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DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 96-024-2]

Cornell University and University of Hawaii; Availability of Determination of Nonregulated Status for Papaya Lines Genetically Engineered for Virus Resistance

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that certain papaya lines developed by Cornell University and the University of Hawaii that have been genetically engineered for virus resistance are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Cornell University and the University of Hawaii in their petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a previous notice announcing our receipt of the Cornell University and University of Hawaii petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: September 5, 1996.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. David Heron, Biotechnology Permits, BBEP, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-7612; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On February 20, 1996, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 96-051-01p) from Cornell University, Geneva, NY, and the University of Hawaii, Honolulu, HI, (Cornell/Hawaii) seeking a determination that papaya lines designated as 55-1 and 63-1 that have been genetically engineered to contain genes that confer virus resistance do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On May 3, 1996, APHIS published a notice in the Federal Register (61 FR 19904-19905, Docket No. 96-024-1) announcing that the Cornell/Hawaii petition had been received and was available for public review. The notice also discussed the role of APHIS and the Food and Drug Administration in regulating the subject papaya lines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether these papaya lines pose a plant pest risk. The comments were to have been received by APHIS on or before July 2, 1996. During the designated 60-day comment period, APHIS received 18 comments on the subject petition from universities; papaya growers, processors, and shippers; a State agricultural experiment station; a papaya industry association; an office of the cooperative extension service; and a State department of agriculture. All of the comments were favorable to the petition.

Analysis

Papaya lines 55-1 and 63-1 have been genetically engineered to express the coat protein gene from papaya ringspot virus (PRV), strain HA 5-1, which confers resistance to PRV. Both the subject papaya lines contain the *nptII* selectable marker gene, and line 55-1 also contains the *gus* selectable marker gene. Expression of the added genes is controlled by the nopaline synthase promoter from *Agrobacterium tumefaciens* and by the 35S promoter and terminator from the plant pathogen cauliflower mosaic virus. The genes used to develop lines 55-1 and 63-1 were transferred into the parental cultivar Sunset through use of the microprojectile process.

The subject papaya lines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences derived from plant pathogens. However, contained field trials of papaya lines 55-1 and 63-1 conducted under APHIS permits indicate that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the field testing of these papaya lines.

Determination

Based on its analysis of the data submitted by Cornell/Hawaii and a review of other scientific data, comments received, and field tests of the subject papaya lines, APHIS has determined that papaya lines 55-1 and 63-1: (1) Exhibit no plant pathogenic properties; (2) will not increase the likelihood of the emergence of new plant viruses; (3) are no more likely to become weeds than papaya developed by traditional breeding techniques; (4) will not increase the weediness potential for any other cultivated or wild species with which they can interbreed; (5) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (6) will not cause damage to processed agricultural commodities. Therefore, APHIS has concluded that the subject papaya lines and any progeny derived from hybrid crosses with other nontransformed papaya varieties will be as safe to grow as papaya in traditional breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that Cornell/Hawaii's papaya lines 55-1 and 63-1 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated

articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject papaya lines or their progeny. However, importation of the subject papaya lines or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969, as amended (NEPA) (42 U.S.C. 4321 *et seq.*), (2) Regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that Cornell/Hawaii's papaya lines 55–1 and 63–1 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 11th day of September 1996.

A. Strating,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96–23663 Filed 9–13–96; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 96-051-01P for the Determination of Nonregulated Status for Transgenic 'Sunset' Papaya Lines 55-1 and 63-1

Environmental Assessment and
Finding of No Significant Impact

September 1996

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment before issuing a determination of nonregulated status for two genetically engineered (transgenic) papaya lines designated as 'Sunset' papaya lines 55-1 and 63-1. APHIS received a petition from Cornell University and the University of Hawaii regarding the status of these papaya lines as regulated articles under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition, supporting documentation, and other relevant scientific information. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that these lines of virus-resistant papaya shall no longer be regulated articles.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: SEP - 5 1996

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an Environmental Assessment (EA) prior to making a determination on the regulated status of two genetically engineered, virus-resistant lines of papaya (*Carica papaya*) designated as 'Sunset' papaya lines 55-1 and 63-1. The developers of these papaya lines, the University of Hawaii and Cornell University, believe that these papayas do not present a plant pest risk, and therefore petitioned APHIS to make a the determination that these lines shall no longer be considered as regulated articles under APHIS regulations (7 CFR Part 340). Under these APHIS regulations, the importation, interstate movements and field tests of these papaya lines have required permits issued by APHIS.

The transgenic 'Sunset' papaya lines 55-1 and 63-1 were developed to resist infection by papaya ringspot virus (PRV), a major limiting factor in papaya production. 'Sunset' papaya lines 55-1 and 63-1 were developed by using genetic engineering (recombinant DNA) techniques to introduce the PRV coat protein (CP) gene into papaya plants of the cultivar 'Sunset.' Incorporation of the PRV CP gene into the papaya plant does not cause plant disease, but rather enables the papaya plants to resist infection by PRV. The PRV CP gene was introduced into the papaya as part of a genetic construct which also included two plant-expressible, genetic marker genes, *nptII* and *uidA* (*gus*)(Line 55-1 contains both *nptII* and *gus*, whereas line 63-1 contains *nptII*). These marker genes enable researchers to easily select those plant tissues that have been successfully transformed with the genetic construct. The construct was introduced into 'Sunset' papaya via a particle bombardment technique.

From 1991 through 1996, APHIS has authorized the University of Hawaii to conduct field tests with these lines of transgenic papaya. Prior to the authorizations for field testing, APHIS prepared an EA that addressed issues pertinent to any plant pest risks associated with a field test conducted under physical and reproductive - confinement. The previous EA did not address several issues relevant to the - unconfined growth of these transgenic papaya lines. In the course of considering the current petition, APHIS has considered potential impacts of the unconfined growth of the transgenic 'Sunset' papaya lines 55-1 and 63-1. APHIS has reached the following conclusions:

1. The transgenic 'Sunset' papaya lines 55-1 and 63-1 exhibit no plant pathogenic properties. Although plant pathogenic organisms were used in the development of these papaya lines, these papaya plants are not infected with PRV, nor can they incite disease in other plants.

2. Cultivation of the transgenic papaya lines 55-1 and 63-1 will not increase the likelihood of the emergence of new plant viruses. In assessing the potential for new plant viruses to appear, APHIS has carefully considered the biology and epidemiology of the plant viruses that infect papaya, and has determined that the unconfined cultivation of these transgenic papaya lines would be no different than nontransgenic, PRV-infected papayas.

3. The transgenic 'Sunset' papaya lines 55-1 and 63-1 are no more likely to become weeds than any other types of papaya. Papaya is not considered to be a weed pest, and there is no reason to believe that the ability of these papayas to resist infection by PRV will lead to them becoming weed pests.

4. The transgenic 'Sunset' papaya lines 55-1 and 63-1 will not increase the weediness potential of any other cultivated plant or native wild species with which they can interbreed. Transfer of the PRV-resistance trait from lines 55-1 or 63-1 to another *Carica* species, although unlikely, would not result in the resulting offspring which are weed pests.

5. The transgenic 'Sunset' papaya lines 55-1 and 63-1 will not harm threatened or endangered species or other organisms, such as bees, which are beneficial to agriculture.

6. The transgenic 'Sunset' papaya lines 55-1 and 63-1 will not cause damage to processed agricultural commodities.

APHIS has also concluded that any new papaya varieties bred from transgenic papaya lines 55-1 and 63-1 should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the papaya lines already field tested, or those observed for papayas in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that the transgenic 'Sunset' papaya lines 55-1 and 63-1 will be just as safe to grow as papaya cultivars developed through traditional breeding practices. The cultivation of these transgenic papaya lines should present environmental impacts that are no different from those associated with cultivating papaya varieties that are not subject to regulation under 7 CFR Part 340 before they enter agriculture. Based upon the analysis documented in this EA, APHIS has reached a finding of no significant impact on the environment from its determination that the transgenic 'Sunset' papaya lines 55-1 and 63-1 will no longer be considered regulated articles under the regulations in 7 CFR Part 340.

