Animal and Plant Health Inspection Service

[Docket No. 96–002–2]

Asgrow Seed Co.; Availability of Determination of Nonregulated Status for Squash Line 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 the Asgrow Seed Company's squash line designed as CZW–3 that has been genetically engineered for virus resistance is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by the Asgrow Seed Company in its 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 Asgrow Seed Company's 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: June 14, 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. James White, 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 December 18, 1995, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 95–352–01p) from the Asgrow Seed Company (Asgrow) of Kalamazoo, MI, seeking a determination that a yellow crookneck squash line designated as CZW–3 (line CZW–3) that has been genetically engineered to contain genes that confer virus resistance does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On February 2, 1996, APHIS published a notice in the Federal Register (61 FR 3899–3900, Docket No. 96–002–1) announcing that the Asgrow 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 squash line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether this squash line posed a plant pest risk. The comments were to have been received by APHIS on or before April 2, 1996. During the designated 60-day comment period, APHIS received four comments on the subject petition from universities, an office of the cooperative extension service, and an agricultural consultant. All of the comments were favorable to the petition.

Analysis

Line CZW–3 has been genetically engineered to contain the coat protein genes from cucumber mosaic virus, watermelon mosaic virus 2, and zucchini yellow mosaic virus for resistance to these viruses. The subject squash line also contains the *nptII* gene from the prokaryotic transposon Tn5, which encodes the enzyme neomycin phosphotransferase II and is used as a selectable marker for transformation. Expression of the added genes is controlled in part by 35S promoters and terminators from the plant pathogen cauliflower mosaic virus. The genes used to develop line CZW–3 were stably transferred into the genome of the yellow crookneck squash parental line through the use of the *Agrobacterium tumefaciens* transformation system.

The subject squash line has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of line CZW–3 conducted in 1993 and 1994 under APHIS permits indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of this squash line.

Determination

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

The effect of this determination is that Asgrow's yellow crookneck squash line CZW-3 is no longer considered a regulated article 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 squash line or its progeny. However. importation of the subject squash line or seeds capable of propagation is 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 (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 Asgrow's yellow crookneck squash line CZW-3 and lines developed from it 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 21st day of June 1996.

Bobby R. Acord,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96-16465 Filed 6-26-96: 8:45 am]

BILLING CODE 3410-34-P

USDA/APHIS Petition 95-352-01P for Determination of Nonregulated Status for CZW-3 Squash

Environmental Assessment and
Finding of No Significant Impact

June 1996

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has conducted an environmental assessment before issuing a determination of nonregulated status for a genetically engineered squash called CZW-3 Squash. APHIS received a petition from the Asgrow Seed Company regarding the status of the CZW-3 Squash as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and 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 virus resistant CZW-3 Squash shall no longer be a regulated article.

John H. Payne, Ph.D.
Acting Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUN 14 1996

TABLE OF CONTENTS

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 a genetically engineered, virus resistant line of yellow crookneck squash (*Cucurbita pepo* subsp. *ovifera* var. *ovifera*) designated CZW-3 squash. The developer of CZW-3 squash, the Asgrow Seed Company, petitioned APHIS requesting the determination on the regulated status of CZW-3, a plant that has been a regulated article under APHIS regulations. Under APHIS regulations importation, interstate movements and field tests of CZW-3 squash have required permits issued by APHIS. Asgrow has petitioned APHIS for a - determination that CZW-3 squash does not present a plant pest risk and should therefore no longer be a regulated article under the APHIS regulations found at 7 CFR Part 340.

The CZW-3 squash has been developed to resist infection by three plant viruses that infect squashes. The genes conferring viral resistance in CZW-3 were introduced via genetic engineering techniques. Those techniques enabled the developer to introduce into yellow crookneck squash viral coat protein genes from cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus (WMV2). Incorporation of these coat protein genes into the squash plants does not cause plant disease, but rather enables CZW-3 squash plants to resist infection by CMV, ZYMV, and WMV2. The genes were introduced into CZW-3 via a well-characterized procedure that utilizes the bacterium *Agrobacterium tumefaciens* to introduce genes into plant genomes.

From 1993 through 1996, APHIS has issued 10 permits to Asgrow to conduct field tests with CZW-3 squash. APHIS prepared EAs prior to granting the field test permits. Previous EAs addressed issues pertinent to plant pest risk issues relative to field tests conducted under physical and reproductive confinement, but they did not address several issues relevant to the unconfined growth of CZW-3 squash. With respect to the unconfined growth of CZW-3 squash, APHIS has reached the following conclusions:

1. CZW-3 squash exhibits no plant pathogenic properties. Although plant pathogenic organisms were used in the development of CZW-3 squash, these squash plants are not infected, nor can they incite disease in other plants.

2. CZW-3 squash is no more likely to become a weed than a virus-resistant squash plant developed by traditional breeding techniques. Squash is not considered to be a weed pest, and there is no reason to believe that the ability of CZW-3 squash to resist infection by CMV, ZYMV, and WMV2 will lead to this squash becoming a weed pest. The introduction of traditionally-bred, improved squash varieties has not resulted in squashes that are considered weeds.

3. CZW-3 squash will not increase the weediness potential for any other cultivated plant or native wild species with which it can interbreed. As with other cultivated squashes, it will be possible for the pollen of CZW-3 squash to pollinate free-living *Cucurbita pepo* (FLCP) plants, the so-called wild relatives of cultivated squash. Although genes can move freely via pollen from CZW-3 squashes to FLCP plants, there is no indication that such cross-pollination will result in hybrid offspring that present any significant increase in their weediness.

4. CZW-3 squash will not cause damage to processed agricultural commodities.

5. CZW-3 will not increase the likelihood of the emergence of new plant viruses. APHIS has carefully considered the biology and epidemiology of the plant viruses that infect squash, and APHIS has determined that the unconfined cultivation of CZW-3 squash would be no different than traditionally bred, virus resistant squash cultivars with respect to the appearance of new plant viruses.

6. CZW-3 squash will not harm threatened or endangered species or other organisms, such as bees, which are beneficial to agriculture.

APHIS has also concluded that there is no reason to believe that new progeny CZW-3 squash varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the CZW-3 squash lines already field tested, or those observed for squashes in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that CZW-3 squash will be just as safe to grow as virus resistant squash cultivars developed through traditional breeding practices. The cultivation of CZW-3 squash should present environmental impacts that are no different from the impacts associated with traditionally-bred squash 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 CZW-3 squash will no longer be considered a regulated article under the regulations in 7 CFR Part 340.

II. BACKGROUND

A. Development of CZW-3 Squash

The CZW-3 squash has been developed to resist infection by three plant viruses that commonly infect squashes. The genes conferring viral resistance in CZW-3 were introduced via recombinant DNA (genetic engineering) techniques rather than conventional breeding techniques. The recombinant techniques enabled the developer to introduce three viral coat protein genes from plant viruses into the yellow crookneck squash. The genes were obtained from cucumber mosaic cucumovirus (CMV), zucchini yellow mosaic potyvirus (ZYMV), watermelon mosaic potyvirus (WMV2). The latter two viruses are members of the potyvirus group of plant viruses while the former is a member of the cucumovirus group. Incorporation of these genes into the squash to yield the CZW-3 plants does not cause plant disease, but rather enables the plants to resist infection by CMV, ZYMV, and WMV2. The genes were introduced into CZW-3 via a procedure mediated by a strain of the plant pathogenic bacterium *Agrobacterium tumefaciens*, which has been disarmed so that it is no longer pathogenic to plants. This procedure is well characterized and has been used widely for over a decade as a means of introducing various genes of interest directly into plant genomes.

CZW-3 squash lines 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. Through the end of 1996, APHIS has issued 10 permits for field tests of CZW-3 squash. The field tests of CZW-3 have been conducted in controlled agricultural settings, under permit conditions that have stipulated physical and reproductive confinement of the CZW-3 plants.

B. 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 squash plants described in the Asgrow petition have been considered regulated articles because noncoding DNA regulatory sequences and portions of the plasmid vector were derived from plant pathogens.

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 CZW-3 squash as a regulated article under APHIS regulations. The developer of CZW-3 squash, Asgrow, submitted a petition to USDA/APHIS requesting that APHIS make a determination that CZW-3 squash shall no longer be considered a regulated article 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

A. No Action

Under the Federal "no action" alternative, APHIS would not come to a determination that CZW-3 squash is no longer a regulated article under the regulations at 7 CFR Part 340. Permits from APHIS would still be required for introductions of CZW-3 squash. APHIS might choose this alternative if there were insufficient evidence to predict the lack of plant pest risk from unconfined cultivation of CZW-3 squash.

B. Determination That CZW-3 Squash is No Longer a Regulated Article

Under the Federal action to render a determination that CZW-3 squash is no longer a regulated article under the regulations at 7 CFR Part 340, CZW-3 squash would be subject to the same regulatory oversight as cultivars that result from traditional breeding practices. As such, permits from APHIS would no longer be required for introductions of CZW-3 squash or its progeny.

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that CZW-3 squash would no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. Previous EAs prepared by APHIS in conjunction with the issuance of permits for field tests of CZW-3 have addressed various attributes of this squash line. This EA discusses the genetic modification of this squash line, the resultant phenotype, and the potential environmental impacts that might be associated with the unconfined cultivation of CZW-3 squash.

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 cucurbits, the genetic components used in the development of CZW-3 squash, and another analysis that supports APHIS' conclusion that CZW-3 squash has no potential to pose plant pest risks.

A. Potential for the Appearance of New Plant Viruses

As mentioned above, CZW-3 squash was developed by engineering the viral coat protein genes of CMV, ZYMV, and WMV2 into a cultivar of yellow crookneck squash, a plant which is frequently infected by these and other plant viruses. As part of its analysis, APHIS evaluated whether the expression of these viral genes in CZW-3 squash 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 CZW-3 squash through the recombination or transencapsidation, and APHIS considered this issue carefully in making its determination. A technical discussion of this issue is found in the Determination document appended to this EA. After careful consideration of the physical and biological properties of CMV, ZYMV, and WMV2, APHIS concluded that it is unlikely that new viruses will appear as a consequence of the widespread cultivation CZW-3 squash. Current control measures are adequate to control any potential new virus.

B. Potential Impacts Based on Increased Weediness of CZW-3 Squash Relative to Traditionally Bred Squash

APHIS evaluated whether the CZW-3 squash itself is likely to present a plant pest risk as a weed. The parent plant in this petition, yellow crookneck squash, is an agricultural crop plant that exhibits no appreciable weedy characteristics. None of the standard texts and lists of weeds indicate that squash 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 CMV, ZYMV, and WMV2, is unlikely to make the CZW-3 squash into a weed. Resistance or tolerance to pests is commonly bred into agricultural crops, including squash. Despite this, improved squash cultivars have not become weeds. Likewise, there is no indication that resistance to ZYMV and WMV2 will result in CZW-3 squash becoming a weed (see the Determination).

