDA) enforce the tolerances set by EPA. EPA has previously granted a tolerance exemption for PRSV coat protein in papaya (EPA, 1997).

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA ensures that human food and animal feed, including those derived from bioengineered sources, are safe and wholesome. University of Florida has submitted a food and feed safety and nutritional assessment summary to FDA for X17-2 papaya in 2007 that is currently under agency review.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would not alter the current regulatory status of the X17-2 papaya. Under this alternative, X17-2 papaya trees would continue to be subject to the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would still be required for introduction of X17-2 papaya trees. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest or environmental risk from the unconfined cultivation of papaya trees engineered to express the coat protein of PRSV. Under this alternative, the petition would be denied.

B. Proposed Action: Determination that X17-2 papaya trees are No Longer Regulated Articles, in Whole (Preferred Alternative)

Under this alternative, X17-2 papayas would no longer be subject to the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of papaya ringspot virus resistant papaya derived from this transformation event. APHIS might choose this alternative if there were sufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of papaya trees engineered to express the coat protein gene of PRSV and marker gene (*nptII*).

APHIS has chosen the proposed action as the preferred alternative. This is based upon the lack of plant pest characteristics of X17-2 papaya. The environmental assessment by APHIS has indicated that neither of the alternatives should significantly impact the environment.

V. Affected Environment

X17-2 papaya likely would have limited use in terms of geographic distribution for effective control of PRSV. The PRSV gene used to engineer X17-2 is from a Florida isolate of the virus and reports have shown that similar gene constructs give resistance to the disease only from highly similar viral strains (OECD 2005). It is likely therefore that X17-2 will only be useful for PRSV disease control in Florida and Caribbean regions. The scope of a determination on X17-2 papaya, however, is considered to cover the entire U.S. and its territories.

Papaya, *Carica papaya* L., is described as an almost herbaceous, typically unbranched small tree in the family Caricaceae (OECD 2005). The fruits are usually consumed fresh or sometimes processed or pressed into beverages. Various plant parts yield latex and the enzyme papain which is used as a meat tenderizer. Commercial production in the United States occurs primarily in Hawaii and secondarily in Florida and Puerto Rico. Papaya is native to the north-tropical Western Hemisphere. Typically the tree will grow 2-10 m in height although commercial growers will remove trees when fruits become difficult to harvest from the ground. The center of origin for the species is believed to be Central America or southern Mexico (OECD 2005). Papaya was probably domesticated in northern tropical America. Feral papayas are documented in tropical habitats of North, Central and South America as well as the Caribbean. In southern Florida there is evidence of pre-Columbian use of papaya (OECD 2005). In the 1500s papaya was moved to the Philippines and India and readily disseminated to tropical Asia, Africa, and the Pacific islands such that it is now cultivated worldwide in tropical and subtropical climates (OECD 2005). A related genus, *Vasconcellea*, the highland papaya is considered the closest relative to *Carica papaya* L. but is not grown in the U.S. Natural hybridization between the two genera is not known to occur. Extensive information on papaya is available in the Organisation for Economic Co-operation and Development (OECD) Consensus Document on papaya (OECD 2005) which is incorporated here by reference.

Depending on the variety and individual seed genetics, papaya plants may be male (producing only anthers and pollen), female (producing only pistils, fruit and seed) or hermaphrodite (producing anthers, pollen, pistils, fruit, and seed). Commercially, hermaphrodite and female trees are preferred in plantings as they are the only trees that produce fruit. Because the sex of trees is not known until a tree flowers, multiple seeds or seedlings are typically planted in a single location and unwanted trees (i.e., male) are removed when the first flowers appear. Commercial plantings of papaya typically begin bearing fruit within the first year of planting. Most commercial plantings will be managed for 3 years, but this time may be shorter or longer, before trees are removed and replanted. Typical planting densities are between ~525 and 875 trees per acre and generally consist of only female and hermaphrodite trees. Producers either sow seed directly in the field or germinate them in a nursery prior to planting. Adequate water and nutrients are critical for optimal plant growth and fruit quality. Typical yields vary widely from location to location. Water availability, soil nutrients, varieties grown, pest and pathogen problems, and management practices all affect yield. The average annual worldwide fruit yield from 1991-2000 was ~15,000 lbs per acre. Typical yields in Hawaii are 19,800 to over 29,000 lbs per acre. The most significant limitations to papaya cultivation result from virus infection. PRSV, a potyvirus, is most problematic but other

viruses impacting yields include papaya mosaic virus, papaya leaf distortion mosaic virus, papaya droopy necrosis virus, papaya leaf curl virus and a number of others. Other pathogens include fungal diseases caused by *Phytophthora*, *Pythium* and *Rhizoctonia* which may be controlled by various fungicides. Pest problems include a number of aphid species, fruit flies, mealy bugs, leafhoppers, mites, and nematodes.