II. BACKGROUND

Development of Papaya Lines 55-1 and 63-1. The transgenic 'Sunset' papaya lines 55-1 and 63-1 have been developed to resist infection by papaya ringspot virus (PRV). The gene conferring viral resistance was introduced via recombinant DNA (genetic engineering) techniques rather than conventional breeding techniques. The recombinant techniques enabled the developer to introduce a viral coat protein gene from a mild strain of PRV into the genome of 'Sunset' papaya. Incorporation of the PRV coat protein gene into papaya to yield the transgenic 'Sunset' papaya lines 55-1 and 63-1 does not cause plant disease, but rather enables the plants to resist infection by PRV. The PRV coat protein gene was introduced into the papaya as part of a genetic construct that also included the *nptII* and *uidA* (*gus*) genes which serve as genetic marker genes. These marker genes are widely used in the development of transgenic plants to enable researchers to easily select those plant tissues that have been successfully transformed with a genetic construct that includes the marker(s) and other genes of interest (A more detailed description of the genetic constructs and other technical aspects of APHIS' review can be found in the appended determination document that is hereby incorporated by reference). The genetic construct containing the PRV coat protein gene and the marker genes was introduced into 'Sunset' papaya tissue via a particle bombardment technique.

The transgenic 'Sunset' papaya lines 55-1 and 63-1 have been evaluated extensively in laboratory, greenhouse, and field experiments to confirm that they exhibit the desired agronomic characteristics and that they do not present a plant pest risk. Researchers have evaluated these lines in field tests conducted continuously from 1991 to the present 1996 field tests. These field tests have been conducted under APHIS permits which stipulate confinement of the transgenic plant material in controlled agricultural settings.

APHIS Regulatory Authority. APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (7 U.S.C. 150aa-150jj), as amended, and the Plant Quarantine Act (7 U.S.C. 151-164a, 166-167), as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. 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 regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. The transgenic papaya plants described in this petition have been considered regulated articles because DNA sequences incorporated into these lines were derived from plant pests, specifically bacterial and viral plant pathogens (see appended determination document for additional details).

An organism is not subject to the regulatory requirements of 7 Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process 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 should no longer be regulated. If the agency determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, APHIS can grant the petition in whole or in part. As a consequence of determining nonregulated status, APHIS permits are no longer required for field testing, importation, or interstate movement of that article or its progeny.

III. PURPOSE AND NEED

APHIS has prepared this EA prior to making a determination on the status of 'Sunset' papaya lines 55-1 and 63-1 as regulated articles under APHIS regulations. The developer of these papaya lines, the University of Hawaii and Cornell University, submitted a petition to USDA/APHIS requesting that APHIS make a determination that 'Sunset' papaya lines 55-1 and 63-1 shall no longer be considered regulated articles under CFR Part 340. This EA was prepared in compliance with: (1) the National Environmental Policy Act of 1969 (NEPA)(42 U.S.C. 4321 et seq.), (2) Regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372; 60 FR 6000-6005, February 1, 1995).

IV. ALTERNATIVES

No Action. Under the Federal "no action" alternative, APHIS would not come to a determination that papaya is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of the transgenic 'Sunset' papaya lines 55-1 and 63-1. APHIS might choose this alternative if there were insufficient evidence to predict the lack of plant pest risk from unconfined cultivation of papaya.

Determination That Papaya Lines 55-1 and 63-1 Are No Longer Regulated Articles. Under the Federal action to render a determination that 'Sunset' papaya lines 55-1 and 63-1 are no longer regulated articles under the regulations at 7 CFR Part 340, these papaya lines would be subject to the same regulatory oversight as papaya cultivars that result from traditional breeding practices. As such, permits from APHIS would no longer be required for introductions of 'Sunset' papaya lines 55-1 and 63-1 or their progeny.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that transgenic papaya lines 55-1 and 63-1 would no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS in conjunction with the issuance of permits for field tests of have addressed various attributes of these papaya lines. This EA discusses the genetic modification of these papaya lines, the resultant phenotype, and the potential environmental impacts that might be associated with the unconfined cultivation of 'Sunset' papaya lines 55-1 and 63-1.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of papayas, the genetic components used in the development of papaya lines 55-1 and 63-1, and the potential plant pest risks associated with a determination that these papaya lines will no longer be regulated articles under 7 CFR Part 340.

Potential for the Introduced DNA Sequences to Cause Disease in the Transgenic Lines 55-1 and 63-1. Although some DNA sequences used in the transformation process were derived from bacterial and viral plant pathogens, these genes do not cause disease in the papaya plant. Once inserted into the genome of the papaya plant, the introduced DNA sequences are maintained and transmitted in the same manner as any other DNA sequences within the plant. Papaya plants pass their genes to their progeny by sexual reproduction that involves self pollination, or pollination of other papaya plants or sexually compatible relatives.

The 'Sunset' papaya lines 55-1 and 63-1 were produced using a microprojectile bombardment protocol to transform papaya with genes designed to confer resistance to PRV. The PRV CP gene that confers this resistance was derived from a strain of the virus originally isolated from infected papayas growing in Hawaii. Expression of this PRV CP gene in the papaya does not cause plant disease, but rather confers resistance to infection by PRV.

The introduced DNA that encodes the CP gene also has accompanying DNA regulatory sequences that modulate the expression of the CP gene in the transgenic lines. The DNA regulatory sequences were derived from plant pathogenic organisms: the bacterium *A. tumefaciens*, cucumber mosaic virus (CMV), and cauliflower mosaic virus (CaMV). Although these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate. During characterization of the performance of the transgenic 'Sunset' papaya lines 55-1 and 63-1 in laboratory,

greenhouse, and field experiments, the plants exhibited the typical agronomic characteristics of the parent papaya cultivar, with the addition of resistance to PRV infection.

Potential for the Appearance of New Plant Viruses. As mentioned above, papaya was developed by engineering the viral coat protein gene of PRV into the 'Sunset' papaya cultivar, a plant which is frequently infected by PRV. As part of its analysis, APHIS evaluated whether the expression of this viral gene in these papayas might present some unusual circumstances that could lead to the appearance of new plant viruses.

In the course of the infection of a plant cell by more than a single type of virus, it is possible for some of the constituents of the viruses to become mismatched. Such occurrences can lead to recombination of the nucleic acid genome or a mixture of the protein subunits (called transcapsidation), which comprise the coat of the virus particle. It is theoretically possible for new plant viruses to arise in the papaya through the recombination or transencapsidation, and APHIS considered this issue carefully in making its determination. A technical discussion of this issue is found contained in the determination document appended to this EA. After careful consideration of the physical and biological properties of PRV, APHIS concludes that it is unlikely that new viruses will arise as a consequence of the widespread cultivation lines 55-1 and 63-1, because no other virus infects papayas in the United States.

Potential Increased Weediness of Papaya Lines 55-1 and 63-1 Relative to Traditionally Bred Papaya. APHIS evaluated whether the papaya itself is likely to present a plant pest risk as a weed pest. The parent plant in this petition, 'Sunset' papaya, is an agricultural crop plant that exhibits no appreciable weedy characteristics. None of the standard texts and lists of weeds indicate that papaya is regarded as a weed (Holm et al., 1979; Muenscher, 1980; Reed, 1970; Weed Science Society of America, 1992).

The relevant introduced trait, resistance to infection by PRV, is unlikely to make the papaya into a weed. Before PRV infection became a commercial limitation for papaya production, nonengineered 'Sunset' papaya cultivars were not considered as weeds. Thus, there is no indication that resistance to PRV will result in papaya becoming a weed pest (see the appended determination document).

No other attributes of the transgenic papaya lines 55-1 and 63-1 suggest that they are any more "weedy" than papaya cultivars that are the result of traditional breeding. The transgenic 'Sunset' papaya lines 55-1 and 63-1 have retained the agronomic characteristics of the parental 'Sunset' papaya.

Potential Impacts on the Free-Living Relatives of Papaya Arising From Pollination by 'Sunset' Papaya Lines 55-1 and 63-1. APHIS evaluated the potential impacts that papaya lines 55-1 and 63-1 might have on the free-living, sexually compatible relatives of papaya. *C. papaya* is usually described as sexually incompatible with other member of the genus. Initial steps have been taken to develop methods for somatic hybridization of *C. papaya* with *C. stipulata* (Litz and Conover, 1979; Litz and Conover, 1980) and with *C. pubescens* (Jordan et al., 1986), but no hybrid plants have been regenerated to date. No *Carica* species is considered a weed, and there is no evidence in the scientific literature to suggest that susceptibility to PRV is the factor that prevents these plants from being weed pests. Therefore, it seems likely that even if the PRV-resistance trait could be transferred from line 55-1 or 63-1 to another *Carica* species, the resultant offspring would not be weed pests.