No other attributes of CZW-3 squash suggest that it is any more "weedy" than squash cultivars that are the result of traditional breeding. The CZW-3 squash has retained the agronomic characteristics of the parental crookneck squash.

C. Potential Impacts on the Free-Living Relatives of Squash Arising From Pollination by CZW-3 Squash

APHIS evaluated two potential impacts that CZW-3 squash might have on the free-living relatives of squash. First, that the traits from CZW-3 squash might cause the free-living relatives to become "weedier." Second, that the pollination of free-living populations of squashes would cause population changes that would lead to reduced genetic diversity.

Successful transmission of genetic material from CZW-3 squash via pollen is possible to a limited number of squash relatives (Wilson, 1993). In the United States, the squash relatives that might be successfully pollinated by CZW-3 squash and produce offspring are *Cucurbita pepo* subsp. *texana* and *C. pepo* subsp. *ozarkana*, plants referred to here and in the Determination as free-living *Cucurbita pepo* (FLCP). In the past, these FLCP plants have been cited as weeds in soybean and cotton fields (Weed Science Society of America, 1992), but the agricultural significance appears to be minimal with the advent of effective control practices over the past decade (see Determination).

It is unlikely that offspring arising from natural crosses of FLCP and CZW-3 will pose a weed problem. Current agricultural production of squash in the United States occurs near the habitats where FLCP plants are found in the Southeastern States. This proximity is sufficient for the pollination of FLCP by squash cultivars, but there has been no apparent emergence of weedy hybrid progeny (see the Determination). This has not occurred even as plant breeders continue to develop cultivated squash varieties with enhanced pest resistance qualities. Typically, breeders seek out free-living (wild) relatives as a source of pest resistance traits to cross into a cultivated crop.

In addition, it is clear that any progeny of cultivated and free-living squashes will receive a set of genetic material from each of the parents. In this case, the cultivated squash parent contributes genetic material responsible for ensuring a plant that produces tender-skinned fruits that have low levels of cucurbitin, a bitter-tasting compound that discourages feeding by herbivores. Invariably, the FLCP is better adapted than commercial squash cultivars to survival in the absence of cultivation. Thus, there has been no report to date of weed problems arising from the possible crosses that might occur between domesticated varieties of squash and their free-living relatives.

Given the available knowledge, it is unlikely that resistance to CMV, ZYMV, and WMV2 infection will confer a selective advantage or be maintained in the FLCP populations. Surveys of natural FLCP populations for the incidence and severity of CMV, ZYMV, and WMV2 infections suggest that resistance to these viruses will confer little, if any, selective advantage, because disease caused by these viruses is apparently not among the factors important to the survival or reproductive success of FLCP (see the Determination).

Based upon our analysis of the biology of cultivated squash and its relatives, APHIS concludes that the environmental impacts of cultivation of CZW-3 squash anywhere in the world will be no different than such impacts attributable to similar varieties produced with traditional breeding techniques. The species *Cucurbita pepo* is native to the North American continent, with a center of biological diversity in northern Mexico, and a center of diversity (probably secondary, though embracing a greater variety) in the Southeastern United States. Cultivated and noncultivated varieties of *C. pepo* have coexisted and co-evolved over millennia. Even if CZW-3 squash were to be cultivated in agricultural regions around centers of *C. pepo* diversity, there is no reason to expect impacts from CZW-3 squash would be significantly different from those arising from the cultivation of any other variety of squash. As discussed above, natural populations of FLCP appear to be largely free of infection by ZYMV and WMV2. It therefore appears that resistance to ZYMV and WMV2 should not provide any selective advantage. Without a selective advantage, this trait is unlikely to persist in the gene pool of FLCP.

There is already considerable cultivation of traditional squash varieties throughout the centers of diversity for *C. pepo*, including virus resistant varieties. Conventionally-bred virus resistant cucurbits that are available for use include: Harris Moran's zucchini squash that is resistant to ZYMV and WMV-2 and zucchini squash (specific virus not described but possibly CMV) and CMV-resistant marrow squash that are available commercially from Thompson and Morgan, Inc. of Jackson, New Jersey. The impact of cultivation of CZW-3 squash on the genetic diversity of FLCP populations is likely to be comparable to that from nontransgenic varieties.

We note also that any international traffic in CZW-3 squash would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December 1992). The treaty, now administered by a Secretariat housed with the United Nations Food and Agriculture Organization in Rome, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines, including Mexico, which has in place a regulatory process that would require a full evaluation of the CZW-3 squash before it could be introduced into their environment. Our decision in no way prejudices regulatory action in Mexico or any other country. The IPPC has also led to the creation of Regional Plant Protection Organizations such as the North American Plant Protection Organization (NAPPO). Our trading partners will be kept informed of our regulatory decisions through NAPPO, and other fora. In addition to the assurance provided by the analysis leading APHIS to a finding of no significant impact for the introduction of this squash variety, it should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new squash varieties internationally apply equally to those covered by this analysis.

D. Potential Impact on Nontarget Organisms, Including Beneficial Organisms Such as Bees and Earthworms

Consistent with its statutory authority, APHIS evaluated whether CZW-3 squash might indirectly harm plants or plant products (such as some agricultural commodities). APHIS considered the potential impact that CZW-3 might exert indirectly on organisms that are recognized as beneficial to agriculture. APHIS concludes that there is no reason to believe that the unconfined growth of CZW-3 squash will pose any deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. The coat proteins expressed in CZW-3 squash are not known to have any toxic properties. In fact, these viral coat proteins are routinely ingested by virtually all animals, including humans, when squash is consumed. Naturally occurring infections of susceptible squash varieties result in concentrations of coat proteins far higher than those that occur in the tissues of the CZW-3 squash (see the Determination).

APHIS believes that CZW-3 squash 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 CZW-3 squash would have any adverse effect on other organisms, including any species recognized as threatened or endangered in the United States. The release of CZW-3 squash from regulation should have no - adverse impact on agricultural commodities.

VI. CONCLUSIONS

APHIS has evaluated information from the scientific literature as well as data submitted by Asgrow that characterize the CZW-3 squash. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that CZW-3 squash would no longer be a regulated article under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of removing CZW-3 from its regulation and reached the following conclusions:

1. CZW-3 squash exhibits no plant pathogenic properties. Although plant pathogenic organisms were used in the development of CZW-3 squash, these squash plants are not infected, nor can they incite disease in other plants.

2. CZW-3 squash is no more likely to become a weed than a virus-resistant squash plant developed by traditional breeding techniques. Squash is not considered to be a weed pest, and there is no reason to believe that the ability of CZW-3 squash to resist infection by CMV, ZYMV, and WMV2 will lead to this squash becoming a weed pest. The introduction of traditionally-bred, improved squash varieties has not resulted in squashes that are considered weeds.

3. CZW-3 squash is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which it can interbreed. As with other cultivated squashes, it will be possible for the pollen of CZW-3 squash to pollinate free-living *Cucurbita pepo* (FLCP) plants, the so-called "wild" relatives of cultivated squash. Although genes can move freely via pollen from CZW-3 squashes to FLCP plants, there is no indication that such cross-pollination will result in hybrid offspring that present any significant increase in their weediness.

4. CZW-3 squash will not cause damage to processed agricultural commodities.

5. CZW-3 will not increase the likelihood of the emergence of new plant viruses. APHIS has carefully considered the biology and epidemiology of the plant viruses that infect squash, and APHIS has determined that the unconfined cultivation of CZW-3 squash would be no different than traditionally bred, virus resistant squash cultivars with respect to the appearance of new plant viruses.

6. CZW-3 squash will not harm other organisms, such as bees, which are beneficial to agriculture.

APHIS has also concluded that new progeny CZW-3 squash varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the CZW-3 squash lines already field tested, or those observed for squashes in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that CZW-3 squash will be just as safe to grow as virus resistant squash cultivars developed through traditional breeding practices. The cultivation of CZW-3 squash should present environmental impacts that are no different from the impacts associated with traditionally-bred squash varieties that are not subject to regulation under 7 CFR Part 340 before they enter agriculture. 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 the CZW-3 squash will no longer be a regulated article 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.

Wilson, H. 1993. Free-living *Cucurbita pepo* in the United States. Viral resistance, gene flow, and risk assessment. Report to USDA Biotechnology, Biologics and Environmental Protection. 24 pp.

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., Staff Biotechnologist
David S. Heron, Ph.D., Staff Biotechnologist
Susan M. Koehler, Ph.D., Staff Biotechnologist
James Lackey, Ph.D., Biological Safety Officer
Vedpal S. Malik, Ph.D., Staff Biotechnologist
Henry K. Reding, Ph.D., Staff Biotechnologist
Sivramiah Shantharam, Ph.D., Team Leader, Microorganisms Branch
James L. White, Ph.D., Team Leader, Plants Branch (Preparer)

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

Response to the Asgrow Seed Company Petition 95-352-01p for Determination of Nonregulated Status for CZW-3 Squash

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

TABLE OF CONTENTS

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, virus resistant line of yellow crookneck squash (*Cucurbita pepo* subsp. *ovifera* var. *ovifera*) designated CZW-3 by the Asgrow Seed Company does not represent a plant pest risk and is therefore not a regulated article 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 CZW-3 squashes or their progeny.

This determination by APHIS has been made in response to a petition received from Asgrow Seed Company, Kalamazoo, Michigan, on December 18, 1995. The petition requested a determination from APHIS that the CZW-3 squash does not present a plant pest risk and is therefore not a regulated article. Based on a review of available scientific information, APHIS has determined that CZW-3 squash does not present a plant pest risk and therefore is no longer a regulated article under the regulations at 7 CFR Part 340. The CZW-3 squash, as defined by its developer, the Asgrow Seed - Company, is a squash line that is designed to resist infection by three plant viruses that frequently infect squash, namely CMV, ZYMV, and WMV2. CZW-3 squash has been modified with genes that express the coat proteins of cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus 2 (WMV2). Expression of these coat protein (CP) genes does not cause plant disease, but rather confers resistance to infection by CMV, ZYMV and WMV2. The introduced DNA that encodes the CP genes also has accompanying DNA regulatory sequences that modulate their expression. The DNA regulatory sequences were derived from plant pathogenic organisms: the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV). Although the regulatory sequences were derived from plant pathogens, the regulatory sequences cannot cause plant disease by themselves or in conjunction with the genes that they regulate in these squash. With respect to *A. tumefaciens*, the genes that cause disease have been removed. The sequences derived from the three plant viruses are only small portions of their genomes and do not encode any pathogenic properties.