A. Papaya Ringspot Virus and Pathogen Derived Resistance

Plant viruses are ubiquitous in the environment and negatively impact global agriculture because of their ability to reduce the quality and, more importantly, the yield of food and fiber crops (Matthews 1991; AIBS 1995; Hadidi et al. 1998; Pappu 1999). Plant virus diseases cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species (OECD 1996). Hundreds of plant viruses have been described, affecting a wide range of plants and trees (ICTV 2005). These viruses infect virtually every plant species, and under natural conditions, certain plant viruses are nearly always present on particular crop or weed hosts (OECD 1996; Waterhouse 2001). The severity of virus infection can vary depending upon location and from one growing season to the next (OECD 1996).

Despite some diversity in size, shape and host range, plant viruses are very simple organisms that have small genomes and contain a small number of genes (Matthews 1991; OECD 1996; Goldbach et al. 2003). Most viruses are composed of proteinaceous coatings called capsids that contain either RNA or DNA genomes. Some capsids may also contain carbohydrates and lipids (Hull 2004; OECD 1996; Goldbach et al. 2003). This proteinaceous coat plays an important role in protecting the genetic material, as well as in insect vector specificity and virus movement inside plants (Callaway et al. 2001; Culver 2002).

Most plant viruses are obligate parasites that move from plant to plant via vector-mediated transmission[4] (Matthews 1991; OECD 1996). Plant viruses can also be spread in a number of other ways, depending upon the virus type, including seed transmission, pollen transmission, and/or mechanical transmission[5] (Matthews 1991; OECD 1996). In some agricultural regions, certain crop species cannot be grown effectively because of the persistent presence of infected plant populations and/or potential virus vectors (OECD 1996). In other areas around the world, chemical pesticide sprays are used to help control insect vectors, but while these pesticide sprays provide the only means of relief, they are both expensive and not very effective in controlling virus disease spread (OECD 1996).

1. Papaya ringspot virus

[4] Vector-mediated transmission can include: insects (e.g., aphids and whiteflies), nematodes, mites, and fungi.

[5] Mechanical transmission can include: intentional transfer of infected plant sap or purified virus in solution, vegetative propagation, infected host tissue, or contaminated equipment.

Papaya ringspot virus (PRSV), a potyvirus, is the causal agent of one of the most damaging viral diseases in papaya. PRSV was first reported and described in Hawaii in 1949 (Jensen 1949). Infection of papaya with PRSV has resulted in major crop losses in Hawaii, Mexico, the Caribbean, South America, Africa and Southeast Asia (OECD 2005). Two strains of PRSV have been identified; type W which infects cucurbits (e.g., watermelon) and type P which primarily infects papayas (Davis and Ying 1999). Researchers working to develop pathogen derived resistance have analyzed genetic differences between PRSV strains from around the world, focusing on differences in virus coat protein[6] sequences (Tennant et al., 1994, Davis and Ying 1999). Pathogen derived resistance to viruses may be most effective using expression of geographically specific coat proteins with high levels of coat protein sequence similarity. Research is continuing to determine how much sequence similarity is required for disease resistance and whether genes can be introduced from multiple viral pathogens for more comprehensive disease control (OECD 2005).

Symptoms of the disease caused by PRSV include dark green rings on fruit, yellow mosaic on leaves, overall stunting of plants and shoestring-like leaves. PRSV is spread by mechanical means and by aphids. Control strategies include removal of infected plants and treatment with insecticides to control aphid populations. Aphids spread PRSV in a non-persistent[7] manner and therefore can acquire the virus from an infected tree and transmit it to a healthy tree in just a few minutes. Once disease is established in an area, however, even these methods are of marginal value for control (OECD 2005).

PRSV infection of commercial papayas in Hawaii in the mid-1990s led to massive losses in production (Gonsalves 2003). Development of genetically engineered papaya varieties resistant to PRSV by Cornell University and University of Hawaii researchers, however, led to recovery of typical yields within just a few years (Gonsalves 2003). Those varieties, “Sunset” and “Rainbow” and their progeny, now represent almost half of the papayas harvested in Hawaii. “Sunset” papaya was the subject of USDA/APHIS petition 96-051-01p which was granted nonregulated status in September 1996. Those papayas were very similar to the plants that are the subject of the current petition. In addition to papaya research conducted in the U.S., work in other countries is progressing and proposing expanded research to address other viral diseases in papaya (Yeh 2004)

2. Pathogen Derived Resistance

In general, the tools available for plant virus disease control are limited, as is their effectiveness in most instances. In cases where plants are susceptible to viruses, common control or management strategies have relied upon ineffective conventional measures of disease control such as use of virus-free planting material, vector control, or eradication

[6] The coat protein serves to surround and protect the genetic material of the virus.

[7] In non-persistent aphid transmission, the viruses are acquired rapidly from plants (i.e., seconds), maintained in the aphid stylet, and can only be transmitted for a very short period of time (usually minutes) (Hull, 2004).

(Gooding 1985; Superak et al. 1993; Swiezynski 1994; OECD 1996; Khetarpal et al. 1998). Unlike other agricultural pests (e.g., insects), there are no chemical control measures that can be used directly to prevent or control plant virus disease outbreaks (OECD 1996; Hadidi et al. 1998; Pappu 1999).