Potential Impacts on Nontarget Organisms, Including Beneficial Organisms Such as Bees and Earthworms. APHIS considered the potential impact that papaya lines 55-1 and 63-1 might exert either directly or indirectly on organisms that are recognized as beneficial to agriculture. APHIS concludes that there is no reason to believe that the unconfined growth of papaya will pose any deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. The coat protein expressed in papaya is not known to have any toxic properties. In fact, this viral coat protein is routinely ingested by virtually all animals, including humans, when papaya is consumed. Naturally occurring infections of susceptible papaya varieties result in concentrations of coat proteins far higher than those that occur in the tissues of the transgenic papaya lines 55-1 and 63-1 (see the determination document).

APHIS believes that the transgenic papaya lines will have no deleterious effects on organisms recognized as beneficial to agriculture (e.g., earthworms, honeybees). In addition, there is no reason to believe that the presence of these transgenic papaya lines would have any adverse effect on other organisms, including any species recognized as threatened or endangered in the United States.

Potential Impact on Processed Agricultural Commodities. Consistent with its statutory authority which defines plant pests as those organisms which cause direct or indirect damage to plants and plant products, APHIS evaluated whether papaya lines 55-1 and 63-1 might indirectly harm plant products such as some agricultural commodities. Analysis of the components and processing characteristics of 'Sunset' papaya lines 55-1 and 63-1 lines reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

VI. CONCLUSIONS

APHIS has evaluated information from the scientific literature as well as data submitted by Cornell University and the University of Hawaii that characterize the papaya lines 55-1 and 63-1. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that papaya lines 55-1 and 63-1 would no longer be regulated articles under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of removing the transgenic 'Sunset' papaya lines 55-1 and 63-1 from its regulation and reached the following conclusions:

1. The transgenic 'Sunset' papaya lines 55-1 and 63-1 exhibit no plant pathogenic properties. Although plant pathogenic organisms were used in the development of these papaya lines, these papaya plants are not infected with PRV, nor can they incite disease in other plants.

2. Cultivation of the transgenic papaya lines 55-1 and 63-1 will not increase the likelihood of the emergence of new plant viruses. In assessing the potential for new plant viruses to appear, APHIS has carefully considered the biology and epidemiology of the plant viruses that infect papaya, and has determined that the unconfined cultivation of these transgenic papaya lines would be no different than nontransgenic, PRV-infected papayas.

3. The transgenic 'Sunset' papaya lines 55-1 and 63-1 are no more likely to become weeds than any other types of papaya. Papaya is not considered to be a weed pest, and there is no reason to believe that the ability of these papayas to resist infection by PRV will lead to them becoming weed pests.

4. The transgenic 'Sunset' papaya lines 55-1 and 63-1 will not increase the weediness potential of any other cultivated plant or native wild species with which they can interbreed. Because no *Carica* species is considered a weed, transfer of the PRV-resistance trait to any other *Carica* species, although unlikely, would not result in the resulting offspring becoming a weed.

5. The transgenic papaya lines 55-1 and 63-1 will not harm threatened or endangered species or other organisms, such as bees, which are beneficial to agriculture.

6. The transgenic 'Sunset' papaya lines 55-1 and 63-1 will not cause damage to processed agricultural commodities.

APHIS has also concluded that any new papaya varieties bred from transgenic papaya lines 55-1 and 63-1 should not exhibit new plant pest properties, i.e., properties substantially different from those observed for the papaya lines already field tested, or those observed for papayas in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that the transgenic 'Sunset' papaya lines 55-1 and 63-1 will be just as safe to grow as papaya cultivars developed through traditional breeding practices. The cultivation of these transgenic papaya lines should present environmental impacts that are no different from the impacts associated with other papaya varieties that are not subject to regulation under 7 CFR Part 340 before they enter agriculture. Based upon the analysis documented in this EA, APHIS has reached a finding of no significant impact on the environment from its determination that the transgenic 'Sunset' papaya lines 55-1 and 63-1 will no longer be considered regulated articles under the regulations in 7 CFR Part 340.

VII. LITERATURE CITED

Holm, L., Pancho, J.V., Herbarger, J.P., and Plucknett, D.L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Muenscher, W.C. 1980. Weeds (2d ed.). Cornell University Press, Ithaca and London. 586 pp.

Reed, C. F. 1970. Selected Weeds of the United States. Agriculture Handbook No. 366. Agricultural Research Service, U.S. Department of Agriculture, Washington, DC. 463 pp.

Weed Science Society of America. 1992. Composite List of Weeds. WSSA. Champaign, Illinois.

VIII. PREPARERS AND REVIEWERS

Biotechnology, Biologics, and Environmental Protection

John Payne, Ph.D., Acting Director

Biotechnology Permits

Arnold Foudin, Ph.D., Deputy Director
Subhash Gupta, Ph.D., Biotechnologist
David S. Heron, Ph.D., Biotechnologist (petition coordinator)
Susan M. Koehler, Ph.D., Biotechnologist
James Lackey, Ph.D., Biological Safety Officer
Vedpal S. Malik, Ph.D., Biotechnologist
Henry K. Reding, Ph.D., Biotechnologist (reviewer)
Sivramiah Shantharam, Ph.D., Team Leader, Microorganisms Branch
James L. White, Ph.D., Team Leader, Plants Branch (reviewer)

Biotechnology Coordination and Technical Assistance

Michael A. Lidsky, J.D., LL.M., Deputy Director
L. Val Giddings, Ph.D., Team Leader, Science Policy Coordination Branch
Shirley P. Ingebritsen, M.A., Regulatory Analyst
Michael Schechtman, Ph.D., Team Leader, Domestic Policy Branch
Frank Y. Tang, Ph.D., J.D., Biotechnologist

Environmental Analysis and Documentation

Carl Bausch, J.D., Deputy Director

IX. AGENCY CONTACT

Ms. Kay Peterson, Regulatory Analyst
Biotechnology Permits, Unit 147
4700 River Road
Riverdale, MD 20737-1237

Phone: (301) 734-7601 Fax: (301) 734-8669

USDA-APHIS Response to Cornell University and the University of Hawaii Petition 96-051-01p for a Determination of Nonregulated Status for 'Sunset' Papaya Lines 55-1 and 63-1

United States Department of Agriculture,
Animal and Plant Health Inspection Service,
Biotechnology, Biologics, and Environmental Protection

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I. SUMMARY

Based on a review of scientific data and public comments, the Animal and Plant Health Inspection Service (APHIS) has determined that the genetically engineered, papaya ringspot virus resistant papaya (*Carica papaya*) lines 55-1 and 63-1 developed by the Cornell University and the University of Hawaii do not present a plant pest risk and are therefore no longer regulated articles under the regulations found at 7 CFR Part 340.6. As a result of this determination, permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of the subject papaya lines or their progeny.

This determination by APHIS has been made in response to a petition received from Cornell University and the University of Hawaii on February 20, 1996. The petition requested a determination from APHIS that transgenic 'Sunset' papaya lines 55-1 and 63-1 should not be considered as regulated articles because they do not present a plant pest risk.

The 'Sunset' papaya lines 55-1 and 63-1, as defined by the developers, Cornell University and the University of Hawaii, have been designed to resist infection by papaya ringspot virus (PRV). Papayas of the cultivar 'Sunset' were modified with a genetic construct that includes the coat protein of the PRV strain HA 5-1. Expression of this coat protein (CP) gene in the transgenic papaya plants does not cause plant disease, but rather confers resistance to infection by PRV. In addition to the PRV CP gene, the genetic construct used in the transformation process included two selectable marker genes, *nptII* and *uidA* (*gus*), that are designed to be expressed in the transformed plant (Line 55-1 contains both *nptII* and *gus*, whereas line 63-1 contains *nptII*). Expression of the PRV CP gene, *nptII*, and *gus* in the papayas is regulated by accompanying DNA regulatory sequences derived from the plant pathogens *Agrobacterium tumefaciens*, cauliflower mosaic virus (CaMV), and cucumber mosaic virus (CMV). Although these DNA sequences were derived from plant pathogens, the sequences can not cause plant disease by themselves nor in conjunction with the genes that they regulate in these papaya lines.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA) (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA) (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations 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 should no longer be regulated. The 'Sunset' papaya lines 55-1 and 63-1 have been considered "regulated articles" under Part 340 of the regulations in part because they have been engineered with a CP gene derived from

PRV. As regulated articles, field tests of these papayas from 1991 to 1996 were done under APHIS permits which stipulated reproductive confinement.

