Finding of No Significant Impact

Animal and Plant Health Inspection Service

Petition for Nonregulated Status for University of Florida X17-2 Papaya

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared a final environmental assessment (EA) prior to approving a petition (APHIS number 04-337-01p) for a determination of nonregulated status received from the University of Florida, under APHIS regulations at 7 CFR part 340. The subject of this petition, papaya (*Carica papaya* L.) line X17-2 (hereafter noted as X17-2), is genetically engineered to express a papaya ringspot virus (PRSV) coat protein, which confers resistance to the virus. On September 2, 2008, APHIS published a notice in the *Federal Register* (73 *FR* 51267-51268, Docket no. APHIS-2008-0054) announcing the availability of the draft EA for public review and comment for a 60-day comment period, ending November 3, 2008. APHIS received over 12,000 comments regarding the EA and petition. APHIS' responses to the issues raised during the comment period are included as an attachment to this document.

In the draft EA, APHIS considered two alternatives: Alternative A – No Action: Continuation as a Regulated Article; Alternative B – Determination that X17-2 papaya is No Longer a Regulated Article, in Whole. APHIS proposed Alternative B as its preferred alternative because X17-2 papaya is unlikely to pose a plant pest risk. APHIS has not identified any greater risk of dissemination of plant pests in this transformed papaya than non-transformed or other non-regulated papayas that would warrant denying the petition for X17-2 papaya. Based upon analysis described in the final EA and in APHIS' response to comments, APHIS has determined that the preferred alternative, to grant the petition in whole, will not have a significant impact on the quality of the human environment and no Environmental Impact Statement will be prepared regarding this decision.

Pursuant to its regulations (7 CFR part 340) promulgated under the Plant Protection Act of 2000, APHIS has determined that X17-2 papaya lacks plant pest characteristics and therefore this determination of nonregulated status will not pose a risk of the introduction or dissemination of a plant pest for the following reasons:

1. APHIS' analysis of data on agronomic performance, disease and insect susceptibility and compositional profiles of X17-2 papaya and non-genetically engineered papayas indicate no significant differences in X17-2 papaya that would be expected to cause either direct or indirect plant pest effects on raw or processed plant commodities.

2. In assessing potential risks associated with gene introgression from X17-2 into its sexually compatible relatives, APHIS considered two primary issues: a) the potential for gene flow and introgression and b) the potential impact of introgression. There are no native or sexually compatible wild relatives of *Carica papaya* L. in the U.S. or its territories; therefore those hybridizations cannot occur. There are commercial

plantings and feral plants of papaya so those hybridizations may occur if X17-2 is planted near enough to those plantings. However, the use of mitigation measures such as planting of buffer rows, increased planting distances, and selection of female or hermaphrodite trees will reduce the likelihood of such hybridizations occurring. These methods to minimize cross pollination can be utilized by both organic and conventional papaya growers who desire not to have their trees receive pollen from X17-2 papaya. Given the size and somewhat spread out nature of the Florida and Caribbean industries and the known effectiveness of using specific methods to exclude papaya pollen (described in the EA and response to comments), significant effects on both organic and conventional growers appear unlikely.

3. APHIS assessed characteristics of X17-2 that might indicate an increased propensity to become weedy or invasive and was unable to identify any. Although there are feral populations of papaya in some locations, hybridizations with X17-2 will not likely result in production of plants with increased weedy or invasive characteristics.

4. Other than resistance to PRSV, X17-2 does not exhibit any changes in disease or pest susceptibility. APHIS reviewed field performance data submitted by the petitioner and these data indicated that the engineered plant is not different in any fitness characteristic (other than resistance to PRSV) compared to control plants that might cause X17-2 to become invasive. Based on the analyses described, there is no apparent potential for significant impacts on the biodiversity of papaya or other organisms in the environment.

5. In assessing risks posed by viral interactions, APHIS considered the potential for recombination, heterologous encapsidation and synergy. Extensive scientific knowledge is available about PRSV, and other members of the potyvirus group, based upon research performed around the world. Analysis of all available scientific information suggests that the likelihood of development of new viruses, or viruses with novel/altered properties is very low to non-existent. The low likelihood of risk posed by viral interactions suggests the lack of a plant pest risk in X17-2 papaya.

6. X17-2 papaya does produce PRSV coat protein and likely relies on a mechanism of RNA gene silencing for resistance to PRSV. Nucleic acids (DNA and RNA) are present in all living organisms and are not known to have toxic properties. PRSV coat protein production from X17-2 papaya does not produce any new environmental exposures compared with PRSV-infected fruit, which is not known to have adverse affects on animals or plants to which they are exposed. Because of these characteristics, there are likely to be no impacts on threatened or endangered species or other non-target organisms.

7. If X17-2 papaya were to be grown commercially, impacts on the environment from agricultural practices would likely be no different than from cultivation of other papayas. PRSV is a devastating disease of papaya worldwide and use of this papaya in Florida and U.S. territories in the Caribbean region would allow growers to better manage their plantings and reduce their costs in these regions.

8. Data supplied by the applicant and reviewed by APHIS indicates that growing of X17-2 papaya for a number of years has not resulted in observable changes to the environment in which they have been grown. Based upon these observations as well as other information provided in the petition and extracted from the relevant literature, APHIS believes that significant cumulative impacts to the human environment are unlikely to occur if X17-2 papaya is granted nonregulated status.

When considered in light of other past, present, and reasonably foreseeable future actions, and considering potential environmental effects associated with the proposed deregulation of X17-2 papaya, APHIS could not identify significant impacts on the quality of the human environment that would result from granting nonregulated status to X17-2 papaya. Granting deregulation, in whole, should not have any significant human health or environmental effects, nor is it expected to establish a precedent for future actions with potentially significant effects. None of the effects on the human environment are highly controversial, highly uncertain, or involve unique or unknown risks. The effects are similar in kind to those already observed for currently commercially available GE papaya varieties widely grown in Hawaii. None of the proposed alternatives are expected to threaten or violate Federal, State, or local law requirements.

Because APHIS has reached a finding of no significant impact, no Environmental Impact Statement will be prepared regarding this decision.

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Animal and Plant Health Inspection Service

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Date: JUL 2 2 2009

Attachment

Finding of no significant impact
Response to Comments
Petition 04-337-01p

On September 2, 2008, APHIS published a notice in the *Federal Register* (73 FR 51267-51268, docket No. APHIS-2008-0054) announcing the availability of the University of Florida petition to grant nonregulated status to papaya ringspot virus resistant papaya X17-2 and of a draft environmental assessment (EA) prepared by APHIS for public review and comment for a 60-day comment period, ending November 3, 2008.

APHIS reviews a petition to determine if the genetically engineered (GE) organism should continue to be considered a regulated article under APHIS biotechnology regulations (7 CFR part 340). In order for a GE organism to be considered a regulated article, the organism must pose a plant pest risk and be modified by recombinant DNA techniques (genetic engineering as defined in the regulation). Prior to making a decision on a petition to grant nonregulated status, APHIS prepares an EA to evaluate the significance of impacts on the environment arising from such a decision. APHIS prepares the EA as part of its obligation, like other Federal agencies, to meet the requirements of the National Environmental Policy Act (NEPA) of 1969. As part of the process, APHIS considers public comments on the proposed deregulation as well as the EA.

APHIS' response to comments below has also been reflected in revisions and clarifications of the draft EA, so that the amended, final EA takes these issues into account.

APHIS received over 12,000 comments on the petition and its draft EA during the comment period. There were 18 comments from scientific organizations or individuals that supported deregulation. One individual supported deregulation as long as the taste of organic papayas was not damaged. Approximately 175 unique comments opposed to deregulation were submitted. The remainder of the approximately 12,000 comments opposing deregulation was form letters generated with essentially identical points compiled by organizations generally opposed to genetic engineering of plants. Several individuals and organizations also submitted other documents, including many popular press articles or documents published by those opposed to genetic engineering of plants, which they believe are relevant to this regulatory decision.

Nine of the commenters writing in support of deregulation were from Hawaii where GE PRSV-resistant papayas have been grown for over 10 years. Commenters noted the benefits to the papaya industry in Hawaii where papaya ringspot virus (PRSV) caused massive losses in papaya plantations in the 1990s up until the introduction of GE papaya there. One commenter from Hawaii is a papaya grower who has "…been growing GE papaya for the last nine years and have had great success." Another commenter from HI commented on the USDA National Organic Standards and the requirements to gain certification. The commenter noted requirements for a source of organic seeds or to know where one's seed comes from if no commercial organic seed is available. The same commenter noted consumption of virus infected papayas for "decades" with no reported adverse (health) effects. Two commenters supporting deregulation believe that the "free-market" should be allowed to sort out the utility of this papaya. Two commenters note that "best management practices" should address issues associated with pollen flow. Several of the commenters are scientists or virologists who are familiar with the science of using a GE viral coat protein approach to developing virus resistant GE plants. One scientist noted the "documented safe release of virus-resistant squash and papaya…" One supporting comment came from a grower/marketer of organic fruit and food products in CA. The commenter notes that the Agency should "…make use of good science." And, "It was a mistake to ban i(t) (*use of genetic engineering in organic production*) in the first place." Another commenter suggests that hand-bagging of fruit to exclude GE pollen is probably not necessary to manage gene flow issues as several studies in Hawaii have demonstrated. Another commenter writes that "(a)ttempts by certain NGOs, who oppose the use of genetic transformation, to impose the will of their minority on the majority of the population…is counter productive to society's needs." One commenter, writing in support of granting nonregulated status, addresses points submitted in many of the form letters related to carpaine production in papaya, colony collapse disorder of honeybees, "serious and mounting concerns about the genetic stability of the artificial gene combinations," and "significant potential for gene flow into native perennial papaya varieties." APHIS agrees with most of the comments submitted in support of deregulation.

Many comments were received that oppose deregulation based on general opposition to development and use of genetically engineered plants without citing specific issues in the EA or petition. Most of these commenters believe that APHIS should prepare an Environmental Impact Statement to fully address all the potential issues associated with a decision to grant nonregulated status to this PRSV papaya. Lengthier comments were received from individuals and organizations that are generally opposed to development of genetically engineered organisms. One letter with extensive comments was received from an organic certifier in the state of Florida. As noted above, a number of commenters submitted popular press articles and other documents that focus on the GE papaya that has been used in HI for over 10 years. APHIS has considered the comments submitted and addressed relevant comments below.

1. Many comments were submitted from organic growers or those who support organic agriculture through either their work or their purchase of organic products. Many commenters raised issues regarding drift of the products of excluded methods onto organic farms. These commenters were concerned that pollen drifting from nearby farms would "contaminate" crops on organic operations and that, as a result, organic farmers could lose the premium for their organic products through no fault of their own. One form letter states that "Genetic contamination is a serious and growing threat." A similar comment related to gene flow into "native" papaya. APHIS considered those comments as a whole to include issues associated with gene flow and potential impacts on organic, as well as conventional, agriculture. One commenter also noted instances in Hawaii where organic papaya growers cut down their trees because they identified the presence of GE materials in their organic plantings.

APHIS addressed issues associated with organic and conventional agriculture in its draft EA (Section VI., #8, pp. 17-19) and referenced relevant, useful and important information for growers of organic papayas (Manshardt R. 2002, added here Manshardt et al., 2007). Information related to gene flow from those references would also be directly applicable to those conventional papaya growers who were concerned with cross pollination of non-GE papaya with GE pollen. Information submitted by one of the commenters fully supports the idea that organic, conventional and GE producers can coexist (Ronald and Fouche 2006). As noted in the Ronald paper, “While 100% purity (zero tolerance for any undesired components) is very difficult to attain for any agricultural commodity, standard procedures involving spatial separation, border rows, planting dates, maturity dates, cleaning of equipment, and post-harvest handling have traditionally been able to provide products that meet diverse market requirements.” Both of the Manshardt papers (Manshardt 2002; Manshardt et al. 2007) also support the idea that growers of GE papayas can easily coexist with those growers who prefer not to grow GE papayas. Recommendations provided are articulated in those papers and simplified here: (1) Use seeds or plants that originate from a known non-transgenic source (presumably all commercial papaya in Florida is currently non-transgenic since seed of transgenic papaya in HI is controlled by a grower organization there and not distributed outside the state), (2) Cover (bag) a few unopened flowers on a known non-GE hermaphrodite tree to produce one’s own self-pollinated seed, (3) Maintain 400 meters isolation from transgenic papayas (although other isolation methods may also be effective), and (4) Grow only hermaphrodite trees. Other guidance is readily available to growers and certifiers desiring to limit the likelihood of cross pollination with GE crops or assist growers in their operations (Riddle 2004; NCAT 2003; Keupper et al. 2007). A document addressing a variety of issues, many of a legal nature, associated with organic agriculture and GE crops is also available (Krueger 2007). That document talks about testing, the presence of excluded methods, tolerance levels for the presence of excluded methods, and other issues relevant to both organic growers and their certifying agents.

One comment did not define what was meant by “genetic contamination” but the implication seemed to be related to cross pollination of organic papaya with pollen (and therefore genetic material) from GE papaya. As previously noted, papaya cross pollination can occur by wind or insects. Also as noted in the EA and above references, methods to minimize the likelihood of this occurrence are well understood.