As an alternative approach, the concept of pathogen-derived resistance (PDR) was described about two decades ago (Sanford and Johnston 1985; Grumet et al. 1987). Pathogen-derived resistance is based upon the use of pathogen-derived genes to generate specific host resistance (Goldbach et al. 2003). One form of PDR is cross-protection which was first identified in 1929 (McKinney 1929) and involves intentional inoculation of crop plants with a closely related mild virus strain (Gooding 1985; Fulton 1986; Sherwood 1987; Beachy 1999; Goregaoker et al. 2000; Culver 2002; Abbas 2005). Prior infection with a protecting or mild strain of a virus can prevent or interfere with infection by a related, more severe strain of the virus (Gooding 1985; Fulton 1986; Sherwood 1987; Beachy 1999; Goregaoker et al. 2000; Culver 2002; Abbas 2005).

The mechanisms for cross protection have been determined to be either RNA-based or protein-mediated. RNA-based cross protection likely results from a gene silencing (post transcriptional gene silencing—PTGS) mechanism that targets viral RNA for destruction (Angell and Baulcombe 1997; Jan et al. 1999; Goregaoker et al. 2000; Savenkov and Valkonen 2001; Culver 2002; Lacomme et al. 2003; Lu et al. 2003; Baulcombe 2004; Chang et al. 2005). Protein-mediated cross protection likely relies upon several different mechanisms, including interference (Sherwood 1987; Beachy 1999; Goregaoker et al. 2000; Culver 2002). This interference relies upon the coat protein of the mild strain of a virus to properly associate with and block disassembly of a more virulent strain of a virus, thus preventing replication and hence infection by the more virulent strain of the virus (Culver 2002).

In recent years, much of the research and development for plant virus disease control has focused on development of transgenic virus resistant plants. Building upon the concept of PDR and mechanisms previously described for cross protection, genetic modifications of host plants and trees are made that allow for expression of viral genes or proteins. Plant expression of viral genes or proteins often acts to delay or prevent infection by the same or related viruses. This form of PDR was first accomplished in 1986 by Roger Beachy and colleagues (Abel et al. 1986) in which tobacco plants engineered to express tobacco mosaic virus (TMV) coat protein were resistant to TMV infection.

Since the initial successful development of a virus resistant transgenic plant, numerous other virus resistant plants and trees have been developed and field tested (Tepfer 2002; ISB 2007). Over the past 15 plus years, nearly 900 virus resistant plants and trees have been authorized by USDA-APHIS for field testing in the United States. Some of these crops have been deregulated by APHIS and grown commercially in the United States, including plants that express viral coat protein genes (e.g., papaya ringspot virus resistant papaya and ZW-20 squash) or a replicase protein gene (potato leafroll luteovirus resistant potato) (EPA 1998; Gonsalves 1998; ISB 2007). Most of this virus resistance is based on the pathogen-derived resistance, and most often using VCP or VCP gene expression as the basis for resistance (Tepfer 2002; ISB 2007).

VI. Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of X17-2 papaya and its progeny in the absence of confinement.

1. Potential impacts from gene introgression from X17-2 papaya into its sexually compatible relatives.

In assessing the risk of gene introgression from X17-2 papaya into its sexually compatible relatives, APHIS considered two primary issues: 1) the potential for gene flow and introgression; 2) the potential impact of introgression.

There is no indication that papaya (*Carica papaya* L.) will hybridize with related *Vasconcellea* species (highland papaya) (OECD 2005). Researchers have attempted to hybridize plants from these genera in the past but most attempts have failed to produce intergeneric hybrids or sterile hybrids (OECD 2005). *Vasconcellea* species are native to central and South America and therefore it is highly unlikely that there would be any interaction between X17-2 papaya and *Vasconcellea* species. Because introgression of genes from X17-2 into *Vasconcellea* species is highly unlikely, impacts relating to gene introgression are also highly unlikely. Therefore, if APHIS chooses either the no action alternative (Alternative A) or the proposed action alternative (Alternative B), there are unlikely to be environmental impacts.

2. Potential impacts based on the relative weediness of X17-2 papaya

APHIS searched numerous scientific databases and could find none that considered papaya to be a weed. Papaya is noted to have feral or naturalized populations in suitable tropical or subtropical locations. Papaya is not listed as a Federal noxious weed or on other weed lists such as:

- Federal Noxious Weed List (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html)
- California Weed Species List (http://www.extendinc.com/weedfreefeed/list-b.htm)
- Hawaii Weed Species List (http://www.hawaiiag.org/hdoa/adminrules/AR-68.pdf)
- Florida Weed Species List (http://plants.usda.gov/java/noxious?rptType=State&statefips=12)

Papayas are sensitive to many herbicides and can be controlled using paraquat, triclopyr or glyphosate (OECD 2005).

The developer analyzed numerous characteristics (Petition, Section V.D. and V.E., pp. 13-16) of X17-2 papaya and noted no significant differences in phenotype compared with non-engineered papaya that would indicate that X17-2 has more weedy characteristics.