After reviewing the available scientific information and public comments on the petition, APHIS has determined that the 'Sunset' papaya lines 55-1 and 63-1 lines do not pose either a direct or indirect plant pest risk and, therefore, will no longer be considered as regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of transgenic 'Sunset' papaya or their progeny. (Importation of 'Sunset' papaya lines 55-1 and 63-1 [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) This determination has been made based on an analysis that revealed that the transgenic 'Sunset' papaya lines 55-1 and 63-1: (1) exhibit no plant pathogenic properties; (2) should not increase the likelihood of the emergence of new plant viruses; (3) are no more likely to become weeds than a virus-resistant papaya developed by traditional breeding techniques; (4) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which they can interbreed; (5) are unlikely to harm other organisms that are beneficial to agriculture, such as bees, and (6) should not cause damage to processed agricultural commodities. APHIS has also concluded that there is no reason to believe that new progeny papaya varieties bred from lines 55-1 and 63-1 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the 'Sunset' papaya lines already field tested, or those observed for papaya derived in traditional breeding - programs.

II. BACKGROUND

APHIS Regulatory Authority. APHIS regulations found at 7 CFR Part 340 (hereafter referred to as the regulations) were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA)(7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended. The regulations pertain to the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

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 there is reason to believe that it is a plant pest. The transgenic 'Sunset' papaya lines 55-1 and 63-1 have been considered "regulated articles" under Part 340 of the regulations because they have been engineered with a coat protein gene from a

strain of PRV and certain additional DNA sequences derived from the plant pathogens CaMV, CMV, and *A. tumefaciens*.

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted information and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. As a consequence of such a determination, APHIS permits would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant a pest or that the plants or genes are pathogenic. The regulations instead have the premise that when plants are developed using biological vectors or material from pathogenic sources, or when pathogens are used as vector agents, they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). APHIS performs a review that allows a verification of the biology and procedures used; assesses the degree of uncertainty and familiarity; and allows the identification of any hazards, should they be present and predictable. The overall aims of APHIS' regulations in the Code of Federal Regulations at 7 CFR Part 340 are to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight, and to enable any issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms to allow for the safe utilization of the technology in agriculture.

A certification that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage either when grown in the field, or when stored, sold, or processed. APHIS' approach to plant pest risk is considerably broader than a narrow definition that encompasses only plant pathogens. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) should not increase the likelihood of the emergence of new plant viruses; (3) is no more likely to become a weed than a virus-resistant plant developed by traditional breeding techniques; (4) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (5) is unlikely to harm other organisms, such as bees, which are beneficial to agriculture and (6) should not cause damage to processed agricultural commodities. Evidence has been presented by Cornell University and the University of Hawaii that bears on these topics. In addition, the current petition seeks a determination regarding the regulated status of any future papaya lines derived from traditional crosses of in which 55-1 or 63-1 is a parent line.

APHIS' decision on the regulatory status of the transgenic 'Sunset' papaya under the regulations at 7 CFR 340, does not release these papaya lines or their progeny from EPA and FDA regulatory oversight.

Regulatory Oversight by Other Federal Agencies. The Environmental Protection Agency (EPA) regulates the use of pesticide chemicals in the environment. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*), the EPA has the authority to regulate the development, sale, distribution, use, storage, and disposal of pesticides. The EPA has published its proposed rule for plant pesticides including an proposed exemption for viral CP produced in plants (59 FR 60495-60547). Their draft proposal has been the subject of three scientific advisory meetings. The material presented at these meetings is available from the EPA's Office of Pesticide Program's Public docket. The proposed exemption of viral CP was supported by EPA's scientific advisory panel.

The USDA Food Safety Inspection Service (FSIS) is responsible for regulation of genetically engineered meat and poultry products (59 FR 12582-83; 56 FR 67054-55). Food safety in the United States, for products other than meat and poultry, is assured by regulation by the Food and Drug Administration (FDA) under the authority of the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 201 et seq.). The FDA's policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Regulatory oversight for the safety of any food or feed products derived from papaya lines 55-1 and 63-1 is under the jurisdiction of the FDA, shared with the EPA when pesticides are involved. Under the FFDCA, the EPA has responsibility for establishing tolerances or exemptions from the requirement of tolerance for pesticide residues on food or feeds, including viral CP.

III. PUBLIC COMMENTS

On May 3, 1996, APHIS published a notice in the Federal Register (61 F.R. 19904-19905, Docket No. 96-024-1) announcing that the petition from Cornell University and the University of Hawaii had been received and was available for public review. In the notice, APHIS solicited written comments from the public as to whether the papaya lines 55-1 and 63-1 posed a plant pest risk. The comments were to have been received by APHIS on or before July 2, 1996. During the designated 60-day comment period, APHIS received a total of 18 comments on the petition from universities, papaya growers and processors, a papaya industry association, an office of cooperative extension service, and a state department of agriculture. All comments were favorable to the petition.

IV. DESCRIPTION OF 'SUNSET' PAPAYA LINES 55-1 AND 63-1

Papaya as a Crop. The papaya is a small, tree-like herbaceous plant that is widely grown in the tropics for its edible fruits. The fruits are usually consumed fresh and locally, or sometimes processed or pressed into a beverage. Latex from various plant parts yields the proteolytic enzyme, papain, which is used as a meat tenderizer. Commercial papaya production in the United States occurs primarily in Hawaii, and secondarily in Puerto Rico and southern Florida (Jewell, 1989; McGregor, 1976).

Taxonomy of Papaya. The Caricaceae is a family of tropical dicot species which encompasses the genera *Carica*, *Jacaratia*, *Cylicomorpha*, and *Jarilla* (*Mocinna*). The papaya (*Carica papaya* L.) is the best known of the 45 species within the genus *Carica* (Willis, 1973). Papaya is native to Central America and is known only as a cultivated species. The phylogenetic origin of *C. papaya* is uncertain. Some botanists have suggested that the species arose from the hybridization of wild relative species (Cobley, 1976), whereas others disagree with this hypothesis (Storey, 1976). Other species of *Carica*, as well as the species of the three remaining genera of the Caricaceae, have little commercial importance and are generally unknown except as horticultural novelties and botanical subjects (Neal, 1965).

Reproduction and Pollination of Papaya. Papaya is a polygamous species, having a mating system that is either dioecious (staminate and pistillate plants) or gynodioecious (hermaphrodite and pistillate plants). Commercially, gynodioecious lines are generally preferred because of their potential for inbreeding and consequent uniformity. Hermaphrodites can be self-pollinated to homozygosity, except for sex characters, yielding gynodioecious lines that segregate in a ratio of 2 hermaphrodites to 1 female. In most Hawaiian hermaphrodites, the position of the anthers relative to the stigma is such that self-pollination occurs at anthesis without

manual assistance or bagging. A low out-crossing rate occurs since hermaphrodites tend to be andromonoecious, producing copious pollen from staminate flowers during most of the year. Pistillate plants never produce anthers and are consequently obligate out-crossers. Each pollination produces hundreds of seeds, which are easily recovered from ripe fruits. In nature, pollination occurs through bees, butterflies, and wind.

Papaya (*Carica papaya* L.) is the best known member of the Caricaceae, a small dicotyledonous family consisting of four genera (Badillo, 1971). *Carica* is the largest genus with 23 described species with overlapping distributions in the foothills of the Andes Mountains in northwestern South America, although members of the genus range from southern Brazil, Argentina and Chile to southern Mexico. Most likely, the center of origin for papaya is the Caribbean coast of Central America (Storey, 1976).

Papaya is a common plant of the lowland tropics where it is grown in gardens and dooryards. It is consumed primarily as a fresh dessert fruit, and it provides a good source of vitamins A and C (de Arriola et al., 1980). Papaya is a perennial with a short juvenile period averaging about six months. The first fruits mature about one year after plantings and then continue to bear fruit more or less continuously. After three to four years, the yield declines and the trees become too tall for efficient harvesting. Thus a new crop has to be planted.

The 'Sunset' variety (the parental variety used for producing the subject transgenic lines) is a gynodioecious line of commercial importance in Hawaii. It has a yellow-fleshed fruit, and approximately 2,250 acres were being harvested in Hawaii in 1990. All Hawaiian cultivars, including 'Sunset' are highly susceptible to PRV.

Biology of the Papaya Ringspot Virus (PRV). PRV (Murphy et al., 1995) is a member of the potyvirus group which consists of about 160 members. The potyvirus group is the largest and most economically important group of plant viruses (Matthews, 1991).