Brookes and Barfoot (2004) studied the extent to which organic soybean, corn, and canola producers in North America have faced difficulties because of the predominant GE production of these crops. These authors found that U.S. organic farmers have had very limited problems coexisting with growers of GE crops, especially given the concentration of organic production in many States with above-average GE crop presence. Survey evidence presented in this study showed that the vast majority of U.S. organic farmers (92 percent) had not incurred any direct additional costs or incurred losses due to GE crops having been grown near their crops. Reportedly, four percent had experienced lost organic sales or downgrading of produce as a result of GE organism presence. The remaining four percent of farmers had incurred small additional costs only for testing.

Brookes and Barfoot (2004) also noted that an examination of trends in the planting of GE and organic crops suggests that the growth of the GE crop area has not impeded the development of the organic sector in North America. Organic corn and organic soybean acreages both more than doubled between 1997 and 2001. However, as observed in Apted and Mazur (2007), Brookes and Barfoot’s study was not able to quantify the impact of measures undertaken by organic producers to avoid GE material coming in contact with organic crops.

Drift[1] has been a difficult issue for organic producers from the beginning. Organic operations have always had to be aware of the potential for drift from neighboring operations, particularly drift of synthetic chemical pesticides. As the number of organic farms increases, so does the potential for conflict between organic and non-organic (which could include conventional, as well as GE, products) operations.

When considering drift issues, it is particularly important to remember that organic standards are process based. Certifying agents attest to the ability of organic operations to follow a set of production standards and practices that meet the requirements of the Organic Foods Production Act and the National Organic Program (NOP) regulations. The regulations prohibit the use of excluded methods in organic operations. As noted in the draft EA (p. 18), the presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of this regulation. As long as an organic operation has not used excluded methods and takes reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan, the unintentional presence of the products of excluded methods should not affect the status of an organic product or operation. This concept is fully supported by documents published by the University of CA at Davis (Ronald and Fouche, 2006).

It has always been the responsibility of organic operations to manage potential contact of organic products with other substances not approved for use in organic production systems, whether from the non-organic portion of a split operation or from neighboring farms. The organic system plan, developed individually by a grower, must outline steps that an organic operation will take to avoid this kind of unintentional contact.

Ultimately, organic producers are obligated to manage their operations so as to avoid unintentional contact with non-organic material. As explicitly affirmed in response to public comment on the establishment of the National Organic Program (NOP), it is the organic producers' responsibility to maintain the organic standards of their operations (Federal Register, Volume 65, p. 80556).

The NOP writes specifically about buffer zones and defines them as areas located between a certified production operation and an adjacent land area that is not maintained under organic management. A buffer zone must be sufficient in size or other features (e.g., windbreaks or a diversion ditch) to prevent the possibility of unintended contact by prohibited substances applied to adjacent land areas with an area that is part of a certified operation. An organic grower may incur costs associated with establishment of these buffer zones as they work toward coexistence with other growers (GE as well as conventional and other organic operations).

A possible cost to organic producers associated with GE agriculture, namely, the presence of GE material in organic products, is dependent upon the acceptable level of GE material that may be inadvertently present and consumers' expectations and perceptions. The NOP identifies four

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[1] Drift is defined here as something moving along in a current of air (e.g., pesticide sprays or pollen are typically noted as being relevant in this discussion).

levels of product composition for organic agriculture: 100 percent organic; 95 percent or more organic; 70 to 95 percent organic; and less than 70 percent organic. A negative public perception of the adventitious presence of GE material in organic products, whether or not factually established, can lower the profitability of organic enterprises through the loss of price premiums earned by products otherwise marketable as organic.

Benefits to organic agriculture from other coexisting agriculture

The demand for organic products by certain consumers is derived from their perceived health, safety, and environmental concerns (Cicia et al. 2006, Durham and Andrade 2005, Naspetti and Zanoli 2006, Zhang et al. 2006). Perceived health concerns regarding GE food crops contribute to this demand and the higher prices certain some consumers are willing to pay for organic food.

Apted and Mazur (2007) note that GE agriculture may also benefit organic producers if, for example, the use of GE crops results in either the use of less persistent agricultural chemicals or a reduction in the volume of agricultural chemicals used, thereby helping to reduce the general level of these chemicals in the environment. As these authors observe, organic producers may then need to implement less costly agricultural chemical contact avoidance measures.

In summary, GE agriculture, as well as conventional agriculture, contributes to the demand for organically produced commodities and the price premiums they earn. At the same time, organic producers may bear costs associated with preventing the adventitious presence of GE organisms in their crops as well as substances used in conventional agriculture but not approved for organic agriculture, given organic agriculture's dependence on some consumer's expectations and perceptions.

Gene flow into "native" papaya

As noted in the draft EA (section V., p. 7), papaya is not native to the U.S. or its territories. The center of origin of the species is believed to be Central America or southern Mexico. While there are feral populations of escaped papaya near areas of papaya cultivation, these do not constitute "native" papaya but rather are feral papaya. The issue of feral papaya is discussed in response to issue 10 in this document.

From the comments, APHIS does note instances where organic papaya growers in Hawaii cut down their trees because there were indications of the presence of GE materials. What is not clear from the articles submitted is whether the growers voluntarily chose to remove trees or whether the organic status of the plantings was at risk because of the presence of GE materials. If entire trees were found to be GE, that would indicate that a grower failed to carefully identify and utilize seed from a known, GE-free seed source. If individual seeds in fruit were identified as containing GE material, it's clear that they were cross-pollinated with pollen from GE trees. Using best management practices and following the grower's organic plan should minimize such exposures.

Conventional agriculture

Similar to organic growers who desire to minimize cross pollination from GE papaya into their plantings, conventional growers have the same basic options. The same methods (increased distance to GE trees, use of buffer trees, selecting for female and hermaphrodite trees, bagging selected fruit to exclude GE pollen to ensure non-GE seed for replanting) can be expected to be just as effective in excluding pollen from X17-2 papaya. (See also our responses in #14 and 19 below)

Brookes, G. and P. Barfoot. 2004. Co-existence in North American agriculture: can GM crops be grown with conventional and organic crops? PG Economics. Dorchester, UK. (http://www.pgeconomics.co.uk/pdf/CoexistencereportNAmericafinalJune2004.pdf)

Cicia, G., T. Del Giudice, I. Ramunno and C. Tagliafierro. 2006. Splitting consumer's willingness to pay premium price for organic products over main purchase motivations. Paper prepared for the 98th EAAE Seminar 'Marketing Dynamics within the Global Trading System: New Perspectives', Chania, Crete, Greece.

Krueger, J. E. 2007. If your farm is organic, must it be GMO free? Organic farmers, genetically modified organisms and the law. Farmer's Legal Action Group, Inc, St. Paul, Minnesota.

Manshardt, R. 2002. Is Organic Papaya Production in Hawaii Threatened by Cross-Pollination with Genetically Engineered Varieties? College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii. (http://www.ctahr.hawaii.edu).

Manshardt, R.M., C.L. Mello, S.D. Lum, and L. Ta. 2007. Tracking papaya pollen movement with the GUS transgene marker. Acta Horticulturae. 740: 183-187.

Riddle, J. A. 2004. Best management practices for producers of GMO and non-GMO crops. University of Minnesota, School of Agriculture.

Ronald, P. and B. Fouche. 2006. Genetic Engineering and Organic Production Systems. Publication 8188. Regents of the University of California, Division of Agriculture and Natural Resources. (http://anrcatalog.ucdavis.edu)

Naspetti, S. and R. Zanoli. 2006. Organic Food Quality & Safety Perception Throughout Europe. Paper prepared for the 98th EAAE Seminar 'Marketing Dynamics within the Global Trading System: New Perspectives', Chania, Crete, Greece.

NCAT. 2003. NCAT's Organic Crops Workbook: a guide to sustainable and allowed practices. National Center for Appropriate Technology. http://attra.ncat.org/attrapub/PDF/cropsworkbook.pdf. Date Accessed: February 24, 2009.

Zhang, F., Huang, C.L., Lin, B-H, and Epperson, J.E. 2006. National Demand for Fresh Organic and Conventional Vegetables: Scanner Data Evidence. Research in Agricultural and Applied Economics. Paper presented at AAEA 2006 annual meeting, July 23-26, Long Beach, CA (http://purl.umn.edu/21107)

Australian Government Office of the Gene Technology Regulator. 2008. The Biology of *Carica papaya* L. (papaya, papaw, paw paw). (http://www.ogtr.gov.au)

2. A comment from a form letter was concerned that the "…approval of perennial GE papaya trees would be a dangerous precedent setting step by USDA…" The comment goes on to indicate that other tree species are also undergoing "gene splicing research" and many are "native species vital to ecosystems…"

X17-2 papaya has undergone an assessment of its potential for increased plant pest risk based upon the information provided by the developer, as well as all other available information. A determination of nonregulated status for X17-2 would not result in predetermination of non-regulated status for other engineered trees in the future by APHIS. Each year, developers apply for permits to conduct field trials on a wide range of plant species (including trees) with a wide range of phenotypes (ISB 2009). However, for a variety of reasons, including economic and marketing factors, only selected products advance to the point where a petition for non-regulated status is submitted by developers. APHIS has no influence on which particular species a developer might choose to develop to the point of attempting to achieve non-regulated status.

Between 1992 and 2009, APHIS granted nonregulated status to a number of genetically engineered plants, including two trees – a papaya ringspot virus resistant papaya in 1996 and a plum pox virus resistant plum in 2007 (USDA/APHIS 2009). Each submission/crop has been reviewed individually and a safety determination for each was based upon the characteristics of that crop/trait combination, and data provided by the developer. Future submissions will be handled in a similar manner. If applications for nonregulated status for other trees are received in the future, APHIS will assess them based upon all available and relevant data that pertains to that specific crop/trait combination. Therefore, there is nothing "precedent-setting" about a decision granting nonregulated status in this case.

ISB. 2009. "Information Systems for Biotechnology - Field Test Release Permits for the U.S." from http://www.isb.vt.edu/cfdocs/fieldtests1.cfm (accessed 1/29/09).

USDA/APHIS. 2009. "Petitions of Nonregulated Status Granted or Pending by APHIS." From http://www.aphis.usda.gov/brs/not_reg.html (accessed 1/29/09).

3. A comment from a form letter was concerned about "…serious and mounting concerns about a broad range of health effects associated with consumption of GE crops, GE pollen, and GE-produced honey." The comment goes on to note possible unintended effects, allergies, unexpected toxins, and "other novel substances…" Also noted in comments was a concern that APHIS had not evaluated "…health effects of alkaloids such as carpaine and related alkaloids on consumers eating GE papaya, pollen, honey or fruit juices…" Other commenters also made reference to the "unpredictability and randomness of genetic engineering utilized in transgenic crops." Other comments noted concern for non-target organisms (e.g., honey bees).

APHIS is aware that papaya naturally produces numerous alkaloids as well as other chemical substances within its tissues (Petition, p. 18; Oloyede 2005; http://www.nal.usda.gov/fnic/foodcomp/search/) and that unpredictable and unintended effects can sometimes occur when using genetic engineering. Much of the data submitted by the developer is designed to address possible unintended effects that might occur as a result of inserting the genetic construct into X17-2 papaya. The developer described data and observations on insertion analysis of the gene construct, gene sequence information about the inserted DNA, genetic inheritance data, protein expression data, disease and pest resistance characteristics, tree growth habit, vegetative vigor, number of days to flowering, fruit maturity, seed and seedling growth, plant survival, pollination, flower morphology, stress adaptation, and nutritional composition of X17-2 papaya. The developer also eliminated over 200 other transgenic lines that did not meet the rigorous standards of his breeding program. He has evaluated the X17-2 PRSV transgenic line for approximately 10 years and has not noted characteristics that might indicate any unintended effects resulting from introduction and expression of the PRSV and NPT II genes. APHIS analyzed the data submitted by the developer to determine if "unintended effects" had been identified. APHIS did not identify any "unintended effects" resulting from insertion of the gene construct and concludes that the likelihood of increased production of new allergens, new toxins, or other "novel substances" in X17-2 is extremely low. PRSV coat protein and NPT II protein are not considered to be toxic to other organisms, including honey bees. The developer also submitted data to APHIS that had also been submitted directly to FDA related to levels of benzyl isothiocyanate (BITC) found in X17-2 papaya. BITC is noted as the likely antihelmintic compound in papaya seeds (Kermanshai et al. 2001), is found naturally in cruciferous vegetables and is also noted as a likely chemopreventive agent (Kassie et al. 1999). He noted that concentrations in X17-2 fell within the range of BITC levels found in non-GE papayas (Addendum 1 to Petition). USDA notes that FDA has completed its review of X17-2 papaya and has indicated that it has no further questions (http://www.cfsan.fda.gov/~lrd/biocon.html, BNF No. 100, completed December 24, 2008). When the developer submits his regulatory data package to EPA (similar to what is submitted to USDA/APHIS), that Agency will conduct further food safety reviews prior to making any registration or tolerance exemption decisions in compliance with FIFRA and FFDCA. Therefore, APHIS concludes that no further assessment or testing is warranted on X17-2 papaya.

Burdick, E.M. 1971. Carpaine: An Alkaloid of Carica Papaya- Its Chemistry and Pharmacology. Economic Botany 25: 363-365.

Kassie, F., Pool-Zobel, B., Parzefall, W. And Knasmuller, S. 1999. Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. Mutagenesis 14: 595-603.