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. CZW-3 squash has been considered a "regulated article" for field testing under Part 340 of the regulations in part because CZW-3 squash has been engineered with CP genes derived from the plant pathogenic viruses CMV, ZYMV, and WMV2. Field testing of the CZW-3 squash has been done under APHIS permits starting in 1993 is continuing in 1996. All field trials were performed essentially under conditions of - reproductive confinement.

APHIS has determined that the CZW-3 squash does not pose a direct or indirect plant pest risk and, therefore, will no longer be considered a regulated article 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 CZW-3 squash or their progeny. (Importation of CZW-3 squash [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 CZW-3 squash: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than a virus-resistant plant developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) should not cause damage to processed agricultural commodities; (5) should not increase the likelihood of the emergence of new plant viruses; and (6) is unlikely to harm other organisms that are beneficial to agriculture, such as bees. APHIS has also concluded that there is no reason to believe that new progeny CZW-3 squash varieties bred from these lines will exhibit new plant pest properties, i.e., properties substantially different from any observed for the CZW-3 squash lines already field tested, or those observed for squashes 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. CZW-3 squash have been considered "regulated articles" under Part 340 of the regulations because they have been engineered with three coat protein genes from plant viruses and certain noncoding regulatory sequences derived from the plant pathogens, cauliflower mosaic virus (CaMV) and *Agrobacterium 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.

APHIS' decision on the regulatory status of CZW-3 squash under the regulations at 7 CFR 340, does not release this squash and its progeny from EPA and FDA regulatory oversight.

III. PUBLIC COMMENTS

On February 2, 1996, APHIS published a notice in the Federal Register (61 FR 3899-3900, Docket No. 96-002-1) announcing that the Asgrow petition had been received and was available for public review. In the notice, APHIS solicited written comments from the public as to whether this squash line posed a plant pest risk. The comments were to have been received by APHIS on or before April 2, 1996. APHIS received a total of 4 comments on the petition from universities, an office of cooperative extension service, and an agricultural consultant. All comments were favorable to the petition.

IV. ANALYSIS OF CZW-3 SQUASH

Biology Of Squash. Plant of the genus *Cucurbita* are members of the family *Cucurbitaceae*. The genus *Cucurbita* includes five domesticated species and 22 wild species (Decker 1988). The genus is indigenous to the Americas with most species coming from North America. The center of distribution is thought to be central or southern Mexico (Whitaker and Davis 1962). *C. maxima* and *C. andreana* are from South America. The common ancestor of all cucurbits is probably an annual gourd-producing plant that was first used in New World agriculture approximately 10,000 years ago. The *C. pepo* lineage appears to be composed of two subsets, formally identified as two subspecies *ovifera* and *pepo*. Subspecies *pepo* includes domesticated types, pumpkins, marrow varieties, and some ornamental gourds, whereas subspecies *oveifera* includes the remaining ornamental gourd varieties and acorn, crookneck, straightneck, scallop, yellow squash (Wilson 1993). The four cultivated cucurbit species includes *C. pepo, C. maxima, C. mixta,* and *C. moschata*. Since all species of *Cucurbita* are monoecious (having both male and female flowers on the same plant) and produce heavy sticky pollen grains, pollination requires an arthropod vector, usually a bee, to transmit the pollen from the staminate to the pistillate flower (Whitaker and Robinson 1986). Interspecific hybridization has been extensively investigated and is well understood in the four cultivated species of *pepo, mixta, moschata*, and *maxima*. F_1 hybrids can be obtained in breeding programs, but only with difficulty and such hybrids are usually sterile. There is no evidence of spontaneous hybridization among these four species despite that fact that they have been grown side by side under cultivation for many generations (Whitaker and Robinson 1986).

There exists in the United States two free-living subspecies (free-living *C. pepo* or FLCP) of *C. pepo* that can cross with cultivated varieties of *C. pepo* without loss of fertility. These include the free living gourds in many states including Texas designated *C. pepo ssp. ovifera var. texana* and free-living gourds in Illinois, Arkansas, and Oklahoma designated *C. pepo ssp. ovifera var. ozarkana*. A detailed analysis of these species and other issue regarding biology of squash was prepared in 1993 by Dr. Hugh Wilson for APHIS.

Biology of the Plant Viruses. CMV is the type member of the cucumovirus group. CMV has been linked to plant disease in all temperate regions of the world. The virus has an extremely wide host range which includes cereals, forages, woody and herbaceous ornamentals, vegetables, and fruit crops. The RNA of CMV consists of four components of different size (Rezaian et al., 1984, 1985; Rizzo and Palukaitis, 1989; Symons, 1985). The three largest RNAs, which are distributed among three separate virion particles, carry all the information needed for successful infection (Peden and Symons, 1973; Lot et al., 1974). A number of experiments and sequencing data has suggested RNAs 1 and 2 are involved in viral RNA synthesis (Gordon et al., 1982); i.e., viral replicase (reviewed by Hull and Maule, 1985). The genetic information for viral coat protein is carried on the dicistronic RNA 3 (Gould and Symons, 1977;1982; Davies and Symons, 1988) and on a coencapsidated subgenomic messenger RNA 4. The subgenomic RNA 4 is mRNA which is translated *in vivo* to give rise to viral coat protein (Kaper and Waterworth, 1981). Beside viral coat protein, RNA 3 codes another that has been implicated to mediate the cell-to-cell movement of the virus (Hull and Maule, 1985). CMV is not seed transmitted in *C. pepo* (Francki *et al* 1979) but is in *C. sativus* (Doolittle and Gilbert, 1919).

Nearly all cucumoviruses are transmitted by aphids in an nonpersistent manner. The main attribute of nonpersistent transmission is that the virus is picked up by the aphid after a brief feeding on the infected plant and can transmit it immediately to one or only a few healthy plants. The rate of transmission is affected by the host on which the aphid species has been raised, the virus source plant, the aphid species, and the virus strain (Garret et al., 1985). One aspect of the specificity and efficiency of the transmission process by aphids is the interaction between the virion and those surfaces in the stylet or foregut of the aphid that are thought to be involved in transmission (Lim et al., 1977). The changes in the efficiency of aphid transmissibility probably results from changes in the amino acid sequences in the coat proteins as a consequence of mutation and the effect that these amino acid substitutions have on the virus-vector interaction.

Some CMV strains contain small RNAs that are not required for virus replication. These small RNAs, called satellites, contain **no significant** homology to the viral genome but are replicated by the CMV replicating enzymes. These small RNAs can often modify the symptoms expressed by infected plants. Depending on the genotype of the host plant, the sequence of the small RNA, the helper virus, and environmental conditions, the symptoms may be attenuated or more severe (Matthews 1991). CMV satellite contain strains have caused serious disease epidemics in Japan, China, Italy, and Spain in the past decade (Tien and Wu, 1991; Kaper et al., 1990). Coat protein gene-mediated protection alone does not protect plants against infection if the virus contains satellite RNAs, so that additional measures are likely to be necessary for engineering effective protection against such satellite-containing viruses (Yie and Tien, 1993).

Watermelon mosaic virus 2 and zucchini yellow mosaic virus. Both of these viruses are members of the genus *Potyvirus*. Potyviruses are filamentous and elongated particles, composed of single coat protein species, and each virus is composed of a single RNA molecule of approximately 10,000 nucleotides in length. The mature protein is a post-translationally modified form of polyprotein precursor encoded its genomic RNA (Yeh and Gonsalves, 1985). These viruses have world wide distribution and are one of the economically important pathogens of cucurbits in the United States (Alderz et al. 1983; Chala et al. 1986; Davis and Mizuki, 1987; Nameth et al. 1985; Provvidenti et al. 1984). The genome of these viruses encode many proteins, some whose function have not been identified. 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. These viruses are apparently not seed transmitted in squash (Lisa and Lecoq, 1984; Purcifull *et al.* 1984; Francki *et al.* 1979).

Rationale for Development of CZW-3 Squash. Commercial production of cucurbits are limited by a number of virus diseases caused by CMV, ZYMV, WMV2, and papaya ringspot potyvirus (PRSV). All four of these viruses are transmitted by the same common aphids (APHIS, 1994). When young plants are infected, they often do not produce marketable fruits. Depending on the year, cultivar, and location, yield losses can range from 20% to 80%. The use of insecticides to kill the insects or stylet oils to inhibit transmission of the virus have been the most common approach to control these diseases. Stylet oils, although more environmentally friendly than insecticides, are not completely effective and require frequent applications. Genes for resistance to WMV2 have been identified in *C. ecuadorenis*, *C. ficifolia*, *C. foetidissima*, *C.pedatifola*, and *C. moschata* (Nigerian squash) and for ZYMV in *C. ecuadorenis*, and *C. moschata*. However, introduction of these resistance genes into domestic cultivars via traditional plant breeding has proven difficult because of genetic incompatibility among species (Provvidenti, 1990). A traditionally bred cultivar that is resistant to ZYMV and WMV-2 is commercially available from Harris Moran Seed Company. The use of genetic based resistance will offer producers another alternative to control these diseases.

In 1986, Powell Abel et al. showed that transgenic plants expressing the CP of tobacco mosaic tobamovirus (TMV) imparted resistance to TMV. Since that time more than 30 different plants have been engineered with 50 different viral genes to impart resistance. With potyviruses a variety of strategies have proven effective in the of resistance to potyviruses. The expression of CP in the sense (Lawson *et al.* 1990) or antisense orientation (Hammond and Kamo 1995), and nontranslatable CP RNAs (Lindbo and Dougherty 1992 a,b; Smith *et al.* 1994), truncated CP mRNAs, or other viral genes (Swaney *et al.* 1995) have all proven effective sources of viral resistance. With CMV, resistance has been obtained with sense or antisense CP constructs (Cuozzo *et al.* 1988) or replicase (Anderson *et al.* 1992). Whether a specific strategy is superior to another strategy in a given virus-plant combination under field conditions is not known.

Development of CZW-3 Squash. The virus resistance genes and a selectable marker gene were engineered into a disarmed *A. tumefaciens* strain as described by Bevin (1984). This bacterial strain in no longer pathogenic since its virulence genes were removed. The WMV-2 coat protein gene was from strain FL-1656 from a watermelon plant from Florida, the ZYMV coat protein gene was from the Florida strain originally isolated from a squash plant from Florida, and CMV coat protein gene was from strain C originally isolated from // plant in //. The coat protein genes synthesis were driven by 35S cauliflower mosaic caulimovirus promoter (Odell *et al.*, 1985) and the termination/polyadenylation signal were also derived from the same virus. Al l three coat protein genes contained the 5' untranslated sequence from cucumber mosaic virus to enhance translation of the transgene mRNA. The selectable marker gene used was neomycin phosphotransferase, which encodes resistance to the antibiotic kanamycin, was isolated from transposon Tn5 from *Escherichia coli*.

V. RATIONAL FOR DEVELOPMENT OF CZW-3 SQUASH

To reach its determination that CZW-3 squash does 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 squash and data presented by Asgrow 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 CZW-3 squash.