Because papaya is not described as a weedy species and there are no sexually compatible species with which it would hybridize, there should be no impact from increased weedy characteristics (beyond those of the non-engineered papaya) from a decision to grant nonregulated status to this variety (Alternative B). There should also be no impact from the no action alternative (Alternative A).

3. Potential impact on non-target organisms, including beneficial organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target organisms from cultivation of X17-2 papaya and its progeny. The subject papaya has been field tested for seven years at the Tropical Research and Education Center, UF/IFAS, near Homestead, FL (petition p. 13). Trees were observed regularly and maintained using standard cultural practices, including use of insecticides and fungicides. The applicant noted common diseases and pests such as powdery mildew, anthracnose, papaya fruit fly, and two-spotted spider mites. Compared to control non-transgenic plants in these trials, no differences in susceptibility or resistance to these organisms were noted (petition, p. 13). A high degree of resistance to infection by PRSV was noted.

APHIS further considered the biology of X17-2 papaya with respect to its potential to affect non-target organisms such as beneficial insects (e.g., honeybees, lacewings, lady beetles, etc.). X17-2 papaya does express detectable PRSV coat protein, but at very low levels compared to papayas infected with PRSV (petition, pp. 11-12). This does not increase the issue of potential impacts to non-target organisms as the PRSV coat protein is not known to have any toxic properties. EPA has established a tolerance exemption for PRSV coat protein and the genetic material necessary for it production (EPA 1997). This exemption eliminates the need to establish a maximum permissible level for residues of PRSV in or on all raw agricultural commodities. The EPA made its determinations about the safety of PRSV coat protein after conducting its own aggregate exposure assessment (EPA 1997). Plant viruses are ubiquitous in the environment and cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species (Matthews 1991; AIBS 1995; Hadidi et al. 1998; Pappu 1999; Gonsalves et al. 2004). Hundreds of plant viruses have been described, affecting a wide range of plants and trees (ICTV 2005). These viruses infect virtually every plant species, and under natural conditions, certain plant viruses are nearly always present on particular crop or weed hosts (OECD 1996; Waterhouse 2001). Viral coat proteins are therefore routinely ingested by virtually all organisms, including humans, when virus-infected fruits and vegetables are consumed. Thus, because of the ubiquitous nature of plant viruses and the likelihood of previous exposure, the likelihood of impact to non-target organisms, including beneficial organisms, is virtually non-existent.

The *npt II* gene is a commonly used marker gene found in soil-inhabiting *E. coli* bacteria. This bacterium is not a plant or human pathogen, and does not cause disease symptoms or the production of infectious agents in plants. In addition, this marker gene is not known to cause adverse effects to non-target organisms and has been granted exemption from the requirement of a tolerance by EPA for use in or on all raw agricultural commodities (EPA 1994).

Finally, based on all the noted considerations, there should be no impact on non-target organisms from a decision to grant nonregulated status to X17-2 papaya (preferred alternative). Similarly, there should be no impact on non-target organisms, including beneficial organisms, if APHIS chooses the no action alternative.

4. Potential impacts on biodiversity

Analysis of available information indicates that, compared with the non-engineered papaya, X17-2 papaya exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other cultivated papaya, and that it is unlikely to harm non-target organisms common to the agricultural ecosystem. Based on this analysis, if APHIS chooses the preferred alternative, there is no apparent potential for significant impact to biodiversity. If APHIS chooses the no action alternative there should also be no impact on biodiversity.

5. Potential for viral interactions and development of new viruses

APHIS has considered the known physical and biological properties of PRSV and its interactions with both its insect vectors and its host plant, papaya. PRSV and the aphids that serve as vectors are widely prevalent in areas of the United States where papayas are grown. PRSV and its aphid vectors are found worldwide where papayas are grown (OECD 2005). Based on the known physical and biological properties of PRSV, the likelihood of the appearance of masked plant viruses or a new plant virus with novel biological properties through field cultivation of transgenic PRSV-resistant X17-2 papaya plants is no greater than the likelihood of novel viruses arising in PRSV-infected papaya cultivars derived through traditional plant breeding practices.

Other viruses have been noted as occurring in Florida and the Caribbean region but have not been considered as significant limiting factors to papaya production compared to PRSV in these areas. These viruses include papaya mosaic virus (a potexvirus that can be mechanically vectored) and papaya droopy necrosis virus (a rhabdovirus, possibly vectored by leafhoppers).

Three phenomena (heteroencapsidation, recombination and synergy) that virologists and ecologists have considered to be issues associated with genetic engineering of virus genes into plants are briefly discussed below. Except in rare instances, these issues have largely been dismissed as having significant ecological risks associated with them when viral coat protein genes are introduced into plants for disease resistance (EPA 2006). Other authors have pointed to a number of publications that provide "…strong evidence of limited, if any, environmental risks, beyond background events…" when addressing issues related to heteroencapsidation and recombination (Fuchs and Gonsalves 2007).