Kermanshai, R., McCarry, B.E., Rosenfeld, J., Summers, P.S., Weretilnyk, E.A., and Sorger, G.J. 2001. Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. Phytochemistry 57: 427-435.

Oloyede, O.I. 2005. Chemical Profile of Unripe Pulp of Carica papaya. Pakistan J of Nutrition 4: 379-381.

4. Some commenters believe that X17-2 papaya should be considered to be a weed and possibly a "noxious weed" as defined in the Plant Protection Act (7 U.S.C. 7702 (10)). They cite international documents (OECD, 2005) and others in support of their argument. One commenter included several sections of the referenced OECD document and also believes that fitness characteristics noted in the petition point to potential increased weediness and associated environmental impacts. The commenter claims that this document is "The most authoritative review of information relevant to papaya biotechnology…" One of the commenters also believes that "…If APHIS wishes not to classify GE papaya as a noxious weed…then it must do so based on sound science which shows definitively that no injury to organic farming and papaya exports will be caused by the nonregulated use of the new crop."

APHIS agrees that the referenced OECD document is an outstanding review of papaya biology. As such, the document considers published information from all over the world and almost all areas where papayas will grow so it would not be surprising that in some regions or localities papaya might be considered by some to be a weed[2]. What is important to making such a determination is the context in which the plant may grow and the various descriptors used by authors in describing how, when, and where a plant will grow as a "weed." Papaya is noted as native to north-tropical Western Hemisphere with a likely center of origin in Central America or southern Mexico (OECD, 2005). The species has been widely distributed and domesticated across the globe in tropical regions. The species does not tolerate freezing temperatures and therefore is relegated to the most southern parts of Florida, Hawaii, Puerto Rico, the U.S. Virgin Islands and other U.S. territories where freezes are rare or do not occur (USDA Hardiness Zones 10b and 11 from http://www.usna.usda.gov/Hardzone/ushzmap.html, accessed 2/4/09). In various sections of the noted OECD document, papaya descriptors include; incidental escapee, opportunist, pioneer species, invasive, "…not…invasive," naturalised, and feral. On the whole, the plant is characterized as an opportunist, pioneer species, mostly in areas where tree vegetation has been lost or vegetation disturbance has occurred. Several citations to these occurrences note specifically post-hurricane studies and cleared and burned areas. In Hawaii, where papaya has been grown for many years, papaya is noted as "sparingly naturalised."

In the U.S., including Hawaii, Puerto Rico, and the Virgin Islands, papaya is noted as "introduced" (http://plants.usda.gov/java/nameSearch?keywordquery=papaya&mode=sciname). As further noted in the OECD document, "Reports are scarce on efforts to reduce feral *C. papaya* in relatively natural habitats…" As noted in the draft EA (part VI., section 2, p. 11), papaya is not recognized as a federal Noxious Weed nor on other state weed lists (updated here with other list sites: http://plants.usda.gov/java/noxious?rptType=State&statefips=12, http://plants.usda.gov/java/noxious?rptType=State&statefips=15, http://plants.usda.gov/java/noxious?rptType=State&statefips=06, accessed 2/4/09).

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[2] A weed is defined variously depending upon many considerations. Some would define it as "a plant growing in a place where a human wants a different kind of plant or no plants at all" (http://www.biology-online.org/dictionary/Weed, accessed 3/6 09). That definition could encompass most fields where crops are grown or the cracks in ones driveway. See also http://www.merriam-webster.com/dictionary/weed (accessed 5/13/09). Considerations of making determinations about weeds are based greatly on context (e.g., one persons' dandelion weed in the lawn is another's source of greens for a salad or wine).

Papaya is also not noted as an invasive species by the National Invasive Species Council (created by Executive Order 13112 in 1999) (http://www.invasivespeciesinfo.gov/plants/main.shtml, accessed 2/4/09). The PIER[3] (Pacific Island Ecosystems at Risk) project assessed papaya, adapting a weed risk assessment model developed in Australia and New Zealand which gave the species a quantitative score of "2" with a qualitative rating of "low risk" to the species (http://www.hear.org/pier/wra/pacific/carica_papaya_htmlwra.htm, accessed 2/4/09) Using this methodology, species assessed to date have ranged from -12 to 19 on this scale and those above a score of ~6 or higher may be considered problematic. The designation of "low risk" is the level of least concern in their assessment process. The model is described by Daehler (Daehler et.al. 2004).

All the above information, along with that included in the draft EA, leads APHIS to conclude that *Carica papaya* has not been considered a significant weed or invasive species in the areas where it has been grown in the U.S. and is unlikely to be a weed in those areas in the future. It would also appear that the scarcity of reports on efforts to reduce feral populations is because it is not considered to be a species that warrants significant efforts in that regard. Nevertheless, APHIS at this time certainly has no reason to consider any papaya, GE or non-GE, to be a noxious weed. APHIS noxious weed regulations (7 CFR part 360) and its list of noxious weeds make it clear that most weeds are not noxious weeds. Noxious weeds are those weeds that are aggressively invasive, have significant negative impacts on agriculture or the environment, and are extremely difficult to manage and control once they are established.

Regarding the comments related to improved fitness of X17-2, the comparator that APHIS uses includes assessment of the transgenic and non-transgenic trees in the absence of disease (i.e., when PRSV is *not* present in the non-engineered trees). The commenter instead infers that APHIS should compare the transgenic trees to non-transgenic trees with disease, an altogether inappropriate method. Using such an inappropriate comparator would obviously lead one to conclude that the transgenic tree is more fit than the non-transgenic. Regardless if one considers the agroecosystem or any natural areas where papayas may grow, the transgenic trees are likely to be more fit, but only where the specific strain of PRSV is present and infecting trees. Prior to the introduction of any disease-causing organism, one could expect that, all things being equal, individual trees would all be equally fit. After introduction of disease, those trees with disease resistance or tolerance (e.g., X17-2 papaya) would be expected to be more fit than trees without that characteristic. That would not, however, equate to *increased* fitness, invasiveness or weediness compared with the baseline trees (i.e., non-transgenic, non-infected trees). The analogy is the same whether one considers plant vigor, increased lifespan, or any resulting increase in fruit or seed. The comparator used by APHIS, and the appropriate one, is the one that considers the transgenic and non-transgenic trees, *in the absence of disease.*

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[3] The PIER project compiles and disseminates information on exotic species of known or potential threat to Pacific island ecosystems. Of primary concern are plant species that are threats to natural or semi-natural ecosystems of all types, but information is also included on species that are agricultural weeds or invaders of other disturbed sites. The project is partially funded by USDA/ Forest Service and is further supported by HEAR (Hawaiian Ecosystems At Risk project), USGS, the National Tropical Botanical Garden, the University of Hawaii, the Nature Conservancy, and others (http://www.hear.org/pier/abtproj.htm, accessed 2/4/09).

Regarding determinations about the Federal Noxious Weed status of a plant species, APHIS/Biotechnology Regulatory Services does not make those specific determinations under its regulations at 7 CFR part 340. A petition submitted to APHIS/BRS only compels the Administrator to make determinations as described in 7 CFR 340.6 and other statutes related to the National Environmental Policy Act, the Endangered Species Act and other relevant Executive Orders, as noted in the draft EA. While APHIS has indicated in a proposed rule (Federal Docket ID: APHIS-2008-0023) that it may consider certain aspects of the Noxious Weed authority in future determinations, those proposed regulations are not currently in place. APHIS/BRS is not aware of any requests or proposals submitted to APHIS to list either papaya or GE PRSV-resistant papaya as a noxious weed pursuant to APHIS' noxious weed regulations.

Daehler, C. C., J. S. Denslow, S. Ansari, and H. Kuo. 2004. A risk assessment system for screening out invasive pest plants from Hawai'i and other Pacific Islands. *Conservation Biology* 18:360-368.

5. At least one commenter believes there are unknown, controversial and unique risks associated with genetic engineering and APHIS failed to assess those in its draft EA. The commenter cites no specific issues associated with X17-2 papaya but instead talks generally about genetic engineering of food crops and mainland cultivation of GE trees. This commenter, along with several others, submitted a number of journal and popular press articles purporting to show unique or unknown changes that might occur using GE technology.

Genetically engineered crops have been grown on increasingly larger acreages worldwide since 1996. Countries continue to adopt the technology in crops such as corn, soybean, cotton, canola, squash, papaya, tomato, carnation, rose, poplar, and petunia such that in 2007, 13 countries were growing over 123,000 acres each these crops. Another 10 countries grow smaller acreages. The estimated total acreage worldwide in 2007 was over 282 million acres (http://www.isaaa.org/resources/publications/briefs/37/executivesummary/default.html, accessed 2/5/09), an increase of over 30 million acres from 2006. Benefits of the adoption of this technology include increased farm incomes, decreased pesticide use (and associated environmental impacts), and decreased greenhouse gas emissions (Brookes and Barfoot, 2008[a]). Additional production arising from use of GE food crops has contributed to being able to feed over 300 million people for a year (Brooks and Barfoot, 2008[b]). GE crops have been planted on over 2,000,000,000 acres cumulatively since 1996 (Marshall 2009) and no verified, documented cases of adverse effects on food or feed safety, damage to the environment or significant effects on non-target organisms have been noted. The National Academy of Sciences noted in their 2002 Report that "…the transgenic process presents no new categories of risk compared to conventional methods of crop improvement…" (NAS 2002). The report also found that "…both transgenic and conventional approaches…can cause changes in the plant genome that result in unintended effects on crop traits." (NAS 2002). These reports, as well as others, recognize that unintended effects (which can be unknown and/or unique) can occur when developing GE crops but that these effects are not significantly different than such effects that can also occur using standard plant breeding methods. Much of the data required and requested of applicants submitting petitions for nonregulated status relates to assessment of such unintended effects and therefore is invaluable to APHIS in making its determinations.

As noted in the EA and in the petition, the applicant conducted numerous analyses on X17-2 papaya. He also eliminated hundreds of other transgenic lines from his program early in development (Petition p. 3) because of likely undesirable qualities or lack of efficacy. He collected data on gene insertion analysis (including gene sequence), information on gene inheritance, protein expression, disease and pest resistance, and numerous characteristics associated with phenotype and agronomic performance (Petition, pp. 6-18 and Appendix I and II). He also collected data on the typical components for a food safety assessment (Petition, Table 6, p. 18; Addendum 1 of Petition). None of the characteristics assessed point to increased risks of this plant leading to adverse environmental or food safety issues. Except for individuals and/or organizations that are fundamentally opposed to use of genetic engineering technology, which many of the commenters appear to be, APHIS cannot identify any scientifically controversial issues associated with a determination of nonregulated status finding for this papaya. Finally, APHIS concludes that no further assessment is warranted at this time.

[a]Brookes, G. and Barfoot, P. 2008. Global Impact of Biotech Crops: Socio-Economic and Environmental Effects, 1996-2006. AgBioForum 11: 21-38.

[b]Brookes, G. and Barfoot, P. 2008. Focus on yield: Biotech crops: evidence, outcomes and impacts 1996-2006. PG Economics Limited. Available at http://www.isaaa.org/.

Marshall, A. 2009. 13.3 million farmers cultivate GM crops. Nature Biotechnology 27: 221.

National Academy of Sciences: Environmental Effects of Transgenic Plants. 2002. National Academy Press, Washington DC.

6. One commenter believes that APHIS is required to address global impacts associated with making a determination of nonregulated status for X17-2 papaya and believes that this papaya "…will have consequences on the human environment worldwide." The commenter further believes that APHIS must consider the "…myriad ethnopharmacological and contemporary medical uses of *Carica papaya* L.…" One commenter also notes a recent paper indicating that extracts of ripe papaya may induce embryonic resorption in pregnant mice.

As the commenter notes from the draft EA, papaya can be cultivated worldwide in tropical and subtropical climates. APHIS also did consider Executive Order 12114 (Environmental effects abroad of major Federal actions) within the draft EA (Section VII., pp. 21-23). It is not entirely clear to APHIS what impacts the commenter believes should be addressed, but it does appear that he would have the Agency address every possible impact anywhere in the world. The OECD document (OECD, 2005) discusses many of the industrial uses, medical uses, and nutrition associated with consumption of or exposure to various parts of the non-GE papaya plant. That document also notes potential adverse consequences of exposure, including allergenicity, possible effects on pregnancy and animal fertility. From the OECD document, APHIS understands that many of the uses of papaya are well known to those who use and consume papaya. There are potential adverse consequences that can be associated with consumption of either ripe or green papaya or exposure to papaya pollen (OECD, 2005). As with many plant pollens, papaya pollen can induce an allergic response in sensitive individuals. Additionally, papaya does produce a milky latex sap that will readily ooze from abraded or damaged green fruits or other plant parts (e.g., leaves, trunk, etc). That sap contains numerous compounds, including papain and chymopapain (used as meat tenderizers and/or digestive aids), chitinases and other proteinases that can irritate or damage unprotected skin. One website noted that those persons with latex allergies may also show allergy to papaya (http://whfoods.org/genpage.php?tname=foodspice&dbid=47#descr, accessed 2/9/09) and that cooking may deactivate those enzymes. One website that notes a number of the compounds that can be found in papaya was submitted as part of a comment to the docket (http://www.ansci.cornell.edu/plants/medicinal/papaya.html, accessed 2/9/09)). The site also notes the myriad of uses for papaya worldwide. Relatively few of the "uses," however, had documented efficacy. That site does indicate that various extracts of the plant may be useful as antihelmintics (de-wormers), antimicrobials or muscle relaxers. Another site that lists various uses of papaya or plant parts also has references to numerous ethnobotanical uses (http://www.ars-grin.gov/duke/, accessed 2/9/09). That site lists over 125 different chemical constituents of papaya, many with reported biological activity. The noted paper on effects of papaya epicarp (skin) extracts on pregnant mice also notes positive effects on skin healing (Anuar et al. 2008). That same paper does note the numerous uses for papaya that are well known in cultures that use and consume papaya across the world. As with other fresh foods, papayas are assumed to have GRAS (generally recognized as safe) status from FDA.