Viruses of the potyvirus group are flexuous rods comprised of a positive-sense RNA genome surrounded by a protein coat (capsid). The protein coat is comprised of repeating subunits of the virus coat protein (CP). The RNA genome is translated in the infected host (plant) cell to yield a polyprotein which is subsequently cleaved into at least a dozen proteins, one of which is the viral CP. Thus, during the infection process, the virus uses the host cell to make copies of the viral genome as well as the viral proteins. Then the viral RNA and CP subunits self-assemble into complete virus particles (virions) which may spread within the plant or to other plants by insect or mechanical transmission. The genome of potyviruses encodes approximately a dozen proteins, some whose functions have not been identified completely. Some proteins encode several diverse functions. Some of the

identified functions include: coat protein, nuclear inclusion body, cylindrical inclusion body, helper component involved in insect transmission, helicase, several proteases, replicase, genome-linked protein, and ATPase.

Potyviruses are easily transmitted mechanically to a relatively narrow range of hosts. The great majority are transmitted by many aphid species in a nonpersistent manner. Viruses transmitted in a nonpersistent fashion by aphids survive in their vectors for only short periods, less than the survival time of the virus in untreated leaf extracts. In nonpersistent transmission the virus is picked up by the insect after a brief feeding on the infected plant and can be transmitted to one or only a few plants immediately and up to several hours. The differences in vector specificity between individual viruses within a group, or even between biotypes of the same virus, imply a mechanism of virus-vector association that goes beyond the general biological properties of the virus and its vector (Harrison and Murant, 1984). There is evidence that two types of virus-encoded proteins, a noncapsid protein (helper component) and the viral CP, play key roles in potyvirus transmission and vector specificity. A role of the HC in aphid transmission was demonstrated when, upon purification, potyviruses lose their aphid transmissibility, although they are still highly infectious when assayed by mechanical inoculation (Pirone and Megahed, 1966). Aphid transmissibility can be restored by the addition of HC which can be extracted from potyvirus infected plants but not healthy plants (Govier and Kassanis, 1974a,b). The way in which helper component makes aphid transmission possible has not been established. Aphids must acquire HC either prior to, or along with, the virus; if they are fed first on virus and then on a source of HC, transmission does not occur (Govier and Kassanis, 1974a,b). This suggests that HC makes it possible for the virus to attach to sites within the aphid in a way that allows it to be transmitted or protects the virus from adverse conditions in the aphid's alimentary tract (Pirone and Thornbury, 1984). Although HC appears essential for aphid transmission of potyviruses, its presence does not guarantee transmission. Recent experiments by Atreya et al. (1991) have demonstrated that certain amino-acid substitutions at specific positions in the N terminus of tobacco vein mottling potyvirus coat protein can reduce or abolish aphid transmissibility of the virus. Lecoq et al. (1991) provide evidence that the lack of aphid transmission of two isolates of the potyvirus zucchini yellow mosaic virus (ZYMV) is due to deficiencies in the HC for ZYMV-PAT and in the CP for ZYMV-NAT.

PRV has been divided into two types: PRV-p type isolates which infect papaya, and PRV-w type isolates which infect watermelon (PRV-w type isolates were previously classed as watermelon mosaic virus, type 1). PRV-p infections of papaya are a major limiting factor in papaya production in Hawaii and other regions where papayas are grown. Attempts to breed for papaya types resistant to PRV-infection have been largely unsuccessful.

Rationale for Development of 'Sunset' papaya lines 55-1 and 63-1. PRV is the most important papaya pathogen and a major limiting factor in commercial papaya production in the world (Gonsalves, 1994). All major production areas in the Western Hemisphere [Brazil, the Caribbean region, Mexico, and USA (Florida and Hawaii)] and Eastern Hemisphere (the Philippines, Taiwan, Thailand, and China) are affected, and the virus is still invading new areas (Hawaii, Israel, Malaysia and Australia) (Gonsalves and Manshardt, Petition 96-051-01p, 1996). Once introduced, PRV has never been successfully eradicated from any region (Gonsalves and Manshardt, Petition 96-051-01p, 1996). Once introduced to a locality, PRV becomes established in weeds or perennial plants and the aphid vectors involved in dissemination of PRV are found worldwide. Thus, even if new PRV-free papaya trees are planted in an area, they soon become infected.

Attempted control measures have met with marginal success. These have included the use of insecticides against insect vectors (aphids), the removal and destruction of diseased plants, and implementation of quarantine regulations to restrict plant movement (Purcifull et al., 1984; Shukla et al., 1994). Other attempts at controlling PRV include cross protection with a mild strain of PRV. Cross protection is very labor intensive, requiring inoculation of each plant with a mild strain of PRV to confer temporary resistance. Plants protected from infection by severe strains, however, still exhibit mild symptoms on that result in a yield reduction of 10-20% (Mau et al., 1989).

Development of 'Sunset' papaya lines 55-1 and 63-1. Transgenic 'Sunset' papaya lines 55-1 and 63-1 were transformed with a genetic construct which includes the coat protein gene from PRV strain HA 5-1, a mild mutant strain (Yeh and Gonsalves, 1994). Strain HA 5-1 was produced by nitrous acid treatment of the severe PRV strain HA which was isolated from papaya in Hawaii (Gonsalves and Ishii, 1980). The PRV CP gene encodes a 36 kDalton protein.

The transgenic papaya lines 55-1 and 63-1 were derived following bombardment of embryogenic cultures of 'Sunset' papaya with tungsten particles coated with DNA of the plasmid pGA482GG/cpPRV-4 (Fitch et al., 1992). This plasmid contains three, plant-expressible coding regions or genes: the PRV CP gene, *nptII*, and *uidA* (also known colloquially as GUS). The plasmid also has two genes which encode tetracycline and gentamycin resistance, but their associated DNA regulatory sequences enable expression only in prokaryotes (bacteria). The plasmid includes the right- and left-border regions (designated as RB and LB, respectively) derived from the *A. tumefaciens* T-DNA. The RB and LB regions are believed to facilitate integration of the genetic construct into the plant DNA. Table 1 lists the genetic components with a brief description of each genetic component used in construction the transgenic papaya lines.

The PRV CP gene construct is comprised of the 35S promoter region derived from CaMV; the 5'-untranslated region, translation initiation codon, and first 39 nucleotides of the CMV CP gene; the PRV CP gene; and the 35S polyadenylation terminator sequences. Because PRV naturally encodes its CP as part of a polyprotein, the CP coding region lacks a 5'-untranslated region and a translation initiation codon. The construct developed for the transformation of the papayas provides these sequences and also preserves the proteolytic cleavage site, Q-S, at the N-terminus of the PRV CP.

The *nptII* gene encodes the enzyme neomycin phosphotransferase which confers resistance to the antibiotic kanamycin. The *nptII* gene was derived from the bacterium *Escherichia coli* then modified with noncoding DNA regulatory sequences that enable expression in plants. The promoter and terminator sequences were from the *nos* gene (nopaline synthase) of *A. tumefaciens*. The *nptII* portion of the genetic construct is a widely used selectable genetic marker in the development of transgenic plants because it enables researchers to select in the laboratory those plant tissues that have been successfully transformed.

The transformation construct utilized a second identifiable marker gene, a plant-expressible *uidA* gene (also referred to as GUS) which encodes the enzyme *beta*-glucuronidase. The *uidA* gene was derived from *E. coli* and modified for plant expression by the addition of 35S promoter region and the *nos* 3'-termination region. The beta-glucuronidase encoded by the *uidA* gene enables a colorimetric assay in the laboratory to identify plant tissues which contain and express the gene.

Transgenic components incorporated into 'Sunset' papaya lines 55-1 and 63-1.

Line 55-1. Southern blot analysis indicates that line 55-1 contains, besides the expected PRV CP gene, intact copies of two plant-expressible marker genes (*nptII* and *uidA*) and a partial copy of the tetracycline resistance marker gene. Genomic DNA did not hybridize with probes to the gentamicin marker genes or to the origin of bacterial replication (Ori V/Tet) region. In addition to being an incomplete copy, the tetracycline resistance gene's prokaryotic regulatory regions would preclude expression in the plant.

Line 63-1. Southern blot analysis indicates that line 63-1 contains intact, functional genes for the PRV CP and *nptII* genes. Unlike line 55-1, Line 63-1 apparently does not contain the *uidA* gene which encodes *beta*-glucuronidase. Genomic hybridization with probes to the gentamicin resistance gene and the Ori T/Tet region indicates that either all or part of the genes for gentamicin and tetracycline resistance have integrated into the papaya genome. However, it is unlikely that these genes are functional since their prokaryotic promoters do not drive expression of these genes in plants (Allmansgberger et al., 1985; An, 1986).