The Introduced Genes, Their Products, and the Added Regulatory Sequences Controlling Their Expression Do Not Present a Plant Pest Risk in CZW-3

The CZW-3 squash plants were derived by transforming yellow crookneck squash via a well-characterized technique that uses DNA sequences from *A. tumefaciens* to introduce genes into the chromosome of the recipient plant (see reviews by Klee and Rogers, 1989; and Zambryski, 1988). Although some DNA sequences used in the transformation process were derived from the plant pathogen *A. tumefaciens* (the causal agent of crown gall disease), the genes that cause crown gall disease were removed, and therefore the squash plant does not develop crown gall disease. Once inserted into the chromosome of the squash plant, the introduced genes are maintained and transmitted in the same manner as any other genes. Squash plants pass their genes to their progeny by sexual reproduction that involves self pollination, or pollination of other squash plants or sexually compatible relatives.

The CZW-3 squash line was produced using an *Agrobacterium*-meditated transformation protocol to transform yellow crookneck squash with genes designed to confer resistance to CMV, ZYMV, and WMV2, three plant viruses that frequently infect squash. The genes that confer this resistance are derived from virus genes that encode the CP of CMV, ZYMV, and WMV2. Expression of these CP genes in the squash does not cause plant disease, but rather confers resistance to infection by these three viruses.

The introduced DNA that encodes the CP genes also has accompanying DNA regulatory sequences that modulate the expression of the CP genes. The DNA regulatory sequences were derived from three plant pathogenic organisms: the bacterium *A. tumefaciens* and CaMV. Specifically, the DNA regulatory sequences associated with the viral CP coding regions comprise promoter and transcriptional termination sequences derived from the 35S gene of CaMV and translational initiation sequences from CMV. 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 CZW-3 squash in laboratory, greenhouse, and field experiments, the plants exhibited the typical agronomic characteristics of the parent crookneck squash, with the addition of resistance to CMV, ZYMV, and WMV2 infection. In APHIS' opinion, the components and processing characteristics of CZW-3 squashes reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. The CZW-3 plants have no plant pest characteristics.

The CZW 3 Squash is No More Likely to Become a Weed Than a Virus-Resistant Plant Developed by Traditional Breeding Techniques

Conclusion: Introduction of virus resistance genes is unlikely to increase the weediness of yellow crookneck squash.

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.

A weed pest is a plant that grows persistently in locations where it is unwanted. Baker (1965) described the ideal characteristics of a weed that include: rapid plant growth to germination in many environments; internally controlled discontinuous germination; long-lived seeds; rapid growth to flowering; continuous seed production; use of wind or unspecialized insects for pollination if outcrossing occurs; high seed production; good competitiveness achieved through for example, allelochemicals or choking growth; and long-lived seeds. None of the characteristics described by Baker involved resistance or susceptibility to pathogens or insects. In 1989, Keeler considered in detail whether genetically engineered crops can become weeds. Her analysis of the closely-related squash, *C. maxima*, stated that squash possesses 3 out of the 15 characteristics of plants that are notably successful weeds. Those are: continuous production of seeds as long as growing conditions permit; use of unspecialized insects as pollinators; and strong competitiveness with other plants. Keeler (1989) and Tiedje *et al.* 1989 have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness - potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes. Although Baker's list has been criticized (Williamson, 1994), no other universally acceptable characters have been defined by ecologists and in our view, there is no formulation that is clearly superior at this time. Ironically, many of these attributes are still being used in 1995 by ecologists to describe characteristic that they use to evaluate commercialized transgenic crops (Purrington and Bergelson 1995).

Yellow crookneck squash 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 Baumman, 1992; Holm *et al.* 1979; Muenscher 1980). Although squash volunteers are not uncommon in areas next to production fields, they do not readily establish feral or free-living populations. Volunteers can still be controlled by mechanical means or herbicides. The CZW-3 squash is likely to be grown mostly in areas that are currently under squash cultivation, i.e., in typical growing regions for the crop. Asgrow has reported that there are no major changes in CZW-3 performance characteristics (except for resistance to CMV, ZYMV and WMV2).

There is no evidence to support the conclusion that introduction of virus resistance genes into squash could increase its weediness potential. Many pathogen and insect resistance genes have been introduced into commercial varieties of squash by conventional means in the past without any reports of increased weediness of squash plants. These include genes for resistance to scab, powdery mildew, downy mildew, cucumber beetle, squashbug, and cucumber mosaic virus. Squash cultivars having ZYMV, PRSV, and CMV resistance genes introduced by conventional plant breeding - techniques are soon to be sold by Asgrow (H. Quemada, personal communication).

The CZW 3 Squash is Unlikely to Increase the Weediness Potential for Any Other Cultivated Plant or Native Wild Species With Which the Organism Can Interbreed

Conclusion: FLCP plants are not serious weeds in unmanaged or agricultural ecosystems. APHIS believes that the virus resistance genes in CZW-3 will be transferred via pollen to FLCP plants. However, since there is no scientific or ancetdotal evidence to suggest the these viruses routinely infect FLCP plants, APHIS believes that will not increase the weediness potential of FLCP plants.

The virus resistance genes in CZW-3 will be transferred via pollen to FLCP plants. APHIS assumes pollen from CZW-3 squash is likely to be carried by bees and successfully pollinate FLCP plants. First, APHIS will discuss whether FLCP plants are weeds and then, whether CMV, ZYMV, and WMV-2 are pests of FLCP plants.

Although FLCP plants were reported to be weeds in cotton and soybean fields during the 1970's, registration of new herbicides now allows effective management of these plants. There are no reports of FLCP plants as significant weeds in any unmanaged ecosystems, but rather, they stably occupy only a particular biological niche along riverbanks. FLCP plants have only been reported to be a serious problem in soybean and cotton fields in the Red River Valley of Arkansas. These reports date from the 1970's, but the FLCP plants continue to be an occasional problem in soybean and cotton fields that are located in flood-prone areas today (F. Baldwin, personal communication). Dr. Baldwin was the representative from Arkansas on the Weed Society of America's 1992 publication entitled "Crop Losses Due to Weeds in the United States" in which FLCP plants were listed as serious weeds in soybean fields. Dr. Baldwin stated that he believed that FLCP plants were not as serious a problem currently as in the past, and he provided APHIS with the names of other persons with up-to-date familiarity with FLCP plants occurrence in Arkansas. A summary of APHIS' discussions with these scientists follows.

Dr. Greg Weidemann (University of Arkansas) conducted research during the 1980's to identify biological control agents to eliminate FLCP plants from soybean fields. He said that the FLCP problem in soybean fields has been controlled in recent years by new herbicides (e.g., Cobra®) that were not available in the 1980's, so that FLCP plants are only a minor problem in soybean fields in the Red River Valley. Joe Vestle, a County Extension agent who works in areas where the FLCP plants were previously serious problems, agreed with Drs. Baldwin and Weidemann that the FLCP plants are less a problem in 1994 than during the 1980's. They also noted that pending registrations of the herbicide bromoxynil for use in conjunction with bromoxynil-tolerant cotton and of the herbicide glyphosate for use in conjunction with glyphosate-tolerant soybeans will further expand the tools for effective control of FLCP plants. They noted that with these additional options FLCP plants should not become a significant weed problem in soybean or cotton fields in Arkansas.

If FLCP plants acquire resistance to CMV, WMV2, and ZYMV from CZW-3 squash, the control of the virus-resistant FLCP plants in soybean or cotton fields should not be more difficult or require new measures than of their nonengineered counterparts. Soybean and cotton crops are not affected by these three viruses (or PRSV) and no viruses are known (with the possible exception of tomato spotted wilt virus) that cause disease in squash, cotton, and soybeans (Matthews, 1991). The most effective means of controlling FLCP plants are herbicide application, rogueing, and collection of the gourds at the end of the season to eliminate the seed source. The effectiveness of all of these methods would be uncompromised by the presence of virus-resistant FLCP plants.

APHIS believes that the historic record and a survey of FLCP plants for viral infection show that these three viruses are not significant pathogens of these plants. FLCP plants are susceptible to CMV, ZYMV, and WMV2 infection but are apparently not infected in the wild (APHIS, 1994). In greenhouse and field tests, *C. pepo* spp. *ovifera* var. *texana* (seed source from Texas) was found to be highly susceptible to CMV, PRSV, and WMV2 when mechanically infected or grown in the presence of aphid vectors and infected plants (Provvidenti et al., 1978). However, the survey of FLCP plants by Asgrow failed to detect seven important cucurbit-infecting viruses including: ZYMV, PRSV, WMV2, CMV, squash mosaic virus, tomato ringspot virus, and tobacco ringspot virus (APHIS, 1994).

APHIS believes that further reviews of the botanical record with respect to FLCP plants in Arkansas supports our conclusion that FLCP plants have not been significantly impacted by CMV or potyviruses. ZYMV first appeared in the mid-1980's in the United States and is now widely prevalent in Arkansas and Texas. It is the most devastating single virus in cucurbit production. Has the appearance of ZYMV in Arkansas affected the FLCP population in soybean fields? The evidence available is qualitative, but has been provided by agricultural experts who have continuously monitored cucurbits in these areas. Dr. Ford Baldwin (Weed Specialist of the USDA, Extension Service) said that FLCP populations in these fields have not noticeably changed since the arrival of ZYMV. Dr. Greg Weidemann (University of Arkansas), who worked during the 1980's to identify biological control agents for FLCP control, potentially including viruses, does not recall any evidence over several years of research of natural viral infections in stands of FLCP plants. (Instead, he selected the fungus *Fusarium solani* to test as a biological control agent (Weidemann and Templeton, 1988). Mr. Joe Vestle also did not recall the presence of viral-infected FLCP plants but did recall the presence of rust (fungus) infection of FLCP plants. Furthermore, these Arkansas scientists indicated that the appearance of ZYMV as a major pest of cucurbits did not affect FLCP populations enough to alter the rate of farmer requests for information on controlling FLCP plants in soybean and cotton fields during the 1980's.

The lack of infection of FLCP plants is not a result of absence of virus or aphid vectors. APHIS believes that the absence of potyviral infection in FLCP plants is not a result of the lack of inoculum for the following reasons: (1) CMV, ZYMV, and WMV2 are widely prevalent in cucurbit crops in many of the major honeydew melon-, cucumber-, pumpkin-, and squash-producing States (APS Virology Committee's List of Widely Prevalent Viruses by State); (2) many potential weed and annual plants that are hosts of CMV, ZYMV and WMV2 are present in these States (APHIS, 1994); and (3) many aphid vectors that can transmit CMV, ZYMV, and WMV2 (APHIS, 1994, Perring et al., 1992) are widely distributed in the States where FLCP have been reported. Wilson (1993) states, "*C. pepo* populations during a given growing season would probably - include every county with the 12 State FLCP distribution. . . ."