In regard to addressing hypothetical worldwide impacts of making a determination of nonregulated status for X17-2 papaya, APHIS does not agree that this is required. One could make a similar argument that any deregulation decision that APHIS made would therefore require such a consideration as most of the commodities (including corn, soybeans, canola, cotton, etc.) that have been granted nonregulated status are traded worldwide. Neither NEPA nor EO 12114 requires that APHIS make such speculations as to what might or could hypothetically happen elsewhere in the world. APHIS granted nonregulated status to a similar PRSV-resistant papaya in 1996 (Petition 96-051-01p, accessed at http://www.aphis.usda.gov/brs/not_reg.html). That papaya has been grown widely only in Hawaii and is available as fresh fruit only in the U.S. and Canada, at this time, where it has received regulatory approvals. Countries have put in numerous regulations relating to plant quarantines as well as those related to international trade and movement of genetically engineered organisms. Phytosanitary standards have been developed under the IPPC (draft EA, p. 21) and a framework for safe transboundary movement of LMOs (living modified organisms) has also been established under the Cartagena Protocol on Biosafety (Petition, p. 22). U.S. exporters who might handle X17-2 papaya will need to comply with and understand the domestic regulations of importing countries. Those countries may or may not have regulations in place that address importation of genetically engineered plants for food, feed, processing or planting. Other considerations are discussed in the draft EA (pp. 21-23).

Anuar, N.S., Zahari, S.S., Taib, I.A., and Rahman, M.T. 2008. Effect of green and ripe *Carica papaya* epicarp extracts on wound healing and during pregnancy. Food and Chemical Toxicology 46: 2384-2389 (doi:10.1016/j.fct.2008.03.025).

7. Two commenters wrote that APHIS did not consider potential effects to threatened and endangered species that should have been considered under the Endangered Species Act (ESA).

APHIS always does prepare an endangered species assessment but inadvertently left that ESA assessment out of the draft EA, It has been included in the final EA.

8. One commenter has "serious concerns about the genetic stability of the artificial gene combinations and the artificially inserted genes used…" The commenter further believes that APHIS should "commission study of the plant's interaction with other viruses in order to avoid this risk of enhancing existing plant pests…" The commenter provides no specific insight or relevant references to instances of genetic instability or virus interaction issues in GE plants being problematic. Another commenter did submit a scientific review paper (Latham and Wilson 2007) that purports to raise new issues surrounding virus interactions that the commenter believes should be considered by regulatory agencies.

APHIS has addressed virus interaction issues associated with transcapsidation (i.e., heteroencapsidation), recombination and synergy in several EAs prepared since 1994 (http://www.aphis.usda.gov/brs/not_reg.html). Those risk assessments were contained in EAs for virus resistant squash (Petitions 92-204-01p and 95-352-01p), virus resistant papaya (Petition 96-051-01p), and virus resistant plum (Petition 04-264-01p). Moreover, APHIS specifically addressed those issues in the draft EA (pp. 13-16) for this petition. The commenters did not note any new issues related to virus interactions that have not been previously thoroughly addressed in all the referenced EAs. Other authors have evaluated issues associated with virus-virus interactions and the likelihood of problems arising from such interactions. Novel issues have not been identified (Turturo et.al. 2008; Quemada 2008; Fuchs 2008) and APHIS is not aware of any of the theoretical issues considered raising any production problems in those crops that have been commercialized. APHIS therefore believes that no further consideration of these issues is warranted at this time.

Regarding gene stability in X17-2 papaya, the commenter provides no specific information that APHIS believes warrants further consideration. Plant breeders deal with gene stability in terms of inheritance patterns and desired traits as parts of their breeding programs as a matter of course. The petitioner indicates that over 250 different lines were eliminated from his program in the course of identifying X17-2 as having the most desired characteristics (Petition, p. 3). He also indicates that he has gone through multiple steps of inbreeding and recurrent selection in order to generate X17-2 (Petition, p. 3) and develop stable characteristics in this line. He is currently in the 6th generation of this process. The commenter provides no relevant references or citations that APHIS feels warrant further consideration of this issue.

Fuchs, M. 2007. Potential for Recombination and Creation of New Viruses in Transgenic Plants Expressing Viral Genes: Real or Perceived Risk? Biotechnology and Plant Disease Management. (eds., Punja et al.) pp. 416-436.

Quemada, H., Strehlow, L., Decker-Walters, D.S., and Staub, J.E. 2008. Population size and incidence of virus infection in free-living populations of *Cucurbita pepo*. Environ. Biosafety Res. 7: 185–196.

Turturo, C., Friscina, A., Gaubert, S., Jacquemond, M., Thompson, J.R., and Tepfer, M. 2008. Evaluation of potential risks associated with recombination in transgenic plants expressing viral sequences. J. Gen. Virol. 89: 327-335.

9. At least one commenter believes that significant similarities exist surrounding possible "genetic contamination" issues highlighted in recent GE alfalfa and rice cases that APHIS should prepare an EIS to explore these issues.

APHIS is aware of issues related to cross pollination (referred to as "genetic contamination" or "genetic pollution" by a number of commenters) as well as those associated with other ways to get seed or product admixtures[4] in many crops, including rice, alfalfa and papaya. APHIS noted methods to address cross pollination issues in its draft EA (p. 18-19). The rice cases noted by the commenter related to the identification of two regulated GE rice lines (LLRICE601 and LLRICE604) being found in the commercial long-grain rice varieties "Cheniere" and "Clearfield 131" in 2006. Low levels of the two GE rice lines were identified both in seed stock for planting and commodity rice. The regulated lines identified were very similar to rice lines that had been granted nonregulated status in 1999 and contained a protein for herbicide tolerance (phosphinothricin acetyl transferase). That same protein had been safely used in other deregulated products for over 10 years and had been evaluated by both USDA and FDA. The findings resulting from the investigation can be found on-line (http://www.aphis.usda.gov/biotechnology/archived_news.shtml). The investigation did indicate, however, that the admixture of LLRICE604 into "Clearfield 131" was likely not from cross pollination. APHIS produced an environmental assessment (EA) for one of the rice lines, LLRICE601, and came to a finding of no significant impact (FONSI). That rice line (LLRICE601) was found not to pose a plant pest risk and given nonregulated status in November 2006 (http://www.aphis.usda.gov/brs/not_reg.html).

The alfalfa case is somewhat different. Two lines of genetically engineered glyphosate tolerant alfalfa were developed by Monsanto Company and Forage Genetics International and were field tested in over 150 trials between 1998 and 2004 (EA for alfalfa events J101 and J163, http://www.aphis.usda.gov/brs/not_reg.html). In June 2005, APHIS granted nonregulated status to those lines based on a prepared EA, FONSI and determination that those alfalfa lines did not pose a plant pest risk. Approximately a year later, a lawsuit was filed making numerous claims about the inadequacy of APHIS' EA and in February 2007, a U.S. District Court in California found that APHIS' EA was inadequate and APHIS was required to prepare an environmental impact statement (EIS). The court vacated APHIS' 2005 deregulation decision for the GE alfalfa lines and issued an injunction limiting or prohibiting further sales of those lines of alfalfa and imposing conditions on how forage derived from fields that had been planted prior to its decision

[4] An admixture here could result from undesired cross pollination between different fields of the same crop followed by seed collection or the unintended physical mixing of seed or product from two or more different sources.

was to be handled. But the court did not overturn federal conclusions regarding the safety of the crop for food and feed purposes. Primary issues relevant to why the court felt that APHIS should prepare an EIS related to the court's view regarding organic farmers' ability to protect their operations from possible gene flow (the transfer of genetic material from one population to another) and the possible economic impacts resulting from such gene flow. The court considered that these and other issues should have been more fully analyzed because the alfalfa crop is grown on large acreages (over 20 million acres, valued at over $7 billion in 2005) across the U.S. In comparison, the most recent information (NASS 2009) indicates that papayas in Florida were grown on less than 183 acres[5]. NASS also published updated papaya production information for Puerto Rico and the U.S. Virgin Islands since the draft EA was published. That information indicated that Puerto Rico had approximately 407 acres of papaya on 76 farms and the U.S. Virgin Islands had just a few acres (exact acreage not reported) on 58 farms (NASS 2009). Wholesale prices of papayas vary considerably depending upon their markets and intended use. Green cooking papayas shipping from Florida often go for ~$20 for a 40 lb box (i.e., 50 cents per lb) while ripe Solo-type papaya from various locations (e.g., Hawaii, Brazil, Guatemala, Belize, etc.) usually get 2-4 times that price (http://agecon-trec.ifas.ufl.edu/TerminalPriceFruit.htm#Papaya). The updated information on papaya prices and estimated economic market values has been included in the final EA. Finally, as documented in the EA and elaborated on above in the response to issue #1, there is extensive information available to papaya growers who would choose to limit cross pollination with GE papaya.

NASS. 2009. 2007 Census of Agriculture. Available at http://www.agcensus.usda.gov/Publications/2007/Full_Report/index.asp. (Website provides separate links to data from U.S. territories).

10. Two commenters believe that APHIS should investigate and document "widespread genetic contamination" of papaya in Hawaii that resulted from the commercialization of GE papaya there. One commenter also believes that APHIS did not adequately consider issues associated with cross pollination with feral papaya. Several documents were submitted that focus on a previous deregulation decision and significant commercial adoption of GE papaya in Hawaii. These documents, generated by those who oppose genetic engineering in general, purport to show that the commercial use of GE papaya in Hawaii has been a failure and has led to problems with "contamination" of other papayas in Hawaii.

USDA/APHIS granted nonregulated status to GE PRSV-resistant papaya lines 55-1 and 63-1 in September 1996 (Petition 96-051-01p, EA, and Finding of No Significant Impact found at http://www.aphis.usda.gov/brs/not_reg.html). Those lines were developed as part of a collaborative effort between Cornell University and the University of Hawaii to address significant disease problems caused by PRSV in Hawaii. APHIS' concluded that those papaya

[5] Using similar methods to a previous assessment of the value of the papaya industry in FL (Degner et al 1997) the value at the time of the 2007 Census would be estimated at $1.9 million (Degner et al estimated 30 cents/lb, 25,000 lbs production per acre, 85% packout and 250 acres). The $1.9 million assumes 50 cents/lb, 25,000 lbs/acre, 85% packout and 183 acres (which is likely an over-estimate since production of 25,000 lbs/acre is rarely achieved and the 183 acres noted (NASS 2009) also includes small acreage from California and Texas).

lines and any progeny derived from those lines would be as safe to grow as papaya from traditional breeding programs that are not subject to regulation. Since that time, those papaya lines have been commercialized and grown extensively in Hawaii, providing significant benefits to growers there (Gonsalves et al. 2004). Some of the submitted documents appear to show that outcrossing or seed dispersal has likely occurred between commercial GE papaya and feral or other papaya in Hawaii. None of the submitted documents, however, seem to demonstrate any increased plant pest risk associated with those papayas. In terms of X17-2 papaya, APHIS understands that it could cross pollinate with other papaya (*Carica papaya*) trees and resulting seed will be transgenic. APHIS also understands that those who plant seed resulting from such cross pollinations will be planting transgenic papayas. Additionally, APHIS recognizes that transgenic trees that are resistant to PRSV may have a fitness advantage over those that are not PRSV resistant in areas where the specific H1K strain of PRSV is present (as human influenza viruses can vary widely, so can plant viruses). Given an understanding of how papayas are pollinated (self, insects, wind), plant scientists recognize "widespread genetic contamination" as just being a natural result of cross pollination. The commenters have not provided any evidence indicating that the GE papaya in Hawaii has caused significant environmental degradation or damage. Issues associated with organic production are discussed elsewhere (see response to comment #1) in this document.

Regarding the purported "failure" of GE papaya in Hawaii, the Hawaii Department of Agriculture (HDOA) generated a market report in 2003 that focused on the fresh market papayas being grown there (http://hawaii.gov/hdoa/add/research-and-outlook-reports/papaya%20outlook%20report.pdf, accessed 2/25/09). The report makes note of numerous market forces impacting the ability to sell and market Hawaii papayas in the U.S. as well as other countries. That report, combined with data from NASS (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113) on papaya production in terms of pounds harvested and market value, shows year to year variability but that a significant industry still exists in Hawaii. The highest production in the last 10 years was in 2001 (~55 million pounds with a value of ~$14.6 million). The lowest production was in 2006 (~28.8 million pounds) and had a value over $11 million. Other economic data regarding papaya markets has been included in the EA (Section VI., #8). The most recent data (2008) indicates ~33.2 million pounds produced with a value of over $14 million. The market report generated by HDOA noted significant competition for market share from other papaya growing countries (e.g., the Philippines, Mexico and Brazil) that has developed over the last 10+ years.