Heteroencapsidation[8]

[8] Previously referred to as transencapsidation, transcapsidation or heterologous encapsidation in older literature

Heteroencapsidation occurs when the coat protein of one virus is able to encapsidate the nucleic acid of a second virus. Heteroencapsidation was first described by Rochow (1970) and has been the subject of numerous reviews (Rochow 1977; Falk and Duffus 1981; Falk et al. 1995; Miller et al. 1997; Tepfer 2002). In some cases, these two or more viruses may be related, while in other scenarios, the viruses may be completely unrelated (Falk et al. 1995; Tepfer 2002). The majority of heteroencapsidation interactions that have been identified involve luteoviruses (Rochow 1977; Falk et al. 1995; Miller et al. 1997). These interactions occur naturally in both agricultural crop and weed plants, and are a natural part of virus-virus and virus-plant interactions (Rochow 1977; Falk and Duffus 1981; Falk et al. 1995). In some cases, heteroencapsidation is a specific interaction between two viruses that plays an important role in both virus biology and survival (Falk et al. 1995).

Heteroencapsidation events are transient and potential impacts would only persist with a single infection in a susceptible host plant (USDA/APHIS 1996; OECD 1996). As an EPA Scientific Advisory Panel has noted regarding heterologous recombination, the likelihood of "novel viral interactions" which would lead to environmental concerns from using plants engineered with viral coat proteins is very low (EPA 2006). The Panel further noted that mixed virus infections in plants are recognized as common, that virus sequences and proteins are in high concentrations in virus infected cells and that viral interactions occur naturally in mixed infections. The Panel concluded that virus resistance resulting from use of viral coat protein engineered plants would result in fewer virus infections and overall lower environmental risk than risks associated with heterologous recombination from naturally occurring mixed infections (EPA 2006). A recent review of studies on transgenic plants expressing viral coat protein genes assessing the significance of heteroencapsidation (Fuchs and Gonsalves 2007) concluded that this phenomenon has been of "limited significance and would be expected to be negligible in regard to adverse environmental effects."

The likelihood of effective heteroencapsidation occurring between products of the *cp* gene and the genomes of infective viruses is greater if the invading virus is a related potyvirus. In Hawaii, PRSV is the only potyvirus and the only reported virus known to infect papaya. APHIS notes that, elsewhere in the world, other viruses have been reported to infect papaya, including papaya mosaic potexvirus, papaya leaf curl geminivirus, and papaya leaf distortion mosaic potyvirus. The latter is found in Japan (Maoka et al. 1995). Thus, APHIS believes that the likelihood of heteroencapsidation occurring in X17-2, when grown in the United States, is highly improbable because no other related potyvirus is likely to infect these lines. Even in the remote possibility that heteroencapsidation could occur with a potyvirus that may be introduced into the United States, the amount of PRSV CP produced by the transgene in these two lines is less than the amount of CP produced in non-transgenic papayas that are naturally infected with PRSV. It is also unlikely that there will be any other novel interactions with the PRSV CP expressed in X17-2, because the protein expressed by the PRSV CP transgene in the transgenic lines is expressed in the same types of tissues where PRSV normally replicates and produces its CP when it infects susceptible papayas.

Recombination

Recombination events in plant viruses contribute to evolution of the viral genome (Falk and Bruening 1994; Gibbs and Cooper 1995; Roossinck 1997; Aaziz and Tepfer 1999; Rubio et al. 1999; Worobey and Holmes 1999; Tepfer 2002). It is theoretically possible for new plant viruses to arise in the X17-2 papaya through recombination and APHIS has considered this issue in its evaluation of this petition. Recombination is defined as the exchange of nucleotide sequences between two nucleic acid molecules (USDA/APHIS 1996; USDA/APHIS 1999). Recombination between viral genomes can result in heritable, permanent change (USDA/APHIS 1996; USDA/APHIS 1999). The persistence of the recombined viral genome depends upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of the parental viruses, its ability to spread systemically within the host, and its successful transmission to other host plants.

Under normal agricultural conditions, plant viruses have numerous opportunities to interact genetically (Falk and Bruening 1994). Multiple or mixed infections, where more than one virus infects a crop or weed host, are common in nature. Some reports have shown five or more different viruses infecting the same plant (Falk and Bruening 1994; Falk et al. 1995; EPA 2004). Falk and Bruening suggest that these mixed infections probably occur more frequently than what has been reported and have likely already brought together numerous combinations of virus genes (Falk and Bruening 1994). Therefore, under natural field conditions, it is possible for viruses that cannot systemically infect a particular plant to interact with viruses that are capable of systemic infection (Falk and Bruening 1994). Although there is potential for these viruses to continuously interact under natural settings, new viral diseases are normally due to minor variants of existing viruses as opposed to new viruses resulting from recombination (Falk and Bruening 1994). The idea of new variants arising from existing viruses, and being responsible for virus diseases is strongly supported by the level of variability that occurs within individual viruses (Falk and Bruening 1994; Gibbs and Cooper 1995; Roossinck 1997; Aaziz and Tepfer 1999; Rubio et al. 1999; Worobey and Holmes 1999; Tepfer 2002).