Why FLCP plants are not infected with common cucurbit-infecting viruses is unknown. If the aphids are to infect FLCP plants they must feed on a CMV-, ZYMV- or WMV2-infected plant immediately before visiting the FLCP plants. The development cycle of the aphids and the maturation of plants in the spring where FLCP grow may not be favorable to viral infection of FLCP. Second, the FLCP plants could produce chemicals that make them unattractive to feeding by the aphid vectors. A *Cucumis melo* genotype has been identified that exhibits this type of resistance (Gray et al., 1986).

It should be noted that the fact that the three cucurbit viruses' CMV, ZYMV, and WMV2 have the cucurbit names "cucumber", "watermelon" and "zucchini" in their titles does not imply that they have ever been pests of FLCP plants in nature. Viruses are given their names after the plant from which they were first isolated and characterized. If the only hosts of these viruses in the eastern United States were squash, these viruses would likely perish. Because these viruses are not seed-transmitted in squash and do not overwinter in squash detritus, squash by itself is a dead-end host. The critical host for survival of these viruses is their overwintering host (usually woody perennials or in dormant seeds). Since the overwintering host for ZYMV in Florida is the wild perennial cucurbit *Melothria pendula* (Adlerz et al., 1983), a more biologically appropriate name for the virus might include *Melothria* in its title. (For additional details on the overwintering hosts of the potyviruses see APHIS (1994)).

The absence of viral infection of FLCP in the survey was not unexpected. FLCP plants have been reported to be highly susceptible to several viruses, yet FLCP plants have survived for decades in areas where these viruses and their aphid vectors are widespread. The virus survey of FLCP plants performed by Asgrow showed that the plants were not infected asymptomatic strains of the selected plant viruses since no viruses were detected. Therefore, FLCP plants are apparently not infected by common viruses that affect **commercial** cucurbit production.

It is true that young FLCP plants deliberately inoculated with ZYMV or WMV2 may die? What happens to the strain of potyviruses that killed the plant? Aphids do not feed on dead and dying plants (Matthews, 1991). Thus, a severe strain of a virus , ie., a virus that kills its host plant, will theoretically be transmitted to other plants less efficiently than less severe strains. Thus, virus strains tend to persist that cause mild symptoms and do not kill plants before seeds are produced. The survival of mild strains is enhanced if there is any genetic variability in the hosts, i.e, if there are some host biotypes present that do not die upon infection and can produce seeds. The association of a moderation of symptoms coupled with maintaining the ability of a virus to be efficiently transmitted has been observed with barley stripe mosaic virus in barley and seed transmission (Timian, 1974), rice stripe virus in rice and leafhopper transmission and lettuce mosaic virus and seed transmission (Matthews, 1991).

Do severe viral strains exist? Yes, but they are predominantly seen in commercial plantings where plants are well maintained and are of one uniformly susceptible genotype, which greatly facilitates transmission via vectors (e.g., aphids) that travel a short distance. In unmanaged ecosystems, plants are not well maintained (i.e., no irrigation, fertilizer, or pesticides) and the aphid vectors may feed on nonhost plants, thus losing the virus.

The movement of the virus resistance genes from CZW-3 to FLCP plants should not have a significant negative impact on FLCP plants. Wilson (1992) states, ". . . any genetically transmissible trait that provides enhanced fitness **in the wild** is cause for concern". Foreign genes (e.g., virus resistance genes) are most likely to be retained in a population if they confer a reproductive advantage to the plant containing the foreign gene over other competitors in the population. Since all evidence supports the conclusion that FLCP populations are **not** under significant environmental stress from viral infection, the resistance genes should not have significant impact on the natural populations of FLCP plants.

Will Hybrids Between CZW-3 Squash and FLCP Plants Persist in the Environment and Become Weeds?

Conclusion: There is no scientific or anecdotal evidence that supports the contention that hybrids between yellow crookneck squash and FLCP plants are weeds and are persistent.

Traditional plant breeding methods have been used for centuries to develop squash varieties that exhibit improved ability to resist environmental stresses, both biotic and abiotic. During the past century many disease resistant varieties have been developed and cultivated throughout the world. FLCP plants have grown in proximity to new, improved cultivars of squash, and yet there have been no reports in the scientific literature to suggest that disease resistance traits have introgressed into FLCP plants to produce hybrid populations that pose increased problems as weeds. There is no reason to believe that the viral resistance associated with CZW-3 squash will impact FLCP populations differently from viral resistance introduced into squash cultivars by traditional breeding. This includes the new Harris Moran zucchini squash, developed by traditional breeding techniques, that is resistant to ZYMV and WMV-2. Virus resistant zucchini squash (specific virus not described but possibly CMV) and CMV-resistant marrow squashes are available commercially from Thompson and Morgan, Inc. of Jackson, New Jersey. Also, several traditionally bred virus resistant cultivars developed by Asgrow or Cornell University are, or shortly will be, on the market.

Asgrow has supplied to APHIS additional information of ongoing field tests with hybrid plants derived from controlled crosses of ZW-20 (a line with resistance to two viruses, ZYMV and WMV-2) with FLCP plants. Based upon the limited observations in field tests, the FLCP x ZW-20 hybrids do not appear to be strong competitors when growing in fields that have not been tilled to remove competing wild plants based on survival of plants and seed set. These field tests have been conducted with several hundred hybrid progeny growing at a single site (data report 93-041-01). Whereas these results cannot predict the behavior of any future hybrid progeny when FLCP plants are pollinated by CZW-3 plants, the evidence supports APHIS' contention that the introgression of the virus resistance from CZW-3 into FLCP plants does not appear to pose a risk of developing a weed pest.

Genes from nonsexually compatible plants have been introduced into *C. pepo*. Genes from nonsexually compatible plants have been previously introduced into *C. pepo*. CMV, WMV2, ZYMV, and PRSV resistance genes from *C. ecuadorensis*, *C. martinezii*, and *C. moschata* (Gilbertalbertini et al., 1993); fruit fly resistance from *C. maxima* (Nath, 1975); trifluralin herbicide tolerance from *C. moschata* (Adenijji and Coyne, 1981); powdery mildew and scab resistance from *C. martinezii* (Kyle et al., 1993) have been or are being introduced into *C. pepo* by classical breeding techniques. *C. martinezii* and *C. ecuadorensis* are not sexually compatible with *C. pepo*, but through a series of bridging crosses (i.e., crosses with other species compatible with both) the genes have been moved into domestic squash plants. The risk of gene pool corruption from CZW-3 is no greater than has been accepted without alarm in the past with no noted ill effects.

The CZW-3 Squash Should Not Cause Damage to Processed Agricultural Commodities

There is no reason to believe that the development of virus-resistant squash plants would result in a change in fresh marketing or processing procedures. Most yellow crookneck squash is consumed as a raw table vegetable or processed for the frozen food market.

The CZW 3 Squash Should Not Increase the Likelihood of the Emergence of New Plant Viruses

Conclusion: Based on the known physical and biological properties of the CMV, ZYMV, and WMV-2, the likelihood of the appearance of masked plant viruses or a new plant virus with novel biological properties through field cultivation of CZW-3 plants is no greater than in naturally occurring potyvirus-infected squash.

A. 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 of coat proteins of both viral strains, the phenomenon is called phenotypic mixing. If the virus is encapsidated of 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 (mixed encapsidation) 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 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 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.

For some viral taxa, a protein other than CP is the primary determinant of whether a specific organism can successfully transmit a virus. These taxa include: potyviruses, caulimoviruses, and waikaviruses (Murphy et al. 1995). This vector transmission protein is called "helper component" in potyviruses and "aphid helper transmission factor" in caulimoviruses (Murphy et al 1995). Unless the appropriate vector transmission protein is present and functional, transcapsidated virions assembled with CP from vector transmissible strain will not be efficiently transmitted by the "heterologous" insect vector (Berger et al., 1989; Atreya et al., 1990). In contrast, viral CPs apparently are the primary determinants for insect-transmissibility for cucumoviruses (Matthews, 1991).

As mixed infections by plant viruses of all taxonomic types are common in nature (Zink and Duffus, 1972; Davis and Mizuki, 1987; Duffus, 1963), it is likely that there are many as yet unrecognized examples of heterologous transcapsidation interaction that naturally occur between plant viruses. However, research thus far indicates that heterologous transcapsidation interactions occur only in specific interactions in most mixed infections. There is evidence for both traditional and transgenic virus resistant plants that transcapsidation may occur (Rochow, 1972; Matthews, 1991; Farnelli et al., 1992; Osbourn et al., 1990; Dalmay et al. 1992; Holt and Beachy, 1992; Candelier and Hull, 1993).

Two issues are important to be addressed in considering the likelihood and significance in any potential instance of transcapsidation in transgenic plants.

- Is there a sufficient amount of coat protein being produced by the transgenic plant to produce a masked virus? Is the CP found in the same or different tissue(s) where the virus is detected in a nontransgenic plant?

- If a masked virus were produced, would it have any new biological properties (vector transmission and host range) and would any effects resulting from transcapsidation be measurable or significant?

One way in which scientists have sought to assess potential transcapsidation frequency in transgenic virus resistant plants has been to compare the amount of the engineered coat protein in the transgenic plants with the amount of coat protein in a similar, but susceptible, nontransgenic plant. One hypothesis has been that comparable or smaller amounts of coat protein would lead to the prediction that the transcapsidation frequency will be comparable or reduced from the frequency that occurs in naturally occurring mixed infections. A second consideration would be whether the transgene CP is synthesized in the same tissues that the virus naturally infects in nontransgenic plants. If CP synthesis takes place in the same tissues then no new interactions with other viruses that may be limited to other plant tissues can occur.

Asgrow has submitted data that show that the levels of the three CP's in healthy CZW-3 is significantly less (approximately 100 fold or more) than the amount of CP found in virus-infected nontransgenic control plants. Farnelli *et al.* (1992) demonstrated that the amount of CP transgene that can be detected in transgenic plants can be higher when the transgenic plants are infected with a taxonomically related virus that they are not resistant to. It is hypothesized that the transgene CP is stabilized in the masked virus particles produced upon infection. Asgrow inoculated CZW-3 with three viruses that provided the CPs of the transgenes and PRSV. As expected, CZW-3 was resistant to infection by CMV, ZYMV, and WMV-2. The amount of CP produced in singly or multiply infected CZW-3 was approximately the same as produced in healthy CZW-3. CZW-3 was susceptible to PRSV infection and the amount of PRSV CP produced in infected plants was approximately the same as was detected in inoculated nontransgenic plants. Thus, CZW-3 produces significantly less CPs than virus-infected nontransgenic plants.