11. One commenter believes that APHIS should follow the "Precautionary Principle" prior to making a determination on X17-2 papaya. The commenter believes that in considering this "Principle," ones obligation is to "…first do no harm to public health and the environment." The commenter also posits a litany of questions that he believes need to be answered prior to APHIS making a decision on X17-2. The questions relate to data collection about cross pollination and seed dispersal over distance and time as well as questions such as "How many fertile transgenic papaya seeds will one…tree produce per year and over its life span?"

Discussions and writings about the use of the "Precautionary Principle" in decision-making continue to influence numerous venues in modern society (http://en.wikipedia.org/wiki/Precautionary_principle; http://www.sourcewatch.org/index.php?title=Precautionary_principle; http://www.heritage.org/Research/Regulation/hl818.cfm, accessed 2/27/09; Foster et al. 2000; Kriebel et al 2001; Gray and Bewers 1996; Sunstein 2002). One can find numerous writings, scholarly articles and books (internet search using Google Scholar) debating the use and value of considering the "Precautionary Principle" in decision-making with no clear consensus either about its value or its exact definition. APHIS always analyzes and takes into consideration environmental issues in its regulatory decisions regarding the regulation of GE organisms. In considering X17-2 papaya, three U.S. government agencies (USDA, FDA, and EPA) ultimately consider extensive scientific data and information produced by the developer regarding this product and make determinations based on that data as well as other relevant data known to the Agencies. The FDA has concluded its food safety analysis of this product and indicated to the developer that it had no further questions (http://www.cfsan.fda.gov/~lrd/biocon.html, BNF No. 100, completed December 24, 2008).

Regarding the lengthy list of questions the commenter believes need to be addressed, APHIS disagrees that these questions need lengthy consideration prior to making a decision about the regulatory status of X17-2 papaya. APHIS reviewed scientific data and information provided by the developer (both quantitative and qualitative) that it believes are adequate to make a regulatory decision about X17-2. APHIS has also addressed relevant environmental issues and believes that specific data collection suggested by the commenter would not provide further useful insight into either relevant plant pest or environmental issues.

Foster, K.R., P. Vecchia, M.H. Repacholi. 2000. Risk Management: Science and the Precautionary Principle. Science 288: 979-981. DOI: 10.1126/science.288.5468.979

Gray, J.S. and J.M. Bewers. 1996. Towards a Scientific Definition of the Precautionary Principle. Marine Pollution Bull 32:768-771.

Kriebel, D., J. Tickner, P. Epstein, J. Lemons, R. Levins, E.L. Loechler, M. Quinn, R. Rudel, T. Schettler, and M. Stoto. 2001. The Precautionary Principle in Environmental Science. Environ Health Perspectives 109: 871-876.

Sunstein, C.R. 2002. The Paralyzing Principle. *Regulation* Winter 32-37.

12. One commenter believes that APHIS should give serious consideration to a 2004/2005 Position Paper generated by the Ecological Society of America (ESA) related to genetically engineered organisms (GEOs) and recommendations. The commenter quotes the seven recommendations made in the paper and notes; "ESA recommendation #(4) underscores the Sierra Club's serious concerns that USDA did not perform an adequate scientific review of the possible risks of *RR sugar beet* prior to release into the environment." The commenter believes that USDA has not taken a "cautious approach" and that "X17-2 clearly fail(s) to be 'designed to reduce environmental risks'" (a recommendation made in the ESA report).

APHIS is familiar with the ESA report from 2005 (Snow et al 2005) and its included recommendations regarding environmental releases of genetically engineered organisms. APHIS disagrees with the commenter that information contained in the Petition and the draft EA are inadequate to make a regulatory decision about X17-2. ESA recommendations are noted here:

> (1) GEOs should be designed to reduce environmental risks. (2) More extensive studies of the environmental benefits and risks associated with GEOs are needed. (3) These effects should be evaluated relative to appropriate baseline scenarios. (4) Environmental release of GEOs should be prevented if scientific knowledge about possible risks is clearly inadequate. (5) In some cases, post-release monitoring will be needed to identify, manage, and mitigate environmental risks. (6) Science-based regulation should subject all transgenic organisms to a similar risk assessment framework and should incorporate a cautious approach, recognizing that many environmental effects are GEO- and site-specific. (7) Ecologists, agricultural scientists, molecular biologists, and others need broader training and wider collaboration to address these recommendations.

As described in the petition and draft EA, insecticides have been commonly used to attempt to control vectors of this disease (Petition, p. 1; draft EA, Section V., p. 9). Use of X17-2 could clearly address recommendation #1 of the ESA report by reducing environmental risks associated with use of these insecticides (also noted in ESA report, Box 1, p. 380, #3). Regarding recommendation #4, while the commenter may believe that scientific knowledge about X17-2 and its possible risks is clearly inadequate, APHIS definitely disagrees. Additionally, none of the seven authors of the ESA report submitted comments that would indicate that they disagree with APHIS' conclusions. APHIS is also not aware of significant environmental degradation or damage that has resulted from its 1996 deregulation decision regarding a previous PRSV resistant papaya. APHIS further believes that the Coordinated Framework has provided adequately for the "cautious approach" recommended by the ESA.

APHIS also notes that this petition and EA deal with PRSV-resistant papaya, not RR sugar beet referenced by the commenter and there are no relevant similarities between these two GE plants.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M., and Wolfenbarger, L.L. 2005. Genetically Engineered Organisms and the Environment: Current Status and Recommendations. Ecological Applications, 15: 377–404.

13. One commenter presents opinions on the "unpredictability and randomness of genetic engineering utilized in transgenic crops" and believes that APHIS (referred to as "defendants" by the commenter) should require complete molecular characterization of X17-2. The commenter then goes on to note at least twelve (12) different types of molecular information that he believes should be required.

APHIS is aware of the sometimes "unpredictable" nature of gene insertions that may occur during processes of *Agrobacterium* transformation or biolistics. These have been noted in a number of petitions that the Agency has evaluated in the past (e.g., soybean 93-258-01p, papaya petition 96-051-01p, sugar beet 98-173-01p, plum 04-264-01p). In each case, researchers characterized and evaluated a number of different lines of the particular crop, typically eliminating many along the way, to identify one or more lines that passed both scientific and regulatory muster. Researchers collected data for at least 2 and up to 10 years prior to submitting petitions to APHIS. X17-2 papaya and other candidate GE papaya lines were evaluated over a period of more than 5 years and the petitioner discarded over 250 other GE papaya lines that, in his professional and scientific judgment, did not meet appropriate standards. APHIS disagrees with the commenter that further molecular analysis is necessary in order to make a decision about the regulatory status of X17-2 papaya.

14. One commenter believes that APHIS must conduct an economic analysis of potential impacts on organic growers.

APHIS discussed potential impacts on organic growers in this document above (#1) and in the draft EA (Section VI., pp. 17-19). APHIS is not aware that a significant organic papaya industry exists where X17-2 would be grown commercially. APHIS did receive a comment from an organic certifying organization in Florida claiming that 10 organic papaya operations exist but APHIS' reading of the certifier's own published list (http://www.foginfo.org/directory.php, accessed 2/27/09) only notes 3 such organic papaya growers. The closest of those growers is noted in Bonita Springs, FL, over 25 miles distant from Dade County where ~90% of commercial papayas are grown in FL (draft EA Section VI., p. 17). The other two growers who list organic papaya as one of their crops are ~100 and over 200 miles away from Dade County. None of the comments received by APHIS appeared to be from growers of organic papayas in FL, Puerto Rico or the US Virgin Islands. As noted elsewhere in this document, organic growers of all organic crops are required to have an organic plan in which they outline methods they will employ to ensure or minimize exposure to or contact with "excluded methods" (which includes "genetically modified organisms"). Given the significant distance between commercial papaya growers and organic papaya growers in FL it appears that organic papaya grower's crops should not be affected by commercial use of X17-2. As noted previously in this document, methods have been described and are available to minimize potential contact if one chooses to do so. Ultimately, the certifying organization should provide adequate education to organic growers of these means (http://www.ams.usda.gov/nop/, accessed 5/18/09). Given that one of the commenters appears to be the only accredited certifying agent in Florida (http://www.ams.usda.gov/nop/), information about methods to minimize potential contact with unapproved methods (GE organisms) should be readily available to organic papaya growers in FL. Further economic impact analysis regarding organic growers in the region would be highly speculative given the small size of both commercial and organic papaya operations in the region (NASS 2009) but information is included both in the EA and #9 above. (See also our response to #1 above).

NASS. 2009. 2007 Census of Agriculture. Available at http://www.agcensus.usda.gov/Publications/2007/Full_Report/index.asp. (Website provides separate links to data from U.S. territories).

15. Two commenters believe that APHIS should not deregulate X17-2 because small interfering (si) RNAs (likely produced by X17-2) should not be considered as safe for consumption. The commenters believe that the interfering RNA should be identified and tested for toxicity in humans and animals that might consume plant material.

The commenters cite a scientific paper that used an AAV8 (adeno-associated virus) gene construct carrying other genes resulting in the production of short hairpin RNAs (shRNAs) when *injected* into mice. They also cite a paper showing that ingestion of gene specific double stranded RNAs can silence a specific gene in cotton bollworms, leading to toxicity of a compound that was previously non-toxic due to the presence of the specific gene product. While the papers cited by the commenter are interesting regarding the use and potential uses of siRNAs (and the mechanism of RNA interference), APHIS does not find them convincingly relevant to APHIS' regulatory deregulation decision for X17-2 papaya. While X17-2 papaya likely does produce siRNAs, siRNAs are likely produced in all plants infected by any virus as RNAi activity is a common cross-kingdom phenomenon (Stram and Kuzntzova 2006; Ghildiyal and Zamore 2009). SiRNA has been found in non-transgenic plum trees infected with plum pox virus (PPV) (Hily et al. 2005). Use of RNAi methods has been demonstrated in sweet potato (Motoyasu et al. 2004) and as a potential method to improve the nutritional value of plants (Tang and Galili 2004). The safety of nucleic acids is widely accepted. Nucleic acids are non-toxic and non-allergenic. Both RNA and DNA are part of all food products that we consume. The EPA has exempted residues of nucleic acids that are part of plant-incorporated protectants (PIPs) from the requirement of a tolerance because of their known safety - they are non-toxic and ubiquitous in all foods and feeds (EPA 2001). Further, given that plant viruses infect a tremendous amount of the fruits and vegetables that we consume, it is highly likely that humans have been exposed to the same or similar viral RNAs that may be expressed in a coat-protein expressing plant, to no apparent harm. (Also see the response to #16 below).

EPA (2001). Exemption From the Requirement of a Tolerance Under the Federal Food, Drug, and Cosmetic Act for Residues of Nucleic Acids that are Part of Plant-Incorporated Protectants (Formerly Plant-Pesticides). 40 CFR Part 174.

Ghildiyal, M. and Zamore, P.D. 2009. Small silencing RNAs: an expanding universe. Nature Reviews/ Genetics. 10: 94-108.

Hily, J-M, Scorza, R., Webb, K., and Ravelonandro, M. 2005. Accumulation of the Long Class of siRNA Is Associated with Resistance to Plum pox virus in a Transgenic Woody Perennial Plum Tree. MPMI. 18: 794-799.

Motoyasu, O., Tatsuro, H. and Takiko, S. 2004. Production of Amylose-free Sweetpotato Plants by RNAi. Hokuriku Crop Science. 39: 44-46.

Stram, Y. and Kuzntzova, L. 2006. Inhibition of viruses by RNA interference. Virus Genes. 32: 299-306.

Tang, G. and Galili, G. 2004. Using RNAi to improve plant nutritional value: from mechanism to application. Trends in Biotechnology. 22: 463-469.

16. One commenter suggested that the nature of the coat protein gene inserted into X17-2 papaya, due to some minor amino acid sequence alterations, would disqualify X17-2 from being eligible for a previous exemption tolerance for PRSV coat protein (EPA 1997). Two

commenters cite discussions in an EPA Scientific Advisory Panel (2005) and a scientific paper from 2002 relating to potential allergenicity of PRSV coat protein.

The determination as to qualifying for EPA tolerance exemption for PRSV coat protein has not been made by EPA at this time. A 2004 EPA scientific advisory panel (SAP) specifically addressed issues of potential allergenicity and viral coat proteins and reported:

> The Panel agreed that (because of the human history of consuming virus infected food), unaltered PVCPs[6] do not present new dietary exposures. There was no clear consensus on how much change would be necessary to invalidate this assumption, although there was general agreement that the appropriate comparison is to the range of natural variation in the virus population. (EPA 2004)
>
> In many cases, there are data showing a high degree of variability in amino acid sequence between the PVCPs of different isolates of the same virus – in the case of potyviruses this is especially true within the N-terminus, as seen with PPV, PRSV and TuMV (Shukla et al. 1994). In this case, anything less than a major mutation is thus unlikely to differ significantly from the variability extant in viral populations. In general, changes that can be considered to be within the bounds normally found in the viral population can probably be considered to be as safe as the initial PVCP. (EPA 2004)

A 2005 EPA SAP also addressed the use of viral coat proteins in plants and included this:

> Historically, virus infected plants have been a part of the human and domestic animal food supply without adverse human or animal health effects. Exposure to PVC proteins generated in PVCP-PIPs that are identical with or within the range of natural variation of plant virus coat proteins would not alter the risk of toxicity and allergenicity. Thus, prior knowledge of natural variation limits of the individual PVC proteins is required. There are no general principles that can be used to determine how relative risk increases with an increasing number of added amino acids. The addition of other amino acids containing reactive side chains that can serve as sites for post-translational modifications may be significant and should be evaluated on a case-by-case basis. It is difficult to identify modifications that would be expected to be "within the range of natural variation for all virus families". This would require prior knowledge of the natural variation limits of the individual PVC proteins, which is not available. Modifications such as single amino acid substitutions with biochemically similar amino acids that do not affect secondary or tertiary structure might be of relatively little concern. Given the possible range of natural variations for PVC proteins, it would be appropriate to assess whether specific modifications are within natural variation limits of the PVC protein case-by-case. (EPA 2006)

The panel commented further on the previously deregulated and commercialized GE PRSV resistant papaya:

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[6] PVCP refers to plant viral coat proteins and PIP refers to plant-incorporated protectant in this section.