According to Bruening (2000), it is highly unlikely, given the high background of recombination known to occur naturally in mixed infections of both crop and wild plants, that the risk of recombination would be any different in transgenic plants (Bruening 2000). Most scientific literature suggests that such an event would be a rare occurrence (Falk and Bruening 1994; USDA/APHIS 1999; EPA 2004). Researchers have looked for viral recombination events in experimental transgenic grapevines, plums and commercial squash (Vigne et al. 2004a; Vigne et al. 2004b; Capote et al., 2007; Lin et al., 2001). plants containing viral *cp* genes and have not found them (Fuchs and Gonsalves 2007). In further considering this issue, one must also consider what risk such a recombination event would pose. Given that recombination is widely accepted as a significant part of virus evolution and that multiple viruses are commonly found in a single plant providing ample opportunity for interaction, the likelihood that transgenic viral coat protein-expressing plants present a greater risk to the environment is low.

Synergy

Synergy occurs when two independent viruses infect a plant simultaneously and the resulting disease symptoms are more severe than when either virus infects the plant individually (Matthews 1991; OECD 1996; Pruss et al. 1997; Tepfer 2002). Synergistic infections typically result in agronomic problems, producing diseased, unmarketable crops, rather than environmental impacts. Their occurrence would not likely be any different in transgenic crops than in naturally mixed infections (USDA/APHIS 1996).

Several naturally-occurring synergistic virus interactions have been described, with the majority of the combinations involving at least one potyvirus (Rochow and Ross 1955; Vance 1991; Vance et al. 1995; OECD 1996; Pruss et al. 1997; Tepfer 2002). Vance and colleagues have shown that when plants are co-infected by both a potyvirus (e.g., potato virus Y virus – PVY; tobacco vein mottling virus – TVMV; pepper mottle virus - PeMV) and potato virus X virus (PVX), the disease symptoms are significantly worse than plants infected with either of the viruses alone (Vance 1991; Vance et al. 1995). In addition to the change in disease symptoms, there was a significant increase in PVX virus particles without any corresponding increase in PVY virus particles (Vance 1991).

While there is potential for synergistic interactions to occur between PRSV and other viruses, there is no evidence to suggest that potyviral coat protein genes alone are involved in synergy. Therefore, it is unlikely that use of X17-2 papaya would increase the potential for synergistic interactions.

Based on these analyses of heteroencapsidation, recombination, and synergy there is no apparent potential for significant impact on the development of new viruses if APHIS chooses either the “no action” alternative or grants nonregulated status to X17-2 papaya.

6. Potential impacts on commercial use

If APHIS takes no action, commercial scale production of X17-2 papaya and its progeny is effectively precluded. These trees could still be grown under APHIS permit as they have been for the past several years. However, widespread, unconfined use of the trees would not be allowed as long as the X17-2 papaya is considered a regulated article. APHIS has evaluated field trial data reports and publications submitted relating to this event and its progeny, and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. The agency expects that if these trees were grown under permit in the future, they would perform similarly. If APHIS were to grant the petition for nonregulated status, X17-2 papaya and its progeny would no longer be considered regulated articles. The unrestricted cultivation and distribution of X17-2 papaya would be allowed and would not be subject to regulation by APHIS under 7 CFR part 340.

From a commercial perspective, current methods for control of PRSV are mostly ineffective and growers who choose to grow X17-2 or its progeny would have less concern for loss of trees and fruit due to PRSV infection. APHIS granted nonregulated status to two lines (55-1 and 63-1) of PRSV-resistant trees in 1996 (USDA-APHIS 1996). The Environmental Protection Agency completed its tolerance exemption for PRSV coat protein in 1997. PRSV-resistant trees have been available to papaya growers in Hawaii

since 1998. Use of these papayas has allowed growers to address the decline of the industry in Hawaii caused by PRSV, which entered the Puna District of Hawaii Island in 1992 (Gonsalves 2003). Use of PRSV-resistant transgenic trees also helped growers of non-transgenic papayas by reducing populations of PRSV in the environment. Transgenic trees are useful as a buffer/barrier to limit virus-carrying aphids from entering a planting of non-GE trees (Gonsalves 2003). As noted (Gonsalves 2003) "…virus-resistant transgenic crops can directly control the virus and also serve as a tool to minimize infection to non-transgenic crops that are grown (in) the area." As the Japanese market (which imports Hawaiian papayas) has not accepted transgenic papaya at this time, Hawaii's industry has implemented a segregation system to separate non-transgenic from transgenic papayas.

Based on all these considerations, there is no apparent potential for significant impact on commercial use if APHIS chooses either the "no action" alternative or grants nonregulated status to X17-2 papaya.

7. Potential Impacts on Agricultural Practices

APHIS considered potential impacts associated with the cultivation of X17-2 papaya on current agricultural practices. As noted previously, current grower practices of vector control for control of PRSV are largely ineffective. The most useful disease control measures have resulted from good isolation of new plantings and strict roguing of apparently PRSV-infected trees (Gonsalves 2003). Isolation results in limiting disease-carrying aphids from entering a planting and roguing removes disease inoculum (i.e., virus) from an area.