CMV, ZYMV and WMV-2 infection of squash results in systemic infection (Lisa and Lecoq 1984; Purcifull *et al.* 1984; Francki *et al.* 1979). Systemic infection results in virus being present in both vascular, root, and leaf tissues throughout the plant. The CP genes of CZW-3 are driven by the cauliflower mosaic 35S promoter that results in production of CP in the same tissues (Benfey et al. 1990).

The three aphid species involved in the dissemination of CMV, ZYMV, and WMV2 are widespread in the United States. Most potyviruses and cucumoviruses are transmitted by many aphid species in a nonpersistent manner. The most important and widespread of these aphid vectors are *Myzus persicae, Aphis gossypii,* and *Macrosiphum euphorbiae* (Lisa and Lecoq, 1984; Purcifull *et al.* 1984; Francki *et al.* 1979; Perring et al., 1992). The main features of non-persistent transmission are that the virus can be picked up by the aphid after as little as 15 seconds on the infected plant and can transmit it immediately to one or only a few healthy plants. These brief acquisition and inoculation times limit the usefulness of insecticides to reduce the spread of these viruses. Most research suggests that viral infections generally originate locally (less than one-quarter of a mile distant) and that long-range emigration of viruliferous aphids is rare (Perring et al., 1992; Adlerz, 1978).

The most likely candidates for masking are other viruses that infect squash including papaya ringspot potyvirus, tomato ringspot nepovirus, tobacco ringspot nepovirus, and squash mosaic comovirus. Only PRSV is likely to be transcapsidated by the potyviral-derived transgenes because the other common viruses are not taxonomically related to the viral transgenes. If masked PRSV or another squash-infecting potyvirus or cucumovirus, containing CP of CMV, ZYMV or WMV-2, was produced, the masked virus would not gain any significant advantage in its ability to be transmitted by aphids or to be transmitted to new plant hosts since the three most common vectors for all three viruses are the same: *A. gossypii*, *M. euphorbiae*, and *M. persicae* (Perring et al., 1992). In fact, these are the most common aphid vectors of potyviruses in temperate regions of the United States. If masked virus were produced, it could only be maintained in the population as long as the virus replicated in CZW-3 plants or plants infected with , CMV, ZYMV and/or WMV2. Once the masked virus was transmitted to a nontransgenic or an uninfected plant, only the original virus would be produced because the CMV, ZYMV and/or WMV2 CP would not be available for the production of masked virus. Secondly, no novel interaction could occur since the CPs in CZW-3 are already present in virus-infected squash plants and real or hypothetical mixed virus could already occur. APHIS would note that in one field study funded under USDA's Risk Assessment grant program addressing the potential for transcapsidation with transgenic plants expressing CMV CP, no masked virus was detected in a several year study even though the experimental design favored transcapsidation events (Gonsalves et al. 1994; Fuchs and Gonsalves, 1995).

Two published reports have reached certain conclusions about the potential risk concerns posed by transcapsidation. In its report to the United Kingdom Ministry of Agriculture, Fisheries, and Food entitled "Risks to the Agricultural Environment Associated with Current Strategies to Develop Virus Tolerant Plants Using Genetic Modification", Henry et al. (1995) state, "The general view is that transcapsidation is not a problem, because it is limited to a single transfer, i.e. once a transcapsidated genome is introduced into a new host, it reverts to using its own CP..". In the report of a workshop on virus resistance prepared by the American Institute of Biological Sciences (AIBS) for the U.S. Department of Agriculture, a similar finding is reached (AIBS, 1995). "Transcapsidation of viral RNAs with coat protein produced by transgenic plants should not have long-term effects, since the genome of the infecting virus in not modified."

In conclusion, the formation of masked viruses in CZW-3 is no more likely than in naturally occurring virus infected squash for the following reasons. The amount of CP produced by CZW-3 is less than in produced in naturally infected squash and the transgene CP are produced in same tissues as they are found in naturally infected squash plants, thus there are no novel viral interactions expected. Even if masked virus is produced, masked virus would have biological properties identical to those that can occur in naturally-infected squash plants.

B. 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. In a large number of these studies (Atebekov and Talinsky, 1990), it has not been determined whether the coat protein is solely responsible for this helper dependent movement, but for viral taxa where a dedicated movement protein has not been described consideration that the coat protein is the primary determinant of movement should be noted. The 3a gene has been identified as the dedicated movement protein for CMV (Hull and Maule, 1985). Although a dedicated movement protein has not been identified in potyviruses, 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 new novel interactions with subliminally-infecting viruses. With CZW-3, if the CP transgenes facilitated the movement of subliminally infecting viruses, the only impact would be diseased CZW-3 plants. Whether the virus whose movement was facilitated could move from CZW-3 to other host plants would depend on its mode of transmission. Since the CP transgenes in CZW-3 are all from viral strains that routinely infect the cucurbit family, it is not expected subliminally infecting viruses will present a problem any more serious than can occur in naturally infected squash plants.

C. 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.

Factors that influence recombination rates and detection of a viable recombinant include: sequence and structural similarity between the nucleic acid molecules, subcellular location and concentration of the nucleic acids, and the number of recombinational events required to form a viable recombinant viral genome (Lai, 1992). The frequency of recombination between two naturally occurring viruses or two viral strains in field-grown plants in the absence of selection pressure has not been determined (Henry et al., 1995) and is difficult or impossible to measure meaningfully. In transgenic plants expressing sequences derived from either a DNA virus (Schoelz and Wintermantel, 1993) or RNA virus (Greene and Allison, 1994), it has been demonstrated that recombination between a viral transgene and a defective challenge virus can restore an functional, infective virus. These results demonstrate that recombinational events occur in plants expressing viral sequences when inoculated with defective viruses but say little about what happens with nondefective viruses replicating in resistant transgenic plants. Recombination is hypothesized as an important mechanism for virus change over evolutionary time frames and may have been quite frequent over this time (Simon and Bujarski, 1994). Recently, the nucleotide sequences of numerous viral strains from many of the known genera have been published. Sequencing data have shown that certain genes in quite different taxa probably arose from recombinational events. In other cases, a single strain of a virus has been found to contain sequences apparently derived from a virus for a different taxa while all other closely related strains do not have these sequences (Koonin and Dolja , 1993, Murphy et al., 1995, Sano et al., 1992; Edwards et al., 1992; LeGall et al., 1995; Pappu et al., 1994; Mayo and Jolly, 1991). Currently, it is not possible to determine whether these recombinational events occurred, for example, since the development of modern agricultural cropping practices or in much longer time frames. However, there is evidence of virus genome stability in shorter time frames, i.e., since the establishment of plant virology as a science . First, the biological properties of TMV have remained remarkably stable over the past century (Ford and Tolin, 1983; Dawson, 1992) and second, the Dutch and Wisconsin (U.S.) substrains of alfalfa mosaic alfalmovirus strain 425 have acquired, in approximately 20 years of laboratory use in each country, several nucleotide changes leading to five amino acid changes with apparently no significant changes in biological properties (Jaspars, 1985).

The use of virus-resistant transgenic plants in agriculture highlights the following questions regarding recombination when transgenic plants are used:

i) What factors may affect the rate of recombination, and will that rate be proportional to the concentrations to transgene RNA molecules?

ii) Are any recombinants thus formed likely to be successful in competition with parental viruses?

CZW-3 squash, like most transgenic plants field tested to date in the U.S. under APHIS oversight, contain CP genes from viruses that regularly infect the host plant, because damage by those viruses poses the most constant potential for loss in the crop species. Sequences from those viruses, when available for recombination, would be unlikely to pose the potential for generating novel recombinants in comparison with natural mixed infections in the recipient plant if the RNA is produced in the same cells as it is found during natural infection and its concentration is less than the amount found in natural infection. As described above in section on transcapsidation, the transgene RNA (and thus the CP) in CZW-3 are produced in the same cells as they would be found in naturally infected squash plants. Thus, the transgene RNA is unlikely to be available to recombine with novel plant or viral RNAs.

With respect to amount of transgene RNA available for recombiantional event, Asgrow has provided data to support that the concentration of transgene RNA in CZW-3 is approximately 100-fold less than the amount CP mRNA in viral-infected nontransgenic plants. APHIS notes a discussion on the this issue in the AIBS (1995), "The implications of these low expression levels for recombination are not clear. Even assuming that the higher concentration of transgene RNA the greater the chance for recombination, we do not know what a meaningful range is; what are low and high concentrations of transgene transcript relative to unacceptable recombination rates?" APHIS believes that the significantly lower concentration of transgene RNA in CZW-3 is reassuring considering recombination has not been detected in transgenic plants with nondefective viruses and the other points raised in this section.

If a recombinant virus is formed in a cell (either in a transgenic plant or during a mixed infection) will that recombinant participate in the replication process in that cell, move systemically in the plant, or cause a new disease? The vast majority of progeny viruses do not apparently function in the replication process. For many viruses, the RNA is encapsidated by CP, viral RNA synthesis in the cell ceases or declines to undetectable levels, and depending on the virus and unless it is transmitted to another plant or via progeny, is degraded when the plant cell dies (Matthews, 1991). The likelihood of a recombinant becoming established depends on many factors, including: its competitiveness with infecting virus and other viruses that naturally infect the plant and by all the additional factors that may affect selection pressure (e.g. temperature, vectors, host plants). Thus, to predict the probability of development of new virus disease resulting from recombination of two viruses or between a virus and a viral derived transgene, requires a considerable level of understanding of the population biology of viruses in cells and virus movement within plants, and a better understanding of the mechanisms of how viruses cause disease.

Thus, based on the above points APHIS believes that because the viral transgene is derived from virus that naturally infects the squash host, is synthesized in the same tissues as in the naturally-infected plants, is produced in less concentration than during natural infections, and if a recombinant was formed would have to be competitive with other squash-infecting viruses. APHIS believes that even if a recombinant virus did occur that this virus could be managed just like the numerous new viruses that are detected every year in the United States.

In a report to Agricultural and Agri-Food Canada on this subject, Rochon et al. (1995) conclude, "It is likely that current means of detecting and controlling new diseases in this country would be adequate to control any new virus resulting from recombination between a transgene and another virus." The AIBS report to USDA (1995) concludes by stating, "With or without the use of transgenic plants, new plant virus diseases will develop that will require attention." **APHIS concurs with their statements.**

D. 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). Synergistic infections can often result in severely diseased, unmarketable crops. Synergy was first described and is best studied with PVX and PVY. The majority of synergistic viral combinations include, as one the viruses, a potyvirus. Expression of portions of the potyviral genome in transgenic plants has allowed identification of the 5'-end of the genome, the N-protease, helper component/protease, and the 50 kilodalton protein of unknown function (Vance et al., 1995) as the sequences involved in potyviral synergism. Since potyvirus CP genes are not involved in synergism, it is unlikely that infection of a transgenic potyvirus-resistant plant with any other virus would result in a synergistic interaction. The identity of that gene is under investigation, and the search has been narrowed to three potential candidate genes on the. (Preliminary indications are that the single gene responsible for the synergism symptom is the helper component-protease gene in PVY and potato X potexvirus and that the <u>same</u> gene is responsible for another synergistic symptom between PVY and tobacco mosaic tobamovirus (Vance, unpublished data)). Synergism has been reported between CMV and ZYMV infections in cucumber (Poolpol and Inouye, 1986).