> There is already extensive experience with PVCP-PIPs, particularly in squash and papayas, and the use of other types of resistance genes without detectable novel or negative effects, which should be considered in future exemption considerations. It is important to build from the positive effects resulting from using those genes in U.S. and worldwide agriculture, and not only the potential negative effects. (EPA 2006)

EPA has not promulgated new regulations relating to the use of viral coat proteins in plants since those two SAP reports were generated. Given what is known about the natural sequence variability in PRSV coat proteins (Bateson et al. 1994; Silva-Rosales et al. 2000; Jain et al. 2004), it would appear that the relatively small changes in amino acid sequence in X17-2 papaya would allow this line to qualify under the current PRSV coat protein exemption. Ultimately, however, the specific decision about X17-2 will be made by EPA.

One scientific paper (Kleter and Peijnenbrug 2002) has theorized on the potential allergenicity of the PRSV coat protein (cp). The paper provides little more than a proposed method for assessing allergenicity (based on short amino acid homology within a protein) of proteins produced in GE plants. Based on this short match (6 amino acids), PRSV coat protein was identified as having similarity to a roundworm allergen. While no definitive publications are known to address this specific issue for PRSV cp, the authors of this paper recognize that "(m)ost identical stretches are likely to be false positives." The most recent and more relevant standards are multi-fold in considering potential allergens. Those typically include consideration of whether the protein is a known allergen (PRSV cp is not); a full length alignment of amino acid homology with a known allergen (none for PRSV cp); an 80 amino acid alignment in a sliding window with a known allergen (none for PRSV cp); an 8 amino acid exact match with a known allergen (none for PRSV cp) (http://www.allergenonline.org/, accessed 3/3/09). This website is authoritative and peer-reviewed by allergen experts and has amassed sequence information on over 1300 allergens. PRSV cp is not noted as an allergen in this database. As demonstrated in the Petition as well as a previous Petition for papaya, the amount of PRSV cp (measured by ELISA) found in non-transgenic PRSV-infected papaya is higher than that found in these transgenic lines (Petition p. 12; Petition 96-051-01p, p. 12). What that means is that anyone who has consumed or otherwise been exposed to PRSV-infected papaya has been exposed to and/or eaten a significant amount of PRSV coat protein (and more than is produced in any GE papaya). APHIS searched the literature and was not able to identify any known allergic reactions from such exposures.

Bateson, M.F., Henderson, J., Chaleeprom, W. Gibbs, A.J., and Dale, J.L. 1994. Papaya ringspot potyvirus: isolate variability and the origin of PRSV type P (Australia). J Gen Virology 75: 3547-3553.

EPA. 1997. Coat Protein of Papaya Ringspot Virus and the Genetic Material Necessary for its Production; Exemption From the Requirement of a Tolerance. 62 FR 44572.

EPA. 2007. Coat Protein of Papaya Ringspot Virus and the genetic material necessary for its production; exemption from the requirement of a tolerance. 72 FR 20434, 20435.

EPA. 2004. FIFRA Scientific Advisory Panel Meeting Minutes October 13-15, 2004. Available at http://www.epa.gov/scipoly/sap/meetings/2004/101304_mtg.htm. (accessed 3/3/09)

EPA. 2006. FIFRA Scientific Advisory Panel Meeting Minutes December 6-8, 2005. Available at http://www.epa.gov/scipoly/sap/meetings/2005/120605_mtg.htm. (accessed 3/4/09)

Jain, R.K., Sharma, J., Sivakumar, S., Sharma, P.K., Byadgi, A.S., Verma, A.K., and Varma, A. 2004. Variability in the coat protein gene of *Papaya ringspot virus* isolates from multiple locations in India. Arch Virol 149: 2435-2442.

Kleter, G.A. and Peijnenburg, A.A. 2002. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE – binding linear epitopes of allergens. BMC Structural Biology. (http://www.biomedcentral.com/1472-6807/2/8).

Shukla D.D., Ward C.W., and Brunt A.A. 1994. The Potyviridae. CAB International.

Silva-Rosales, L., Becerra-Leor, N., Ruiz-Castro, S., Téliz-Ortiz, D. and Noa-Carrazana, J.C. 2000. Coat protein sequence comparisons of three Mexican isolates of papaya ringspot virus with other geographical isolates reveal a close relationship to American and Australian isolates. Arch Virol 145: 835-843.

17. One commenter took issue with the use of a 1993 reference in the Petition relating to a safety assessment of the NPT II protein and felt reference to a more recent publication should have been used. The commenter provided no further insight into what that citation should be or that the 1993 citation was not still relevant or accurate in its conclusions.

APHIS does not take issue with the use of the Fuchs 1993 paper on the safety assessment of NPT II protein and considers it still relevant to this petition.

18. One commenter submitted an entire doctoral dissertation written in 2005 that focuses on issues associated with scientific dissent and a myriad of issues (e.g., politics, the public trust, intellectual property rights, the "university-industrial complex", etc) related to agricultural biotechnology. The dissertation author writes extensively about three particular cases that have been widely publicized and analyzes how those cases developed over time. Nothing in the dissertation specifically addresses issues in either the Petition or the draft EA related to the deregulation of GE papaya line X17-2. The commenter also writes about field trials of eucalyptus and those who have "vested interests in promoting their own research careers…" He also believes that a great deal more research needs to be done on GE trees.

APHIS understands that some in the scientific community may have dissenting opinions about the appropriate uses for certain technologies, including biotechnology ones, and how they are managed in a societal context. The commenter believes that the science surrounding uses of GE trees is inadequate to allow the widespread use of GE trees at this time. He provides no compelling documents or references, however, that data presented to APHIS is inadequate. The agency disagrees with the commenter that scientific data and information from the petition and generally available in the literature are inadequate to make a regulatory decision on the deregulation of X17-2.

19. One organization submitted reports from another group that focus on "contamination" of various non-GE seeds with GE seed and the possibility of contamination of food supplies by crops grown for pharmaceutical or industrial uses. The reports make a number of recommendations that the organizations believe the regulatory agencies and others involved in the seed and commodity business should undertake in order to safeguard and preserve the seed and food supplies. The commenter believes that APHIS should complete an EIS in order to analyze potential gene flow issues that might be relevant.

The commenters consider normal biological and physical processes that result in seed mixing as fundamentally undesirable or unacceptable. They characterize cross-pollination and seed mixing as "contamination" and fail to consider that almost all seed and commodity products contain some level of contaminants in their products (i.e., absolute zero contamination is virtually not achievable). The Federal Seed Act (7 CFR part 201) refers to noxious weed seeds and inert matter (including stones, fungus bodies, etc). Grain standards have similar allowances for impurities, including insects, insect parts, stones, etc (http://archive.gipsa.usda.gov/reference-library/standards/810101.pdf or http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&rgn=div5&view=text&node=7:7.1.2.8.4&idno=7, accessed 3/6/09). The Federal Food, Drug and Cosmetics Act (FFDCA) similarly has allowances or sets tolerances for impurities in food products (http://www.fda.gov/opacom/laws/fdcact/fdcact4.htm#sec406, accessed 5/19/09). The presence of undesirable materials in seed or other products is recognized and noted in these various standards. Contaminants may be stones, weed seeds, fungi, insect or insect parts, or are unknown and typically are allowable up to some very low level in commodities. These are acceptable and the industry and consumers tolerate these low levels because they are acceptably safe and do not result in significant impacts (in terms of costs, impacts to health, etc.) to the public. Higher standards ultimately result in higher costs to producers and that cost could be expected to be passed along to consumers.

In the case of X17-2 papaya, there is no indication that commercial growers of conventional or organic papaya will be significantly impacted by deregulation of this product. It seems more likely that those who have had to manage PRSV in their plantings would use these GE papaya trees to their best advantage and as they see fit. Growers of organic papaya in FL (and their organic certifiers), as a result of the publication of the draft EA and Petition, are now aware that X17-2 papaya may be grown in the region in the near future. One commenter on the draft EA appears to be the only organic certifying organization in the State of FL and is in the position to educate and assist any organic papaya growers that exist in the State. That commenter gave no indication as to the size and location of organic papaya growers in FL, the U.S. Virgin Islands or Puerto Rico, which might have been useful in assessing those issues. But given the likely very small number, size and location of those organic papaya growers, APHIS believes that any significant impact to those growers is unlikely. The draft EA and this document have described methods to address issues associated with cross-pollination of papaya that are straightforward and can be accomplished with minimal effort. APHIS believes that no further assessment is warranted. (See also our responses in #1 and 14 above).

20. One commenter believes that APHIS should analyze potential socio-economic impacts from deregulation of X17-2 papaya. The commenter also notes a decline in papaya production and prices in Hawaii and ties that to introduction of GE papaya there. The commenter also believes that APHIS must prepare an EIS to analyze impacts on consumer choice and biodiversity.

The Council on Environmental Quality (CEQ) NEPA implementing regulation addressing when socio-economic impacts need to be analyzed in NEPA documentation makes clear that socio-economic impacts need only be addressed if and only if the "economic or social and natural or physical environmental effects are interrelated." 40 C.F.R. 1508.14. Thus, there must be a **causal interrelationship** between a specific change in the natural or physical environment resulting from the proposed federal action (in this case the deregulation of papaya line X17-2) and the claimed socio-economic effects resulting from the same proposed federal action. In the X17-2 papaya deregulation actions, as in other APHIS deregulation decisions of GE crops, there are no specific economic impacts directly interrelated with any specific ***physical*** environmental change resulting from a proposed deregulation decision itself--- if there are any potential economic impacts at all, they would be the result of **human**, not natural or physical, changes resulting from either the choices of certain farmers to grow or not grow certain types of papaya lines (GE, conventional, or organic lines) and/or of certain consumers' preferences to buy or not buy certain types of grown papaya lines.

Nevertheless, as we have explained in other response above, based on the information we have reviewed and the analyses we have done, we do not expect there to be any significant impacts on growers of organic or conventional papaya lines resulting from APHIS' regulatory decision to deregulate papaya line X17-2. None of the commenters provided insight into the size and location of the organic papaya industry in Florida or U.S. Caribbean territories. It appears, based on one comment provided and an analysis of the commenter's published list of possible (certified organic) growers that the industry, almost entirely, is remote from commercial papaya growers (who would likely use X17-2) in Florida (Dade County to the largest extent) and is therefore unlikely to be significantly impacted by a decision to deregulate X17-2 papaya. (See also our responses to #1, 14 and 19 above).

The decline in papaya production in Hawaii likely has resulted from a number of different factors (a major one being competition from other countries), introduction of GE papaya being only one of those (see response to comment #10 above). The HDOA market report and NASS statistics (http://hawaii.gov/hdoa/add/research-and-outlook-reports/papaya%20outlook%20report.pdf, accessed 2/25/09), (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113) note several of the likely and possible factors that relate to that decline. Regardless, papayas are still being grown in and exported from Hawaii. (See also our response to #10 above).

The commenter makes note of a 2007 court ruling related to GE alfalfa in making his assertions related to biodiversity and consumer choice. We don't believe that ruling regarding alfalfa is relevant to APHIS' comprehensive evaluation of X17-2 papaya and our decision to deregulate papaya line X17-2. Moreover, in Hawaii, where the largest papaya industry exists in the U.S., growers and exporters have adapted to inclusion of GE papaya into the commerce stream. GE papayas exist and are consumed locally as well as being shipped to mainland U.S. and Canada. Additionally, a market exists for non-GE papayas which are shipped to Japan and elsewhere. It appears that biodiversity has increased (i.e., a new genetic background providing robust resistance to PRSV has been introduced into papaya germplasm) and consumer choice has expanded (both GE and non-GE papayas are currently available to the consumer) because of the introduction of GE papaya to Hawaii. This leads APHIS to conclude that significant impacts related to both biodiversity and consumer choices are unlikely to occur as a result of introduction of X17-2 papaya.

USDA/APHIS Final 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

June 2009

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 nonregulated status for genetically engineered 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 acknowledged 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*[7]) 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 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[8] 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[9] through the unconfined cultivation and use in agriculture of the regulated article.

III. PURPOSE and NEED

The developer of 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.

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

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

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

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 part 372).

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. A genetically engineered 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 will be required to submit 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. The University of Florida completed their food and feed safety and nutritional assessment review of X17-2 papaya (BNF No. 100) with FDA's Center for Food Safety and Applied Nutrition in December 2008 (http://www.cfsan.fda.gov/~lrd/biocon.html, accessed 3/10/2009).