If growers can maintain plantings longer by using X17-2 trees that do not get PRSV, they may be able to leave trees in the ground longer and therefore replant less often. For growers who have not had to manage PRSV in their plantings, there would be no change to their practices. In either case, impacts on agricultural practices would be comparable to growing papayas in a location where PRSV was not a major disease.

Given the above considerations, there is no apparent potential for significant impact on agricultural practices if APHIS chooses either the "no action" alternative or grants nonregulated status to X17-2 papaya.

8. Potential Impacts on Conventional and Organic Farming

APHIS searched a number of databases and identified data from 1996 indicating a relatively small papaya industry in Florida amounting to approximately 250 acres (Dade County, FL) (Degner et al., 1997). That acreage was down from 1990 when acreage was estimated at 375 acres (Degner et al., 1997). The total crop value in Dade County in 1996 was approximately $1.6 million. At the time, Dade County production was estimated to produce approximately 90% of the papayas grown in FL (Mossler and Nesheim 2002). APHIS was unable to identify more recent data regarding papaya production in Florida. USDA/NASS only tracks papaya production from Hawaii. Comparing estimated acreages from Hawaii and Florida, it appears that the industry is between five and ten times larger

in Hawaii than Florida. In terms of shipping of papayas, Hawaiian papayas of the Solo type are shipped frequently around the country while Florida ships green cooking type papayas much less often (Accessed 9/28/2007: http://marketnews.usda.gov/portal/fv;jsessionid=WMMRIP1GAIAMYCQKAFOSFEQ). Importation of papayas from Mexico, Belize, Brazil, Dominican Republic, and Jamaica is common. APHIS also notes specific tracking of papaya imports and exports by the University of Florida using USDA/ERS data (Accessed 9/28/2007: http://agecon-trec.ifas.ufl.edu/papaya.htm). There is no indication that Florida papayas are exported.

Organic farming operations, as described by The National Organic Program administered by USDA's Agricultural Marketing Service, requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that farmers, including organic farmers, who choose not to plant transgenic papaya varieties or sell transgenic papaya, will be significantly impacted by the expected introduction of this product. Non-transgenic papaya will likely still be sold and will be readily available to those who wish to plant it. The papaya industry in Hawaii, in response to market needs associated with exports of non-GE papayas to Japan and growers of organic papayas, has successfully implemented identity preservation programs that segregate GE and non-GE papayas. If market needs exist to do the same where X17-2 papaya would be grown, these protocols could be similarly implemented (Fuchs and Gonsalves 2007).

Papaya trees are normally propagated by seed and methods to exclude unwanted pollen from a papaya flower are as easy as placing a bag over a developing flower, allowing it to self-pollinate, and collecting seeds from developed fruit. If the University of Florida receives regulatory approval from all appropriate agencies, it will likely make X17-2 papaya and derived varieties available to growers or breeders. Growers of organic

papayas in Hawaii have been coexisting with conventional and GE papaya growers for a number of years and have information available to them to guide them in their continuing operations (Manshardt 2002).

It is important to note that the flesh of papaya fruit is exclusively derived from the maternal tree and the cells of the flesh are genetically identical to the cells of the maternal tree (Esau 1965). Therefore, even in the instance that cross pollination was to occur between a transgenic X17-2 tree and a receptive non-transgenic tree, the resulting edible portion of the papaya fruit (i.e., flesh) of the non-transgenic tree would contain no transgenic cells. The papaya seed resulting from the cross pollination described above, would be transgenic.

Finally, given the above considerations, there is no apparent potential for significant impact on conventional or organic farming if APHIS chooses either the "no action" alternative or grants nonregulated status to X17-2 papaya.

9. Potential Impacts on Raw or Processed Agricultural Commodities

APHIS analysis of data in the Petition (Section V.D., V.E., Table 6, P. 18) on agronomic performance, disease and insect susceptibility, and compositional profiles of X17-2 papayas indicate no significant differences between X17-2 papaya and non-transgenic counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of X17-2 papaya. In addition, as discussed earlier, the only additions to the X17-2 papaya are the coat protein gene from PRSV and the *nptII* selectable marker gene. These nucleic acids are not unlike all other nucleic acids that are considered to be "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA) (FDA 1992) and both the *nptII* and PRSV genes are exempt from the requirement of a tolerance under the Federal Food Drug and Cosmetic Act by the U.S. Environmental Protection Agency (EPA) (EPA 1994; EPA 1997). Finally, the X17-2 is currently undergoing review by the FDA for use in food and feed (http://www.cfsan.fda.gov).