Even though there is no evidence to suggest that potyviral CP are involved in synergy, Asgrow inoculated CZW-3 with several common squash-infecting viruses. No synergistic symptoms were seem in virus infected plants. Even if highly unlikely occurrence, synergistic symptoms occurred in CZW-3 plants, this would only be an agronomic phenomenon and have no longer term environmental impact (AIBS, 1995).

With respect to FLCP plants and the viral events described in this section, there is no evidence that their susceptibility or resistance to common squash-infected viruses are significantly different than domesticated squashes (Provvidenti 1990; Provvidenti *et al.* 1978). Thus, APHIS believes that outcomes of these events in FLCP plants would not be significantly different than in domesticated squashes.

The CZW-3 Squash 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 CZW-3 squashes, based on two lines of reasoning:

(1) No direct pathogenic properties, nor any hypothetical mechanisms for pathogenesis toward beneficial organisms, such as bees and earthworms, were identified for CZW-3 squash. APHIS also cannot envision any plausible mechanisms for any hypothetical pathogenetic effect since the two proteins engineered in CZW-3 are already present in high concentration in naturally infected squash plants.

(2) Cucurbitin, a naturally occurring toxicant in squash plants, levels are likely to be unchanged and can be easily identified in cucurbit fruits by its bitter taste. Cucurbitin was not detected by taste-testing of fruits from CZW-3 and its descendants.

The definition of CZW-3 squash encompasses not only squash lines that already have been field tested, but also new squash lines that may be produced through conventional breeding using CZW-3 squash as one or both parents. APHIS believes that the analysis applied to CZW-3 squash already field tested will apply equally well to these new squash lines, and that the data provided by Asgrow justifies the conclusion that such new CZW-3 squash will not present a plant pest risk. The variation in agronomic characteristics among the CZW-3 squash lines that have been field tested does not differ significantly from that seen in commercial cultivars of squash 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 CZW-3 squash and another squash - cultivar, cross-breeding between well-characterized squash varieties is the traditional means by which new and improved squash varieties are created. These crosses have often used as one-parent squash cultivars that are considerably more genetically different from standard commercial cultivars than are CZW-3 squashes, i.e., other members of the genus *Cucurbita*.

The CZW-3 Squash May Reduce Insecticide Usage Under Certain Environmental Conditions

If virus resistant squashes, developed by genetic engineering or by traditional breeding techniques, become widely accepted, the use of certain agricultural chemicals may be reduced. Whether there is or is not a reduction at any specific production site probably depends on whether whiteflies are a major problem at that site. Because precise data on the amount of insecticide used exclusively on yellow crookneck squash is not available, APHIS cannot hypothesize on the absolute amount of any reduction in use but we do not think that speculation on the possible reduction in pesticide use will have any bearing on our determination or FONSI. We would hypothesize that a reduction, if any, in insecticide use would be minor relative to total insecticide use on U.S. crops.
A reduction in usage would be most likely in States where whiteflies are only a minor pest. In States where whiteflies are a major problem we might predict that insecticide use would be unlikely to change. Whiteflies are a major problem in the Southern tier of States from North Carolina to California. Cucurbit viruses are problems in many of these States (APHIS, 1994). If whiteflies are a major problem at the site, chemicals will probably still be applied. Without whiteflies, genetic resistance will probably be sufficient since aphids alone usually do not cause sufficient damage to warrant chemical application.

VI. CONCLUSION

In response to a petition from the Asgrow Seed Company, APHIS has evaluated information regarding the potential plant pest risks presented by the transgenic squash line designated as CZW-3. CZW-3 squashes have been transformed via an *Agrobacterium*-mediated protocol with the viral CP genes of cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus, type 2 (WMV2). Expression of the CMV, ZYMV, and WMV2 CP genes in CZW-3 squash does not cause plant disease, but rather confers resistance to infection by CMV, ZYMV, and WMV2. APHIS has determined that the CZW-3 squash does not pose a direct or indirect plant pest risk and therefore will no longer be considered a regulated article 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 those squashes or their progeny. (Importation of CZW-3 squashes [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 CZW-3 squash: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than a virus-resistant plant developed by traditional breeding techniques; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) should not cause damage to processed agricultural commodities; (5) should not increase the likelihood of the emergence of new plant viruses; and (6) is unlikely to harm other organisms, such as bees, which are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny CZW-3 squash varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the CZW-3 squash lines already field tested, or those observed for squashes in traditional breeding programs.

John H. Payne, Ph.D.
Acting Director Biotechnology,
Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date JUN 1 4 1996

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.

Adeniiji, A., and Coyne, D. P. 1981. Inheritance of resistance to trifluralin toxicity in Cucurbita moschata Poi. Horticultural Science 16:774-775.

Adlerz, W. C. 1978. Watermelon mosaic virus 2 epidemics in Florida 1967-1977. Journal of Economic Entomology 71:596-597.

Adlerz, W. C., Purcifull, D. E., Simone, G. W., and Hiebert, E. 1983. Zucchini yellow mosaic virus: A pathogen of squash and other cucurbits in Florida. Proceedings of Florida State Horticultural Society 96:72-74.

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.

Anderson, J. M., Palukaitis, P., Zaitlin, M. 1992. A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants. Proceeding of the National Academy of Sciences (USA) 89:8758-8763.

APHIS. 1994. Response to the Upjohn Company/Asgrow Seed Company Petition 92-204-01 for Determination of Nonregulated Status for ZW-20 Squash.

Atebekov, J.R., Takiansky, M. E. 1990. Expression of plant-virus encoded transport function by different viral genomes. Advances in Virus Research 38:201-248.

Atreya, C. D., Raccah, C. D., and Pirone, T. P. 1990. Amino acid substitutions in the coat protein results in loss of insect transmissibility of a plant virus. Proceedings of National Academy of Sciences (USA) 88:7887-7891.

Baker, H. G. 1965. Characteristics and modes of origin of weeds. *In*: The genetics of colonizing species, pp. 147-168. Baker, H. G., and Stebbins, G. L. (eds.) Academic Press, New York.

Benfey, P. N., Ren, L., and Chua, N-H. 1990. Tissue-specific expression from CaMV 35 S enhancer subdomains in early stages of plant development. The EMBO Journal 9:1677-1684.

Berger, P. H., Hunt, A. G., Domier, L. L., Hellman, G. M., Stram, Y., Thornbury, D. W., Pirone, T. P. 1989. Expression in transgenic plants of a viral gene product that mediates insect transmission of potyviruses. Proceedings of National Academy of Sciences (USA) 86:8402-8406.

Bourdin, D., and Lecoq, H. 1991. Evidence that heteroencapsidation between two potyviruses is involved in aphid transmission of a non-aphid-transmissible isolate from mixed infection. Phytopathology 81:1459-1464.

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

Candelier, H. P., Hull,. R. 1993. Cucumber mosaic virus genome is encapsidated in alfalfa mosaic virus coat protein expressed in transgenic tobacco plants. Transgenic Research 2:227-285.

Chala, V. H., Harrison, C. W., and Halliwell, R. S. 1986. Identification of two distinct strains of watermelon mosaic virus 2 affecting cucurbits in Texas. Plant Disease 71:750-752.

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.

Cuozzo, M., O'Connell, K. M., Kaniewski, W., Fang, R.-X., Chua, N.-H., Tumer, N. E. 1988. Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. Bio/Technology 6:549-557.

Dalmay, T., Rubino, L., Burgyan, J., Russo, M. 1992. Replication and movement of a coat protein mutant of cymbidium ringspot tombusvirus. Molecular Plant-Microbe Interactions 5: 379-383.

Davies, C., Symons, R. H. 1988. Further implications for the evolutionary relationships between tripartite plant viruses based on cucumber mosaic virus RNA 3. Virology 165:216-224.

Davis, R. F., Mizuki, M. K. 1987. Detection of cucurbit viruses in New Jersey. Plant Disease 71:40-44.

Dawson, W. O. 1992. Tobamovirus-plant interactions. Virology 186:359-367.

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.

Decker, D. S. 1988. Origin(s), evolution, and systematics of *Cucurbita pepo* (*Cucurbitaceae*). Economic Botany 42:4-15.

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.

Duffus, J. E. 1963. Incidence of beet viruses in relation to overwintering beet fields. Plant Disease (Reporter) 47:428-431.

Edwards, M. C., Petty, I. T. D., Jackson, A. O. 1992. RNA recombination in the genome of barley stripe mosaic virus. Virology 189:389-392.

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.

Falk, B. W., and Bruening, G. 1994. Will transgenic crops generate new viruses and new diseases. Science 263:1395-1996.

Farnelli, L., Malone, P., and Collet, G. F. 1992. Heterologous encapsidation of potato virus Y strain O (PVY-O) with the transgenic coat protein of PVY strain N (PVY-N) in *Solanum tuberosum* cv Bintje. Bio/Technology 10:1020-1025.

Ford, R., Tolin, S. A. 1983. Genetic stability of tobacco mosaic virus in nature. Tobacco Science 27:14-17.

Francki, R. I. B., Mossop, D. W., Hatta, T. 1979. Cucumber mosaic virus. CMI/AAB Description of Plant Viruses, number 213. Association of Applied - Biologists, Warwick, United Kingdom.

Fuchs, M, Gonsalves, D. 1995. Risk assessment of virus spread and gene introgression using transgenic vegetable crops expressing viral coat protein. Abstracts of the Annual Meeting of the American Phytopathology Society, p. 186.

Gilbertalbertini, F., Lecoq, H., Pitrat, M., and Nicolet, J. L. 1993. Resistance of *Cucurbita moschata* to watermelon mosaic virus type 2 and its genetic relationship to resistance to zucchini yellow mosaic virus. Euphytica 69:231-237.

Gonsalves, D., Fuchs, M., Klas, F., Tennet, P. 1994. Field assessment of risks using papaya, cucurbits, and tomatoes expressing novel coat protein genes. pp. 117-127. In:

Proceedings of the 3rd International Symposium on "The Biosafety of Field tests of genetically modified plants and microorganisms. Monterey, California.

Gordon, K. H. J., Gill, D. S., Symons, R. H. 1982. Highly purified cucumber mosaic virus- induced RNA-dependent RNA polymerase does not contain any of the full length translation products of the genomic RNAs. Virology 123:284-295.

Gould, A. R., Symons, R. H. 1982. Cucumber mosaic virus RNA 3: Determination of the nucleotide sequence provides the amino acid sequences of protein 3a and viral coat protein. European Journal of Biochemistry 126:217-226.