V. PLANT PEST ANALYSIS OF 'SUNSET' PAPAYA LINES 55-1 AND 63-1.

To reach its determination that the transgenic 'Sunset' papaya lines 55-1 and 63-1 do not present a plant pest risk, APHIS has addressed not only issues raised in public discussions about virus resistant plants and the movement of pest resistance genes to free-living plants, but also considered basic information on the biology of papaya and data presented by Cornell University and the University of Hawaii or otherwise available to APHIS that are relevant to consideration of plant pest risk. Based on the data described, APHIS has arrived at a series of additional conclusions regarding the properties of 'Sunset' papaya lines 55-1 and 63-1.

The Introduced Genes, Their Products, and the Added Regulatory Sequences Controlling Their Expression Do Not Present a Plant Pest Risk in 'Sunset' Papaya Lines 55-1 and 63-1. Although some DNA sequences used in the transformation process were derived from bacterial and viral plant pathogens, these genes do not cause disease in the papaya plant. Once inserted into the genome of the papaya plant, the introduced DNA sequences are maintained and transmitted in the same manner as any other DNA sequences within the plant. Papaya plants pass their genes to their progeny by sexual reproduction that involves self pollination, or - pollination of other papaya plants or sexually compatible relatives.

'Sunset' papaya lines 55-1 and 63-1 were produced using a microprojectile bombardment protocol to transform papaya with genes designed to confer resistance to PRV. The gene that confers this resistance was derived from a coat protein (CP) gene of a mild strain (HA 5-1). Expression of this CP gene in the papaya does not cause plant disease, but rather confers resistance to infection by PRV.

The introduced DNA that encodes the CP gene also has accompanying DNA regulatory sequences that modulate the expression of the CP genes. The DNA regulatory sequences were derived from plant pathogenic organisms: the bacterium *A. tumefaciens*, CMV, and CaMV. Specifically, the DNA regulatory sequences associated with the viral CP coding regions comprise promoter and transcriptional termination sequences. Although these regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or with the genes that they regulate. During characterization of the performance of transgenic 'Sunset' papaya lines 55-1 and 63-1 in laboratory, greenhouse, and field experiments, the plants exhibited the typical agronomic characteristics of the parent papaya, with the addition of resistance to PRV infection. In APHIS' opinion, the components and processing characteristics of 'Sunset' papaya lines 55-1 and 63-1 lines reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. These transgenic papaya plants have no plant pest characteristics.

The Transgenic 'Sunset' Papaya Lines 55-1 and 63-1 Should Not Increase the Likelihood of the Emergence of New Plant Viruses. APHIS has considered the known physical and biological properties of PRV and its interactions with both its insect vectors and its host plant, papaya. PRV and the aphids that serve as vectors are widely prevalent in areas of the United States where papayas are grown. Indeed, PRV and its aphid vectors are found worldwide where papayas are grown. Based on the known physical and biological properties of PRV, the likelihood of the appearance of masked plant viruses or a new plant virus with novel biological properties through field cultivation of transgenic PRV-resistant 'Sunset' papaya lines 55-1 and 63-1 plants is no greater than the likelihood of novel viruses arising in PRV-infected papaya cultivars derived through traditional plant breeding practices.

Similar issues were addressed previously when APHIS made a determination on the regulated status of a transgenic line of virus resistant squash. The squash line, ZW-20, was genetically engineered with CP genes from two potyviruses, zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus, type 2 (WMV2) (see USDA/APHIS document "Response to the Asgrow Seed Company Petition 95-352-01p for Determination of Nonregulated Status for ZW-20 Squash").

Transcapsidation. When a single plant cell is simultaneously infected by two different strains of a virus (or two viruses), it may be possible for the genome of one virus to become encapsidated by coat protein of the second virus. If the virus is encapsidated by coat proteins of both viral strains, the phenomenon is called phenotypic mixing (mixed encapsidation). If the virus is encapsidated by only one of the coat proteins, it is termed genomic masking or transcapsidation (for simplicity, it will be assumed that the terms transcapsidation and genomic masking include the phenotypic mixing phenomenon since the issues for all are identical). Transcapsidation has been reported to be important in only a few instances in field situations in insect transmission of viruses (Falk et al., 1995), even though field grown plants and trees are known often to be infected with multiple viruses (Abdalla et al., 1985; Falk and Bruening, 1994). Transcapsidation has been best studied with infections with different strains of the barley yellow dwarf virus (a luteovirus), where the phenomenon can be important in field situations, in that coat protein determines which specific aphid vector transmits the virus (Matthews, 1991). This phenomenon has also been detected with potyviruses (Bourdin and Lecoq, 1991; Lecoq et al., 1993). The result of transcapsidation, a "masked" virion, has a mismatched coat that may or may not be sufficiently functional to allow transmission of the viral genome it contains to another host plant. The "mismatched" or heterologous viral coat is not maintained in subsequent rounds of viral infection, because subsequent production of coat protein subunits is directed by the viral coat protein gene carried in the genome. Therefore, transcapsidation events are transient and any potential impacts can only persist with the first round of infection of the masked virus if it infects a susceptible host plant. As stated previously, for potyviruses including PRV the CP is the secondary determinant of

aphid transmissibility. Whether a masked virus is transmitted would be mainly determined on whether the virus had a functional helper component.

The likelihood of effective transcapsidation occurring between products of the CP gene and the genomes of infective viruses is greater if the invading virus is a related potyvirus. In Hawaii, PRV is the only potyvirus and the only reported virus known to infect papaya. APHIS notes that, elsewhere in the world, other viruses have been reported to infect papaya, including papaya mosaic potexvirus, papaya leaf curl geminivirus, and papaya leaf distortion mosaic potyvirus. The latter is found in Japan (Maoka et al., 1995). Thus, APHIS believes that likelihood of transcapsidation occurring in lines 55-1 and 63-1 when grown in the United States is highly improbable because no other virus is likely infect these lines. Even in the remote possibility that transcapsidation could occur with a potyvirus that may be introduced into the United States, the amount of PRV CP produced by the transgene in these two lines is less than the amount of CP produced in nontransgenic papayas that are naturally infected with PRV. It is also unlikely that there will be any other novel interactions with the PRV CP expressed inlines 55-1 and 63-1, because the protein expressed by the PRV CP transgene in the transgenic lines is expressed in the same types of tissues where PRV normally replicates and produces its CP when it infects susceptible papayas.

Coat protein and the movement of subliminally infecting viruses. The movement of a virus from the initial site of infection throughout a plant, called systemic infection, requires expression of one or more viral genes (a dedicated movement protein, coat protein, and/or other viral proteins), and a permissible host plant (Hull, 1989; Maule, 1991; Dawson et al., 1988; Dolja et al., 1995; Cronin et al., 1995). If a virus is unable to move from the initial site of infection, these infections are called subliminal. In a limited number of cases, viruses that cause subliminal infections in a host species may no longer be restricted when the host is infected by a second virus. At least two genes, the coat protein and the helper component/protease genes are involved in intracellular movement of potyviruses (Dolja et al., 1995; Cronin et al., 1995). If the coat protein expressed in the transgenic plant can facilitate the movement of viruses that cause subliminal infections, this would be a significant concern only if that CP was from a virus that rarely or never infects the recipient host plant. If CP is derived from a virus that is widely prevalent in the recipient plant, there would be no novel interactions with subliminally-infecting viruses. With 'Sunset' lines 55-1 and 63-1, if the CP transgenes facilitated the movement of subliminally infecting viruses, the only impact would be diseased 'Sunset' 55-1 and 63-1 plants. Whether the virus whose movement was facilitated could move from 'Sunset' lines 55-1 and 63-1 to other host plants would depend on its mode of transmission. Since the CP transgene in 'Sunset' papaya lines 55-1 and 63-1 is from a viral strain that routinely infects papaya, it is not expected subliminally infecting viruses will present a problem any more serious than can occur in naturally infected papaya plants.

Recombination. Recombination is defined as an exchange of nucleotide sequences between two nucleic acid molecules. Recombination between viral genomes results in heritable, permanent change. The persistence of a recombined viral genome will depend upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of parental viruses, its ability to spread systemically within the host, or its successful transmission to other host plants. As stated above, there are no other known viruses that infect papaya besides PRV. Thus, the likelihood of recombination between the viral transgene and another virus is virtually nil. The amount of PRV CP and its mRNA in the transgenic papaya is lower than in naturally infected non-transgenic plants. Thus, even if another virus was introduced into the United States and infected the papaya plants, the likelihood of recombination is no greater with the transgenic plants that would occur in naturally PRV-infected papaya.