APHIS further considered potential effects on human health from a decision to grant nonregulated status to X17-2 papaya. As noted previously, X17-2 does produce NPT II and PRSV proteins. Both of these proteins have been granted tolerance exemptions by the EPA (EPA 1994 and 1997). FDA further considers genetic material (nucleic acids) to have GRAS (generally recognized as safe) status (FDA 1992). APHIS searched numerous databases and found no indication that the introduced nucleic acids or the resulting proteins produced have any effect on organisms, including humans, which might consume them. Based on data submitted in this Petition (Table 4, p. 12), as well as a previous papaya submission (http://www.aphis.usda.gov/brs/aphisdocs/96_05101p.pdf), the amount of virus coat protein in PRSV-infected papayas is many times higher than the amount of virus coat protein produced in X17-2 papaya. Therefore, any organism that consumes papayas naturally infected with PRSV ingests higher amounts of viral coat protein than if they were to consume X17-2 papayas.

Given the above considerations, there is no apparent potential for significant impact on raw or processed agricultural commodities if APHIS chooses either the "no action" alternative or grants nonregulated status to X17-2 papaya.

10. Cumulative Impacts

APHIS considered whether the proposed action could lead to significant cumulative impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions. Typically, papaya production occurs on land that can be dedicated to similar production for many years. As with most agricultural production, continuous production of papaya would normally include the use of resources to limit the growth of weeds, limit the potential impact caused by insects, animals or disease, and to maximize production. Widespread use of X17-2 papaya is expected to have an insignificant impact on typical papaya production. The virus resistance trait of these trees will help limit the impact of PRSV in Florida areas where this virus is a problem. Other than PRSV coat protein, the CP gene (nucleic acid) of PRSV and *nptII* gene, X17-2 papaya will not produce any other substance that is not normally produced by papaya trees, nor is the composition of the fruit produced by these trees significantly different from unmodified papaya. Therefore, APHIS does not expect accumulation of a novel substance in soil, nor does APHIS expect impacts on organisms living in and around these orchards because of exposure to X17-2 papaya.

Data supplied by the applicant, including results of several years of field tests in Florida, suggest that the X17-2 papaya trees have not had observable or measurable impacts on the ecosystems in which they have been allowed to grow. Based upon available information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create significant cumulative impacts or significantly reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the ecosystem in which X17-2 papaya is planted.

11. Highly uncertain, unique or unknown risks

NEPA implementing regulations require consideration of the degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risk (40 CFR § 1508.27(b)(5)). None of the effects on the human environment identified above are highly controversial, highly uncertain, or involve unique or unknown risks. The effects are similar in kind to (and no worse than) those already observed for currently commercially available and widely planted genetically engineered papaya varieties in agriculture production systems. APHIS is not aware of any means by which the proposed action (a determination of nonregulated status for X17-2 papaya) would threaten or violate Federal, State, or local law requirements.

VII. Consideration of Executive Orders, Standards, and Treaties Relating to Environmental Impacts

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also requires federal agencies to conduct their programs in a manner that will prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency’s mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionately adverse human health or environmental effect on minorities, low-income populations, or children.

EO 13112, “Invasive Species”, requires that Federal agencies take action to prevent the introduction of invasive species, to provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Both non-engineered and deregulated engineered PRSV papayas are grown in the United States. Based on historical experience with these papayas and the data submitted by the petitioner and reviewed by APHIS, these engineered papaya plants are very similar in fitness characteristics to other papaya varieties currently grown. Due to the fact that papayas have never been weedy or invasive species, they are not expected to have an increased invasive potential.

EO 12114, “Environmental Effects Abroad of Major Federal Actions” requires Federal officials to take into consideration any potential significant environmental effects outside the United States, its territories, and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should nonregulated status be determined for X17-2 papaya or if the no action alternative is chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new papaya cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR part 340. Any international traffic of X17-2 papaya subsequent to a determination of nonregulated status for X17-2 papaya would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC “is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control” (https://www.ippc.int/IPP/En/default.jsp). The protection it affords extends to natural flora and plant products and includes both direct and indirect

damage by pests, including weeds. The IPPC set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (169 countries as of August 2008). In April 2004, a standard for pest risk analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the Plant Protection Act as well as with guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003, and 147 countries are Parties to it as of August 2008 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with domestic regulations that importing countries that are Parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation.

LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the United States Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). These data will be available to the Biosafety Clearinghouse. APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines, and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States, and within the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In

addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea.

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Appendix I: Summary table of data submitted with petition 04-337-01p for X17-2 papaya

Schematic diagram of PRSV-cp gene cassette	Figure 1, page 6
Description of DNA components inserted into X17-2 papaya	Table 2, page 7
Plant/ T-DNA right border gene sequences	Figure 2, page 9
Plant/ T-DNA left border gene sequences	Figure 4, page 10
Mendelian inheritance data collected from breeding work with X17-2	Table 3, page 11
PRSV coat protein expression analysis (ELISA)	Table 4, page 12
PRSV coat protein analysis (Western blots)	Figures 5A and B, page 13
Comparative PRSV field infection assessment (transgenic and non-transgenic lines)	Table 5, page 14
Comparative nutritional composition analysis of transgenic and non-transgenic papaya lines	Table 6, page 18
Plasmid map of gene construct used in transformation and Southern blots for *nptII* gene and plasmid backbone	Appendix I, following references
PRSV coat protein gene sequence inserted into X17-2 papaya	Appendix II, following Appendix I