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 and environmental 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 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 (H1K) and reports have shown that similar gene constructs give resistance to the disease only from highly similar viral strains (OECD 2005). As noted in the Petition, GE papaya developed for Hawaii has been shown to be susceptible to a number of PRSV strains outside of Hawaii (Bau et al. 2003; Chiang et al. 2001; Gonsalves 1998; Tennant et al. 1994; Tennant et al. 2001). 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 it 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[10] (Matthews 1991; OECD 1996). Plant viruses can also be spread in a number of other ways, depending upon the virus type, including seed transmission,

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

pollen transmission, and/or mechanical transmission[11] (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

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[12] 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[13] 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

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

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

[13] 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).

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 (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 over 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 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, 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. Hybridization with other commercially grown or feral papayas may occur as a result of this deregulation, depending on the distance to such plants (i.e., a further distance will result in less cross pollination). Careful selection of fruit/seeds (either from commercial suppliers or within ones own plantings) should allow growers to manage such hybrids, if they choose to do so. Further discussion of issues associated with cross pollination and hybridization with other papayas is included in the response to comments attached to this document (#s 1 and 4). APHIS concludes that by choosing the proposed action (Alternative B, deregulation in whole), there are unlikely to be significant 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/plant_health/plant_pest_info/weeds/, accessed 3/10/2009)
- California Weed Species List (http://www.extendinc.com/weedfreefeed/list-b.htm, accessed 3/10/09)
- Hawaii Weed Species List (http://www.hear.org/hawaiinoxiousweeds/index.html, accessed 3/10/09), (http://plants.usda.gov/java/noxious?rptType=State&statefips=15, accessed 3/10/09)
- Florida Weed Species List (http://plants.usda.gov/java/noxious?rptType=State&statefips=12, accessed 3/10/09)

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, uninfected papaya that would indicate that X17-2 has more weedy characteristics. Further discussion of issues associated with potential weediness of X17-2 papaya is included in the response to comments document attached to this EA (# 4).

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).

3. Potential impact on non-target organisms, including beneficial organisms and threatened and endangered species

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; EPA 2007). This exemption eliminates the need to establish a maximum permissible level for residues of PRSV in or on all raw agricultural commodities[14]. 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).

In addition to the analysis of potential impact to non-target organisms described above, APHIS also considered the potential impact on federally listed threatened or endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. In this analysis, APHIS considered the biology of X17-2 papaya, as well as typical agricultural practices associated with its cultivation.

There are 114 species of threatened and endangered species noted as being present in Florida, 78 in Puerto Rico, and 17 in the U.S. Virgin Islands (http://ecos.fws.gov/tess_public/StateListing.do?state=all, accessed and updated 2/12/09). In total, 101 threatened or endangered animals are noted in these areas (some are duplicative as they may occur in more than one state or territory). Many of these animals reside in water environments: they include various corals, mussels, fish, sea turtles, seals, the West Indian manatee, a cave shrimp, and several whales. It is unlikely that any of these species would be exposed to X17-2. Other animals which could be exposed to this

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[14] Although EPA has received comments from scientific advisory panels regarding possible changes to their regulations that might modify this exemption (EPA 2004; EPA 2006), they have not, to date, promulgated new rules that would invalidate this exemption.

papaya include a bat, beetles, butterflies, crocodiles, a number of bird species, deer, mice, a rabbit species, rats, skinks and other amphibians, snakes, a vole species, and the gray wolf.

As far as plant species are concerned, there are 108 threatened or endangered species noted for Florida, Puerto Rico and the U.S. Virgin Islands (as with the animals noted above, some of these are also duplicative). The applicant notes that typical cultivation practices associated with growing X17-2 papaya are likely to remain the same as those employed for non-transgenic papaya (Petition, p. 17). As such, potential effects on threatened or endangered plant species would likely be the same as, and no worse than, those that would occur regardless of a determination of nonregulated status for X17-2 papaya.

Although it is unlikely that X17-2 papaya would be useful, and therefore grown, in Hawaii, APHIS also considered threatened and endangered species that are present there. APHIS notes 329 TES in Hawaii (http://ecos.fws.gov/tess_public/pub/stateListingIndividual.jsp?state=HI&status=listed, accessed 5/19/09). Animal species include various bats, birds (honeycreepers, creepers, a crow, ducks, finches, etc), pomace flies, turtles, a seal, snails, a spider, thrushes, the humpback whale, and others. Plant species include numerous species of *Melicope*, *Isodendrion, Poa, Cyanea*, *Cyrtandra*, *Labordia*, *Pritchardia*, *Dubautia*, *Lipochaeta*, *Gouania*, *Hedyotis, Hesperomannia*, *Phyllostegia*, and many others. Most of the species exist only in specialized habitats such as caves, particular forest habitats (e.g., tropical rain, arid scrub, temperate subalpine, cloud) the ocean, adjacent to the shore, or in areas designated as critical habitat. Many of the listed animal species also do not consume papaya and would not be expected to frequent papaya groves; thus their possible exposure to papaya would be unlikely. Additionally, none of the plant species would likely be found in the highly managed environment of a papaya grove.

As noted previously, X17-2 does express two proteins not found in non-transformed uninfected papaya; the PRSV coat protein (cp) and neomycin phosphotransferase (NPT II). NPT II protein and the *npt II* gene are not known to cause adverse effects to non-target organisms and have been granted exemption from the requirement of a tolerance by EPA for use in or on all raw agricultural commodities (EPA 1994). The PRSV coat protein is also present in X17-2 papaya as noted in the Petition (pp. 11-13). Regarding consumption of PRSV coat protein, as noted here and elsewhere in the draft EA (Section VI., part 9), APHIS has searched numerous databases looking for information that would indicate that consumption of any plant virus coat protein would have any effect, adverse or otherwise, on an organism that might consume it. We could identify no data that indicates any effect of consuming plant viral coat proteins on any organism. Regardless of exposure to X17-2 papaya (whether incidental or by consumption), APHIS has not identified any exposure that would be hazardous or have any significant impact on any of these species due to the presence of NPT II or PRSV coat protein.

APHIS also notes that any threatened and endangered species that might be exposed to X17-2 papaya has likely been exposed to papaya as long as they have been grown. As such, when an organism consumes a papaya that is infected with PRSV, it is consuming a large quantity of PRSV coat protein. To APHIS' knowledge, no adverse effects of consuming such infected fruit, due to the presence of the virus (and therefore viral coat protein), have ever been documented. An assessment similar to what has been noted above regarding the non-toxic nature of X17-2 papaya and expressed proteins would be applicable wherever X17-2 might be grown (e.g., Hawaii).

After reviewing possible effects of granting nonregulated status to X17-2, APHIS has not identified any stressor that would affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. Consequently, an exposure analysis for individual species is not necessary. APHIS has considered the effect of X17-2 production on designated critical habitat or habitat proposed for designation and could identify no difference from affects that would occur from the production of other papaya varieties. APHIS has reached a conclusion that the release of X17-2, following a determination of nonregulated status, would have no effect on federally listed threatened or endangered species or species proposed for listing, nor is it expected to adversely modify designated critical habitat or habitat proposed for designation, compared to current agricultural practices. Consequently, a written concurrence or formal consultation with the USFWS is not required for this action. Based on this analysis, there is no apparent potential for significant impact on threatened or endangered species if APHIS were to grant this petition for nonregulated status to X17-2 papaya.

Finally, based on all the noted considerations, there should be no impact on non-target organisms, including beneficial organisms and threatened and endangered species from a decision to grant nonregulated status to X17-2 papaya (preferred 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. Given that X17-2 papaya has no increased toxic properties (noted directly above in Part 3 of this section), compared to non-engineered papaya, and that agricultural practices associated with growing X17-2 papaya are expected to remain unchanged, there is no apparent potential for significant impacts to biodiversity if APHIS chooses the preferred alternative.

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[15]

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

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

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. 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 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 different 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 (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 the preferred alternative of granting 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 X17-2 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 and Ferreira 2003; 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 and Ferreira 2003). As noted (Gonsalves and Ferreira 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 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 and Ferreira 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 grants nonregulated status to X17-2 papaya.

8. Potential Impacts on Conventional and Organic Farming

After publication of the draft EA, USDA/NASS published updated information on the papaya industry in the U.S., including Florida, the U.S. Virgin Islands and Puerto Rico. Information cited in the draft EA indicated that planted papaya acreage in Florida likely amounted to approximately 250 acres in Dade County, FL in 1996 (Degner et al., 1997). More recent data from 2007 (NASS 2009) indicates that papaya acreage in FL has decreased to less than 183 acres[16]. The total crop value in Dade County in 1996 was approximately $1.6 million. Using a similar estimation, (Degner et al. 1997), and considering 2007 data, the 2007 crop could be estimated at ~$1.9 million (see also response to comments document attached to this EA, #s 9 and 10). In 1996, Dade County production was estimated to produce approximately 90% of the papayas grown in FL (Mossler and Nesheim 2002). Currently, Dade County has 62% of papaya growers (42/68) and over 90% of the papaya acreage (167/183) in the state. The remaining reported papaya operations in FL (26) are spread across 10 different counties (NASS 2009). Comparing estimated acreages from Hawaii and Florida, the industry is over 12 times larger in Hawaii than Florida. The U.S. Virgin Islands (USVI) and Puerto Rico (PR) also have papaya industries and some information on them is also captured (NASS 2009). The USVI industry is very small and only harvested ~20,390 lbs of papaya in 2007. In some locations, that much papaya could be harvested from 1 acre or less per year but the USVI reports 58 farms growing papaya. USVI can be adversely affected by hurricanes, disease and other issues and that could account for the relatively small production. Puerto Rico reports 76 papaya growers and ~407 acres in 2007. Although papaya growers are noted in 31 municipalities (similar to counties) in PR, the bulk of production takes place in three or four (NASS 2009). They also reported ~9.2 million lbs harvested in 2007 (NASS 2009). In the same year, HI production amounted to ~33.4 million lbs (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113). Estimated total papaya acres where X17-2 would be useful to combat PRSV (FL, PR, USVI) amount to ~591 while papayas are grown on ~2,318 acres in HI.

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[16] The entire U.S. papaya industry was estimated at 2,501 acres. Of that, 2,318 are in Hawaii while the other 3 states (FL, TX, and CA) do not report specific information. TX and CA industries are likely very small, however, and only have 3 reported growers of papaya while FL reported 68 growers and 515 in HI (NASS 2009). USVI does not report specific papaya acreage while acreage is estimated at ~407 in PR.

In terms of shipping of papayas, Hawaiian papayas of the Solo type are shipped frequently around the country while Florida and Puerto Rico ship green cooking type papayas much less often (http://www.marketnews.usda.gov/portal/fv, accessed 3/10/2009). 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 (http://agecon-trec.ifas.ufl.edu/papaya.htm, accessed 3/11/09). There is no indication that Florida, USVI or PR 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. 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. One fruit typically will produce hundreds of seeds, thereby giving a grower a ready seed supply for many acres from bagging just a few flowers. Alternatively, if a grower desires to have trees from an open-pollinated source (while minimizing the likelihood of cross-pollination with a GE tree), he/she should choose and collect fruit from within a papaya planting, avoiding fruit from perimeter rows which would be more likely to have been pollinated from outside the planting. 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; Manshardt et al. 2007). Following recommended good management practices can assist all growers in producing marketable crops (Ronald and Fouche 2006).

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 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. As noted 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 University of Florida completed their food and feed safety and nutritional assessment review of X17-2 papaya (BNF No. 100) with FDA’s Center for Food Safety and Applied Nutrition in December 2008 (http://www.cfsan.fda.gov). The FDA had “no further questions” regarding X17-2 papaya.

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; EPA 1997; EPA 2007). 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 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 does 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 (Petition p. 18, Petition addendum 1). Therefore, APHIS does not expect accumulation of novel substances 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 non-GE and GE 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. Granting nonregulated status to X17-2 is not 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-GE and deregulated GE 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 GE 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. 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 155 countries are Parties to it as of March 2009 (see http://www.biodiv.org/biosafety/default.aspx , accessed 3/11/09). 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 of 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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USDA/APHIS Final 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

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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 nonregulated status for genetically engineered 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 acknowledged 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 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*

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

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

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 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 part 372).

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. A genetically engineered 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.

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[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).

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 will be required to submit 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. The University of Florida completed their food and feed safety and nutritional assessment review of X17-2 papaya (BNF No. 100) with FDA's Center for Food Safety and Applied Nutrition in December 2008 (http://www.cfsan.fda.gov/~lrd/biocon.html, accessed 3/10/2009).

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 and environmental 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 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 (H1K) and reports have shown that similar gene constructs give resistance to the disease only from highly similar viral strains (OECD 2005). As noted in the Petition, GE papaya developed for Hawaii has been shown to be susceptible to a number of PRSV strains outside of Hawaii (Bau et al. 2003; Chiang et al. 2001; Gonsalves 1998; Tennant et al. 1994; Tennant et al. 2001). 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 it 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

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

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

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

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

[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).