Synergy. Occasionally, when two viruses simultaneously **naturally** infect a plant, the symptoms can be more severe than when either of the viruses infects the plant singly. This phenomenon is called synergy (Matthews, 1991). First, there is no evidence to suggest that potyviral CPs are involved in synergy. Second, there are no reports of other viruses infecting papayas in the United States. Even in the unlikely event that plants of lines 55-1 or 63-1 became infected with another virus, any potential synergistic effects would be limited to the growing cycle or season and have no long term environmental impact (AIBS, 1995).

The Transgenic 'Sunset' Papaya Lines 55-1 and 63-1 Are No More Likely to Become Weed Pests Than the Nontransgenic Parent. A study (National Research Council, 1989) entitled "Field Testing Genetically Modified Organisms: Framework for Decisions," identified the potential to inadvertently produce a new weed or increase the aggressiveness of existing weeds as "perhaps the single most commonly voiced concern about the introduction of genetically modified plants." In their summary in the chapter on weediness, the authors conclude, "However, genetically modified crops are not known to have become weedy through the addition of traits such as herbicide and pest resistance". APHIS could not find any current research that would contradict the Council's conclusion.

Papaya is not listed as a weed in the Federal Noxious Weed Act (7 U.S.C. 2801-2813) and is not reported by the Weed Society of America to be a common or troublesome weed anywhere in the United States (Bridges and Bagman, 1992; Hold *et al.* 1979; Muenscher 1980). Papaya was not considered a weed in Hawaii before PRV became a limitation to commercial production; therefore, it seems unlikely that resistance to PRV through genetic engineering would result in weediness.

Hybrids Between Transgenic 'Sunset' Papaya Lines 55-1 and 63-1 and Other *Carica* Species Are Unlikely to Persist in the Environment and Become Weeds. The genus

Carica includes 22 species of herbaceous, tree-like dicots (Badillo, 1971). Only *C. papaya* is important economically. Resistance to PRV has been reported in *C. candicans*, *C. cauliflora*, *C. pubescens*, *C. quercifolia*, *C. stipulata*, and *C. x heilbomii nm. pentagona* ('babaco') (Conover, 1964; Horovitz and Jimenez, 1967). *C. papaya* is usually described as sexually incompatible with other member of the genus. Initial steps have been taken to develop methods for somatic hybridization of *C. papaya* with *C. stipulata* (Litz and Conover, 1979, 1980) and with *C. pubescens*, (Jordan et al., 1986) but no hybrid plants have been regenerated to date. No *Carica* species is considered a weed, and there is no evidence in the scientific literature to suggest that susceptibility to PRV is the factor that prevents these plants from being weed pests. Therefore, it seems likely that even if the PRV-resistance trait could be transferred from line 55-1 or 63-1 to another *Carica* species, the resultant offspring would not be weed pests.

The 'Sunset' Papaya Lines 55-1 and 63-1 Should Not Be Harmful to Beneficial Organisms, Including Bees. There is no reason to believe deleterious effects on beneficial organisms could result specifically from the cultivation of 'Sunset' papaya lines 55-1 and 63-1. No direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis toward beneficial organisms, such as bees and earthworms, were identified for 'Sunset' papaya lines 55-1 and 63-1. APHIS also cannot envision any plausible mechanisms for any hypothetical deleterious effect since the levels of PRV CP expressed in lines 55-1 and 63-1 is already present in relatively high concentrations in papaya plants naturally infected with PRV.

APHIS' determination regarding the regulated status of transgenic 'Sunset' papaya lines 55-1 and 63-1 encompasses not only papaya lines that already have been field tested, but also any new papaya lines that may be produced through conventional breeding using transgenic 'Sunset' papaya lines 55-1 and 63-1 as one or both parents. APHIS believes that the analysis applied to 'Sunset' papaya lines 55-1 and 63-1 already field tested will apply equally well to these new papaya lines, and that the data provided by Cornell University and the University of Hawaii justifies the conclusion that such new 'Sunset' papaya lines 55-1 and 63-1 will not present a plant pest risk. The variation in agronomic characteristics among the 'Sunset' papaya lines 55-1 and 63-1 lines that have been field tested does not differ significantly from that seen in commercial cultivars of papaya that have never been considered regulated articles. While it is impossible to predict the exact agronomic characteristics of the progeny of a cross between a 'Sunset' papaya lines 55-1 and 63-1 and another papaya cultivar, cross-breeding between well-characterized papaya varieties is the traditional means by which new and improved papaya varieties are created. These crosses have often used as one-parent papaya cultivars that are considerably more genetically different from standard commercial cultivars than are 'Sunset' papaya lines 55-1 and 63-1.

The 'Sunset' Papaya Lines 55-1 and 63-1 Should Not Cause Damage to Processed Agricultural Commodities. APHIS can find no reason to believe that these PRV resistant papaya plants would adversely affect processed agricultural commodities. This conclusion is based upon the nature of papayas and their use as fresh fruit or in food processing. Most papaya fruit is consumed when ripe as a fresh dessert fruit. In southeast Asia, immature fruits are grated to produce a salad. The papaya latex, which contains the proteolytic enzyme papain, is frequently stewed with meat as a tenderizer. Papain is also extracted for commercial production. There is no reason to believe that the addition of PRV resistance would alter these properties.

VI. CONCLUSION

In response to a petition from The University of Hawaii and Cornell University, APHIS has evaluated information regarding the potential plant pest risks presented by the transgenic papaya lines designated as 'Sunset' line 55-1 and 63-1. The 'Sunset' papaya lines 55-1 and 63-1 lines have been transformed via a microprojectile bombardment protocol with a genetic construct that includes the PRV CP gene. Expression of the PRV CP gene in 'Sunset' papaya lines 55-1 and 63-1 does not cause plant disease, but rather confers resistance to infection by PRV. APHIS has determined that the 'Sunset' papaya lines 55-1 and 63-1 do not pose a direct or indirect plant pest risk and therefore will no longer be considered as regulated articles under APHIS regulations found at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those papaya lines or their progeny. (Importation of 'Sunset' papaya lines 55-1 and 63-1 [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the - Foreign Quarantine Notice regulations at 7 CFR Part 319.). This determination has been made based on an analysis that revealed that 'Sunset' papaya lines 55-1 and 63-1: (1) exhibit no plant pathogenic properties; (2) should not increase the likelihood of the emergence of new plant viruses; (3) are no more likely to become weed pests than a virus-resistant plant developed by traditional breeding techniques; (4) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (5) are unlikely to harm other organisms, such as bees, which are beneficial to agriculture, and (6) should not cause damage to processed agricultural commodities. APHIS has also concluded that there is a reasonable certainty that new progeny 'Sunset' papaya lines 55-1 and 63-1 varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the 'Sunset' papaya lines 55-1 and 63-1 already field tested, or those observed for papayas developed in traditional breeding programs.

John H. Payne

John H. Payne, Ph.D.
Acting Director Biotechnology,
Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date SEP - 5 1995

VII. REFERENCES

Abdalla, O. A., Desjardins, P. R., and Dodds, J. A. 1985. Survey of pepper - viruses in California by the ELISA technique. Phytopathology 75:1311.

Bourdin, D., and Lecoq, H. 1991. Evidence that heteroencapsidation between two potyviruses is involved in aphid transmission of a nonaphid transmissible isolate from mixed infection. Phytopathology 28.81:1459-1464.

Falk, B. W., Bruening, G. 1994. Will transgenic crops generate new viruses and new diseases? Science 263:1395-1396.

Falk, B. W., Passmore, B. K., Watson, M. T., Chin, L.-S. 1995. The specificity and significance of heterologous encapsidation of virus and virus-like RNA's. pp. 391-415. In: Biotechnology and Plant Protection: Viral pathogenesis and disease resistance. Bills, D. D. and Kung, S.-D. (eds.). World Scientific, Singapore.

Allmansberger, R., Brau, B., and Piepersberg, W. 1985. Genes for gentamycin(3)-N-acetyl-transferases III and IV. II. Nucleotide sequences of three AAC (3)-III genes and evolutionary aspects. Molecular and General Genetics 198: 514-520.

American Institute of Biological Sciences (AIBS). 1995. Transgenic virus-resistant plants and new plant viruses. Meeting report from AIBS workshop sponsored by U.S. Department of Agriculture. 47pp.