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 (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 over 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 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, 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. Hybridization with other commercially grown or feral papayas may occur as a result of this deregulation, depending on the distance to such plants (i.e., a further distance will result in less cross pollination). Careful selection of fruit/seeds (either from commercial suppliers or within ones own plantings) should allow growers to manage such hybrids, if they choose to do so. Further discussion of issues associated with cross pollination and hybridization with other papayas is included in the response to comments attached to this document (#s 1 and 4). APHIS concludes that by choosing the proposed action (Alternative B, deregulation in whole), there are unlikely to be significant 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/plant_health/plant_pest_info/weeds/, accessed 3/10/2009)
- California Weed Species List (http://www.extendinc.com/weedfreefeed/list-b.htm, accessed 3/10/09)
- Hawaii Weed Species List (http://www.hear.org/hawaiinoxiousweeds/index.html, accessed 3/10/09), (http://plants.usda.gov/java/noxious?rptType=State&statefips=15, accessed 3/10/09)
- Florida Weed Species List (http://plants.usda.gov/java/noxious?rptType=State&statefips=12, accessed 3/10/09)

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, uninfected papaya that would indicate that X17-2 has more weedy characteristics. Further discussion of issues associated with potential weediness of X17-2 papaya is included in the response to comments document attached to this EA (# 4).

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).

3. Potential impact on non-target organisms, including beneficial organisms and threatened and endangered species

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; EPA 2007). This exemption eliminates the need to establish a maximum permissible level for residues of PRSV in or on all raw agricultural commodities[8]. 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).

In addition to the analysis of potential impact to non-target organisms described above, APHIS also considered the potential impact on federally listed threatened or endangered species (TES) and species

[8] Although EPA has received comments from scientific advisory panels regarding possible changes to their regulations that might modify this exemption (EPA 2004; EPA 2006), they have not, to date, promulgated new rules that would invalidate this exemption.

proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. In this analysis, APHIS considered the biology of X17-2 papaya, as well as typical agricultural practices associated with its cultivation.

There are 114 species of threatened and endangered species noted as being present in Florida, 78 in Puerto Rico, and 17 in the U.S. Virgin Islands (http://ecos.fws.gov/tess_public/StateListing.do?state=all, accessed and updated 2/12/09). In total, 101 threatened or endangered animals are noted in these areas (some are duplicative as they may occur in more than one state or territory). Many of these animals reside in water environments: they include various corals, mussels, fish, sea turtles, seals, the West Indian manatee, a cave shrimp, and several whales. It is unlikely that any of these species would be exposed to X17-2. Other animals which could be exposed to this papaya include a bat, beetles, butterflies, crocodiles, a number of bird species, deer, mice, a rabbit species, rats, skinks and other amphibians, snakes, a vole species, and the gray wolf.

As far as plant species are concerned, there are 108 threatened or endangered species noted for Florida, Puerto Rico and the U.S. Virgin Islands (as with the animals noted above, some of these are also duplicative). The applicant notes that typical cultivation practices associated with growing X17-2 papaya are likely to remain the same as those employed for non-transgenic papaya (Petition, p. 17). As such, potential effects on threatened or endangered plant species would likely be the same as, and no worse than, those that would occur regardless of a determination of nonregulated status for X17-2 papaya.

Although it is unlikely that X17-2 papaya would be useful, and therefore grown, in Hawaii, APHIS also considered threatened and endangered species that are present there. APHIS notes 329 TES in Hawaii (http://ecos.fws.gov/tess_public/pub/stateListingIndividual.jsp?state=HI&status=listed, accessed 5/19/09). Animal species include various bats, birds (honeycreepers, creepers, a crow, ducks, finches, etc), pomace flies, turtles, a seal, snails, a spider, thrushes, the humpback whale, and others. Plant species include numerous species of *Melicope*, *Isodendrion, Poa, Cyanea*, *Cyrtandra*, *Labordia*, *Pritchardia*, *Dubautia*, *Lipochaeta*, *Gouania*, *Hedyotis*, *Hesperomannia*, *Phyllostegia*, and many others. Most of the species exist only in specialized habitats such as caves, particular forest habitats (e.g., tropical rain, arid scrub, temperate subalpine, cloud) the ocean, adjacent to the shore, or in areas designated as critical habitat. Many of the listed animal species also do not consume papaya and would not be expected to frequent papaya groves; thus their possible exposure to papaya would be unlikely. Additionally, none of the plant species would likely be found in the highly managed environment of a papaya grove.

As noted previously, X17-2 does express two proteins not found in non-transformed uninfected papaya; the PRSV coat protein (cp) and neomycin phosphotransferase (NPT II). NPT II protein and the *npt II* gene are not known to cause adverse effects to non-target organisms and have been granted exemption from the requirement of a tolerance by EPA for use in or on all raw agricultural commodities (EPA 1994). The PRSV coat protein is also present in X17-2 papaya as noted in the Petition (pp. 11-13). Regarding consumption of PRSV coat protein, as noted here and elsewhere in the draft EA (Section VI., part 9), APHIS has searched numerous databases looking for information that would indicate that consumption of any plant virus coat protein would have any effect, adverse or otherwise, on an organism that might consume it. We could identify no data that indicates any effect of consuming plant viral coat proteins on any organism. Regardless of exposure to X17-2 papaya (whether incidental or by consumption), APHIS has not identified any exposure that would be hazardous or have any significant impact on any of these species due to the presence of NPT II or PRSV coat protein.

APHIS also notes that any threatened and endangered species that might be exposed to X17-2 papaya has likely been exposed to papaya as long as they have been grown. As such, when an organism consumes a papaya that is infected with PRSV, it is consuming a large quantity of PRSV coat protein. To APHIS' knowledge, no adverse effects of consuming such infected fruit, due to the presence of the virus (and therefore viral coat protein), have ever been documented. An assessment similar to what has been noted above regarding the non-toxic nature of X17-2 papaya and expressed proteins would be applicable wherever X17-2 might be grown (e.g., Hawaii).

After reviewing possible effects of granting nonregulated status to X17-2, APHIS has not identified any stressor that would affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. Consequently, an exposure analysis for individual species is not necessary. APHIS has considered the effect of X17-2 production on designated critical habitat or habitat proposed for designation and could identify no difference from affects that would occur from the production of other papaya varieties. APHIS has reached a conclusion that the release of X17-2, following a determination of nonregulated status, would have no effect on federally listed threatened or endangered species or species proposed for listing, nor is it expected to adversely modify designated critical habitat or habitat proposed for designation, compared to current agricultural practices. Consequently, a written concurrence or formal consultation with the USFWS is not required for this action. Based on this analysis, there is no apparent potential for significant impact on threatened or endangered species if APHIS were to grant this petition for nonregulated status to X17-2 papaya.

Finally, based on all the noted considerations, there should be no impact on non-target organisms, including beneficial organisms and threatened and endangered species from a decision to grant nonregulated status to X17-2 papaya (preferred 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. Given that X17-2 papaya has no increased toxic properties (noted directly above in Part 3 of this section), compared to non-engineered papaya, and that agricultural practices associated with growing X17-2 papaya are expected to remain unchanged, there is no apparent potential for significant impacts to biodiversity if APHIS chooses the preferred alternative.

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[9]

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. 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 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

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

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 different 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 (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 the preferred alternative of granting 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 X17-2 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 and Ferreira 2003; 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 and Ferreira 2003). As noted (Gonsalves and Ferreira 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 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 and Ferreira 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 grants nonregulated status to X17-2 papaya.

8. Potential Impacts on Conventional and Organic Farming

After publication of the draft EA, USDA/NASS published updated information on the papaya industry in the U.S., including Florida, the U.S. Virgin Islands and Puerto Rico. Information cited in the draft EA indicated that planted papaya acreage in Florida likely amounted to approximately 250 acres in Dade County, FL in 1996 (Degner et al., 1997). More recent data from 2007 (NASS 2009) indicates that papaya acreage in FL has decreased to less than 183 acres[10]. The total crop value in Dade County in 1996 was approximately $1.6 million. Using a similar estimation, (Degner et al. 1997), and considering 2007 data, the 2007 crop could be estimated at ~$1.9 million (see also response to comments document attached to this EA, #s 9 and 10). In 1996, Dade County production was estimated to produce approximately 90% of the papayas grown in FL (Mossler and Nesheim 2002). Currently, Dade County has 62% of papaya growers (42/68) and over 90% of the papaya acreage (167/183) in the state. The remaining reported papaya operations in FL (26) are spread across 10 different counties (NASS 2009). Comparing estimated acreages from Hawaii and Florida, the industry is over 12 times larger in Hawaii than Florida. The U.S. Virgin Islands (USVI) and Puerto Rico (PR) also have papaya industries and some information on them is also captured (NASS 2009). The USVI industry is very small and only harvested ~20,390 lbs of papaya in 2007. In some locations, that much papaya could be harvested from 1 acre or less per year but the USVI reports 58 farms growing papaya. USVI can be adversely affected by hurricanes, disease and other issues and that could account for the relatively small production. Puerto Rico reports 76 papaya growers and ~407 acres in 2007. Although papaya growers are noted in 31 municipalities (similar to counties) in PR, the bulk of production takes place in three or four (NASS 2009). They also reported ~9.2 million lbs harvested in 2007 (NASS 2009). In the same year, HI production amounted to ~33.4 million lbs

[10] The entire U.S. papaya industry was estimated at 2,501 acres. Of that, 2,318 are in Hawaii while the other 3 states (FL, TX, and CA) do not report specific information. TX and CA industries are likely very small, however, and only have 3 reported growers of papaya while FL reported 68 growers and 515 in HI (NASS 2009). USVI does not report specific papaya acreage while acreage is estimated at ~407 in PR.

(http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113). Estimated total papaya acres where X17-2 would be useful to combat PRSV (FL, PR, USVI) amount to ~591 while papayas are grown on ~2,318 acres in HI.

In terms of shipping of papayas, Hawaiian papayas of the Solo type are shipped frequently around the country while Florida and Puerto Rico ship green cooking type papayas much less often (http://www.marketnews.usda.gov/portal/fv, accessed 3/10/2009). 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 (http://agecon-trec.ifas.ufl.edu/papaya.htm, accessed 3/11/09). There is no indication that Florida, USVI or PR 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. 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. One fruit typically will produce hundreds of seeds, thereby giving a grower a ready seed supply for many acres from bagging just a few flowers. Alternatively, if a grower desires to have trees from an open-pollinated source (while minimizing the likelihood of cross-pollination with a GE tree), he/she should choose and collect fruit from within a papaya planting, avoiding fruit from perimeter rows which would be more likely to have been pollinated from outside the planting. 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; Manshardt et al. 2007). Following recommended good management practices can assist all growers in producing marketable crops (Ronald and Fouche 2006).

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 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. As noted 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 University of Florida completed their food and feed safety and nutritional assessment review of X17-2 papaya (BNF No. 100) with FDA's Center for Food Safety and Applied Nutrition in December 2008 (http://www.cfsan.fda.gov). The FDA had "no further questions" regarding X17-2 papaya.

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; EPA 1997; EPA 2007). 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 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 does 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 (Petition p. 18, Petition addendum 1). Therefore, APHIS does not expect accumulation of novel substances 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 non-GE and GE 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. Granting nonregulated status to X17-2 is not 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-GE and deregulated GE 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 GE 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. 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 155 countries are Parties to it as of March 2009 (see http://www.biodiv.org/biosafety/default.aspx , accessed 3/11/09). 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 of 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

Appendix II: Determination of nonregulated status for X17-2 papaya

In response to petition 04-337-01p from the University of Florida, APHIS has determined that X17-2 papaya and progeny derived from it are no longer regulated articles under APHIS regulations at 7 CFR part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for X17-2 papaya and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR part 319 and the Federal Seed Act regulations at 7 CFR part 201. This determination is based on APHIS' analysis of field, greenhouse and laboratory data, references provided in the petition, and other relevant information as described in this environmental assessment that indicate that X17-2 papaya poses no more risk of dissemination of plant pests than its non-engineered counterpart. The transgenic event found in X17-2 papaya will not increase that risk for the following reasons: (1) disease susceptibility and compositional profiles of X17-2 are similar to other papaya varieties, therefore no direct or indirect effects on raw or processed plant commodities are expected; (2) X17-2 will not hybridize with any native papaya species, although it may hybridize with feral or other *Carica papaya* plants; known mitigation methods to exclude GE pollen are described and lead APHIS to conclude that significant effects on both organic and conventional growers are unlikely; (3) it exhibits no characteristics that would cause it to be more weedy than the non-genetically engineered papaya from which it was developed or other papayas; (4) X17-2 does not exhibit changes in pest or disease susceptibility (other than resistance to PRSV) therefore significant impacts on biodiversity of papaya or other organisms in the environment are unlikely; (5) in assessing viral interaction issues, APHIS considered the potential for recombination, heteroencapsidation and synergy and concluded that the likelihood of development of new viruses or viruses with novel/altered properties is very low; (6) the anti-viral activity of the inserted genes does not pose risks to non-target organisms, including beneficial organisms and threatened and endangered species; (7) compared to current papaya PRSV management practices, cultivation of X17-2 should not significantly impact standard agricultural practices or commercial uses of papaya; (8) multiple years of growing X17-2 papaya has not resulted in observable changes to the environment, therefore APHIS concludes that significant cumulative impacts resulting from granting X17-2 nonregulated status are unlikely to occur.

In addition to our finding that a determination of nonregulated status for X17-2 papaya is unlikely to result in dissemination of plant pests, there will be no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitat, resulting from a determination of nonregulated status for X17-2 papaya and its progeny. APHIS also concludes that new varieties bred from X17-2 are unlikely to exhibit new plant pest properties, i.e. properties substantially different from any observed for X17-2, or those observed for other papaya varieties not considered regulated articles under 7 CFR part 340.

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