An, G. 1986. Development of plant promoter expression vectors and their use for analysis of differential activity of nopaline synthase promoter in transformed tobacco cells. Plant Physiology 81:86-91.

Arriola, M. d., Calzada, J., Menchu, J., Rolz, C., Garcia, R., and Cabrera, S. d. 1980. Papaya. In: NagyB S. and Shaw, P. (e, Is.), Tropical and subtropical fruits. AVI, Westport, pp, 316-340.

Atreya, P.L., Atreya, C.D., Pirone, T.P. 1991. Amino acid substitutions in the coat protein result in loss of insect transmissibility of a plant virus. Proceedings of the National Academy of Sciences. USA 88:7887-7891.

Badillo, V. 1971. Monographia de la familia Caricaceae. Maracay. Venezuela.

Bridges, D. C., and Baumann, P. A. 1992. Weeds causing losses in the United States. Weed Science Society of America. 404 pp.

Cobley, L. S. 1976. An Introduction to the Botany of Tropical Crops. Longman. London and New York. 371 pp.

Conover, R. A. 1964. Distortion ringspot, a severe virus disease of papaya in Florida. Proceedings of the Florida State Horticultural Society 77: 440-444.

Cronin, S., Verchot, J., Halderman-Cahill, R., Schaad, M. C., Carrington, J. C. 1995. Long-distance movement factor: A transport function of the potyvirus helper component. The Plant Cell 7:549-559.

Dawson, W. O., Bubrick, P., Grantham, G. L. 1988. Modification of tobacco mosaic virus coat protein gene affecting replication, movement, and symptomatology. Phytopathology 78:783-789.

Dolja, V. V., Halderman-Cahill, R., Montgomery, A. E., Vandenbosch, K. A., Carrington, J. C. 1995. Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. Virology 206:10007-1016.

Fitch, M. M. M., Manshardt, R. M., Gonsalves, D., Slightom, J. L., and Sanford, J. C. 1992. Virus resistant papaya derived from tissues bombarded with the coat protein gene of papaya ringspot virus. Bio/Technology 10: 1466-1472.

Gonsalves, D. and Ishii, I. 1980. Purification and serology of papaya ringspot virus. Phytopathology 70: 1028-1032.

Gonsalves, D. 1994. Papaya Ringspot Virus. In: Compendium of Tropical Fruit Diseases APS Press: 67-68.

Govier, D. A., Kassanis, B. 1974b. A virus-induced component of plant sap needed when aphids acquire potato virus Y from purified preparations. Virology 61:420-426.

Govier, D. A., Kassanis, B. 1974a. Evidence that a component other than the virus particle is needed for aphid transmission of potato virus Y. Virology 57:285-286.

Harrison, B. D., Murant, A. F. 1984. Involvement of virus-coded proteins in transmission of plant viruses by vectors. In: Vectors in Virus Biology, pp. 1-36. Mayo, M. A., Harrap, K. A. (eds.). Academic Press, London.

Holm, L., Pancho, J. V., Hewrberger, J. P., and Plucknett, D. L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Horovitz, S. and Jimenez, H. 1967. Cruzamientos interespecíficos e intergenéricos en Caricaceas y sus implicaciones fitotécnicas. Agronomía Tropical (Maracay) 17: 323-343.

Hull, R. 1989. The movement of viruses in plants. Annual Review of Phytopathology 27:213-240.

Isherwood, M. O. 1994. Status of papaya ringspot virus program. Proc. 30th Annual Hawaii Papaya Industry Association Conference, pp. 1-3.

Jewell, D. L. 1989. Agricultural Statistics, 1988. U.S. Government Printing Office, Washington, D.C. 544 pp.

Jordan, M., Ciudad, G., Rojas, M., and Valverde, F. 1986. Isolation, culture and fusion of *Carica candamarcensis* and *C. papaya* protoplasts. Gartenbauwissenschaft 51: 175-178.

Lecoq, H., Bourdin, D., Raccah, E., Hiebert, E., Purcifull, D.E. 1991. Characterization of a zucchini yellow mosiac virus isolate with a deficient helper component. Phytopathology 81:1087-1091.

Litz, R. and Conover, R. 1980. Somatic embryogenesis in cell cultures of *Carica stipulata*. HortScience 15: 733-735.

Litz, R. and Conover, R. 1979. In vitro improvement of *Carica papaya* L. Proceedings of the Tropical Region, American Society for Hortorticultural Science 23: 157-159.

Maoka, T., Kawano, S., and Usugi, T. 1995. Occurrence of the P strain of papaya ringspot virus in Japan. Annals of the Phytopathology Society of Japan. 61: 34-37.

Matthews, R.E.F. 1991. Plant Virology, third edition. Academic Press, New York. 835 pp.

Mau, R. F. L., Gonsalves, D., and Bautista, R. 1989. Use of cross protection to control papaya ringspot virus at Waianae. Proceedings of the 25th Annual Papaya Industry Association Conference, 1989, pp. 77-84.

Maule, A. J. 1991. Virus movement in infected plants. Critical Reviews in Plant Sciences 9:457-473.

McCammon, S. L., and Medley, T. L. 1990. Certification for the planned - introduction of transgenic plants in the environment. *In*: The Molecular and

Cellular Biology of the Potato, pp. 233-250. Vayda, M. E., and Park, W. D. (eds.). CAB International, Wallingford, United Kingdom.

McGregor, S. E. 1976. Insect Pollination of Cultivated Crop Plants. Agriculture Handbook No. 496. U.S. Government Printing Office. Washington, DC. 411 pp.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, Ithaca and London. 586 pp.

Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., Martelli, G. P., Mayo, M. A., Summers, M D. 1995. Virus Taxonomy. Classification and nomenclature of viruses. Springer-Verlag Wien, New York.

National Research Council. 1989. Field Testing Genetically Modified Organisms: Framework for Decisions. National Academy Press, Washington, D.C.

Neal, M. C. 1965. *In* Gardens of Hawaii. Bishop Museum Press. Honolulu, Hawaii. 924 pp.

Pirone, T. P., Megahed, E.-S. 1966. Aphid transmissibility of some purified viruses and viral RNAs. Virology 30:631-637.

Pirone, T. P., Thornbury, D. W. 1984. The involvement of a helper component in nonpersistent transmission of plant viruses by aphids. Microbial Science 1:191.

Purcifull, D., Edwardson, J., Hiebert, E., and Gonsalves, D. 1984. Papaya ringspot virus. CMI/AAB Descriptions of plant viruses. No. 292. (No. 84 Revised, July 1984), 8 pp.

Shukla, D.D., Ward, C.W., and Brunt, A.A. 1994. The Potyviridae. CAB International, Wallingford, UK, 516 pp.

Storey, W. B. 1976. Papaya. pp. 21-24 in Simmonds, N. W. Evolution of Crop Plants. Longman Scientific and Technical. Essex, England. 339 pp.

Willis, J. C. 1973. A Dictionary of the Flowering Plants and Ferns. Eighth Edition. Cambridge University Press, Cambridge et alibi. 1245 pp.

Yeh, S.-D. And gonsalves, D. 1994. Practices and perspective of control of papaya ringspot virus by corss protection. In: Advances in Disease Vector Research. Vol. 10. Springer-Verlag, NY, Inc. 237-257 pp.

Table 1. Components of the plasmid (pGA482GG/PRV-4) used in the development of the transgenic papaya lines 55-1 and 63-1 (Gonsalves and Manshardt, Petition 1996).

Item	Brief description (Reference)
nos	nopaline synthase promoter (An, 1986; Bevan et al., 1983), originally from *Agrobacterium tumefaciens*
nptII	neomycin phosphotransferase (Topfer et al., 1980), originally from *Escherichia coli*
35S Pro	promoter from cauliflower mosaic virus (Odell et al., 1985; Pietrzak et al., 1986)
5'-UT	70 bp 5' untranslated region of cucumber mosaic virus RNA 3 (Quemada et al., 1991)
CMV-PRV Fusion Coat	coat protein gene of PRV HA 5-1 which has codons specifying the first 16 amino acids of CMV coat protein at its N-terminus (Ling et al., 1991)
35S Poly (A)	poly (A) terminator from cauliflower mosaic virus (Odell et al., 1985; Pietrzak et al., 1986)
uidA (GUS)	*beta*-glucuronidase (Jefferson, 1987), originally from *E. coli*
Gent	Gentamycin resistance gene (Allmansberger et al., 1985), originally from *E. coli*
Tet	Tetracycline gene (An, 1986), originally from *E. coli*