Gray, S. M., Moyer, J. W., Kennedy, G. G., and Campbell, C. L. 1986. - Virus-suppression and aphid resistance effects on spatial and temporal spread of watermelon mosaic virus 2. Phytopathology 76:1254-1259.

Greene, A. E., and Allison, R. F. 1994. Recombination between viral RNA and transgenic plant transcripts. Science 263:1423-1425.

Hammond, J., Kamo, K. K. 1995. Effective resistance to potyvirus infection conferred by expression of antisense RNA in transgenic plants. Molecular Plant-Microbe Interactions 8:674-682.

Henry, C. M., Barker, I., Pratt, M., Pemberton, A. W., Farmer, M. J., Cotten, J., Ebbels, D., Coates, D., Stratford, R. 1995. Risks associated with the use of genetically modified virus tolerant plants. A report to Ministry of Agriculture Fisheries and Food (MAFF), United Kingdom.

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.

Holt, C. A., Beachy, R. N. 1992. *In vivo* complementation of infectious transcripts from mutant tobacco mosaic virus cDNA's in transgenic plants. Virology 181:109-117.

Hooker, W. J. 1981. Compendium of potato diseases. American Society of Phytopathological Society, St. Paul, Minnesota. 125 pp.

Hull, R., Maule, A. J. 1985. Virus Multiplication. pp. 83-116. In: The Plant Viruses, vol. 1. Francki, R. I. B. (ed.). Plenum Press, New York.

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

Jaspars, E. M. J. 1985. Interaction of alfalfa mosaic virus nucleic acids and protein. pp. 155-221. In: Molecular Plant Virology, vol. 1. Davies, J. W. (ed.). CRC Press, Boca Raton, Florida.

Kaper, J. M., and Waterworth, H. E. 1981. Cucumoviruses. *In*: Handbook of Virus Infections: Comparative Diagnosis. E. Kurstak, ed. pp. 257-332. Elsiever-North Holland, Amsterdam.

Keeler, K. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Klee, H. J., and Rogers, S. G. 1989. Plant gene vectors and genetic transformation: plant transformation systems based on the use of *Agrobacterium tumefaciens*. Cell Culture and Somatic Cell Genetics of Plants 6:1-23.

Koonin, E. V., Dolja, V. V. 1993. Evolution and taxonomy of positive-stranded RNA viruses: Critical Reviews in Biochemistry and Molecular Biology 28:375-430.

Kyle, M., Moriarty, G., and Munger, H. M. 1993. Cucumber, melon, and squash germplasm from the Cornell collection. Cucurbit Genetics Cooperative 16:90-91.

Lai, M.M.C. 1992. RNA recombination in animal and plant viruses. Microbiological Reviews 56:61-79.

Lawson, C., Kaniewski, W., Haley, L., Rozman, R., Newell, C., Sanders, P., Turner, N. 1990. Engineering resistance to mixed virus infection in a commercial potato cultivar : Resistance to potato virus X and potato virus Y. Biotechnology 8:127-134.

Lecoq, H., Ravelondandro, M., Wipf-Schelbel, C., Monslon, M., Raccah, B., Dunez, J. 1993. Aphid transmission of a non-aphid transmissible strain of zucchini yellow mosaic potyvirus from transgenic plants expressing the capsid protein of plum pox potyvirus. Molecular Plant-Microbe Interactions 6:403-406.

Lecoq, H., Pitrat, M., and Clemant, M. 1981. Identification et caracterization d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. Agronomie 1:827-834.

LeGall, O., Lanneau, M., Candresse, T., Dunez, J. 1995. The nucleotide sequence of the RNA-2 of an isolate of the English serotype of tomato black ring virus: RNA recombination in the history of nepoviruses. Journal of General Virology 76;1279-1283.

Lindbo, J. A., Dougherty, W. G. 1992b. Untranslatable transcripts of the tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology 189:725-733.

Lindbo, J. A., Dougherty, W. G. 1992a. Pathogen-derived resistance to a potyvirus: Immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence. Molecular Plant-Microbe Interactions 5:144-153.

Lisa, V., Lecoq, H. 1984. Zucchini yellow mosaic virus. CMI/AAB Description of Plant Viruses, number 282. Association of Applied Biologists, Warwick, United Kingdom.

Lot, H., Marchoux, G., Marrou, J., Kaper, J. M., West, C. K., van Volten-Doting, L., Hull, R. 1974. Evidence for three functional RNA species in several strains of cucumber mosaic virus. Journal of General Virology 22:81-93.

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

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

Mayo, M. A., Jolly, C. A. 1991. The 5'-terminal sequence of potato leafroll RNA: evidence of recombination between virus and host RNA. Journal of General Virology 72:2591-2595.

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.

Nath, P. 1975. Breeding vegetable crops for resistance to diseases in India. Sabrao Journal 7:7-11.

National Research Council. 1989. Field Testing Genetically Modified Organisms: Framework for Decisions. National Academy Press, Washington, DC. 170 pp.

Osburn, J. K., Sarkar, S., Wilson, T. M. A. 1990. Complementation of coat protein-defective TMV mutants in transgenic plants expressing coat protein. Virology 179:921-925.

Pappu, H. R., Karasev, A. V., Anderson, E. J., Pappu, S. S., Hilf, M. E., Febres, V. J., Eckloff, R. M. G., McCaffery, M., Boyko, V., Gowda, S., Dolja, V. V., Koonin, E. V., Gumpf, D. J., Cline, K. C., Garnsey, S. M., Dawson, W. O., Lee, R. F., Niblett, C. L. 1994. Nucleotide sequence and organization of eight 3' open reading frames of the citrus tristeza closterovirus genome. Virology 199:35-46.

Perring, T. M., Farrar, C. A., Mayberry, K., and Blua, M. J. 1992. Research reveals pattern of cucurbit virus spread. California Agriculture 46: 35-40.

Poolpol, P., and Inouye, T. 1986. Enhancement of cucumber mosaic virus multiplication by zucchini yellow mosaic virus in doubly infected cucumber plants. Annals Phytopathology Society of Japan 52:22-30.

Powell Abel, P., Nelson, R. S., De, B., Hoffmann, N., Rogers, S. G., Fraley, R. T., and Beachy, R. N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

Provvidenti, R., Robinson, R. W., and Munger, H. M. 1978. Resistance in feral species to six viruses infecting *Cucurbita*. Plant Disease (Reporter) 62:326-329.

Provvidenti, R. 1990. Viral disease and genetic sources of resistance in *Cucurbita* species. pp. 427-435. *In*: The Biology and Utilization of Cucurbitacae. Bates, D. M., Robinson, R. W., and Jefferys, C. (eds.). Cornell University Press, Ithaca, New York.

Provvidenti, R., Gonsalves, D., and Humaydan, H. S. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida and California. Plant Disease 68:443-446.

Purcifull, D., Hiebert, E., Edwardson, J. 1984. Watermelon mosaic virus 2. CMI/AAB Description of Plant Viruses, number 293. Association of Applied - Biologists, Warwick, United Kingdom.

Purrington, C. B., Bergelson, J. 1995. Assessing weediness of transgenic crops: Industry plays plant ecologist. Trends in Ecology and Evolution 10:340-342.

Register, J. C., and Nelson, R. S. 1992. Early events in plant virus infections: relationships with genetically engineered protection and host gene resistance. Seminars in Virology 3:441-451.

Rezaian, M. A., Williams, R. H. V., Gordon, K. H. J., Gould, A. R., Symons, R. H. 1984. Nucleotide sequence of cucumber mosaic virus RNA 2 reveals a translation product significantly homologous to corresponding proteins of other viruses. European Journal of Biochemistry 143:277-284.

Rochon, D. M., Ellis, P. E., Martin, R. R., Sanforn, H. 1995. Transgenic plants expressing viral genes: Issues related to field releases. A report of Agricultural and Agri-Food Canada. 24pp.

Rochow, W. F. 1972. The role of mixed infections in the transmission of plant viruses by aphids. Annual Review of Phytopathology 10:101-1124.

Sano, Y., Van der Vlugt, P., de Haan, P., Takahashi, P., Goldbach, R., Kojima, M. 1992. On the variability of the 3'-terminal sequences of the turnip mosaic virus genome. Archives of Virology 126:231-238.

Schoelz, J. E., Wintermantel W.M.. 1988. Expansion of viral host range through complementation and recombination in transgenic plants. Plant Cell 5:1669-1679.

Shew, H. D., and Lucas, G. B. 1991. Compendium of tobacco diseases. American Phytopathological Society, St. Paul, Minnesota. 68 pp.

Simon, A. E., Bujarski, J. J. 1994. RNA-RNA recombination and evolution in virus-infected plants. Annual Review of Phytopathology 32:337-362.

Smith, H. A., Swaney, S. L., Parks, T. D., Wernsman, E. A., Dougherty, W. G. 1994. Transgenic plant virus resistance mediated by untranslatable sense RNAs: Expression, regulation, and fate of nonessential RNAs. Plant Cell 6:1441-1453.

Swaney, S., Powers, H., Goodwin, J., Rosales, L. S., Dougherty, W. G. 1995. RNA-mediated resistance with nonstructural genes from the tobacco etch virus genome. Molecular Plant-Microbe Interactions 8:1004-1011.

Symons, R. H. 1985. Viral Genome Structure. In: The Plant Viruses, Vol. 1. pp. 57-82. Francki, R. I. B. (ed.). Plenum Press, New York.

Symons, R. H. 1985. Viral Genome Structure. In: The Plant Viruses, Vol. 1. pp. 57-82. Francki, R. I. B. (ed.). Plenum Press, New York.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and Regal, P. J. 1989. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70:298-314.

Tien, P., Wu, G. S. 1991. Satellite RNA for the biocontrol of plant disease. In: Advances in Virus Research, vol. 39. pp.321-339. Maramorosch, K., Murphy, F. A., Shatkin, A. J. (eds.). Academic Press, London.

Timian, R. G. 1974. The range of symbiosis of barley and barley stripe mosaic virus. Phytopathology 64:342-345.

Vance, V. B., Berger, P. H., Carrington, J. C., Hunt, A. G., Shi, X. M. 1995. 5' proximal potyviral sequences mediate potato virus X/potyviral synergistic disease in transgenic plants. Virology 206: 583-590.

Wang, H. L., Gonsalves, D., Provvidenti, R., and Lecoq, H. L. 1991. Effectiveness of cross protection by a mild strain of zucchini yellow mosaic virus in cucumber, melon, and squash. Plant Disease 75:203-207.

Weidemann, G. J., and Templeton, G. E. 1988. Efficacy and soil persistence of *Fusarium solani* f. sp. *cucurbitae* for control of Texas gourd (*Cucurbita pepo*). Plant Disease 72:36-38.

Williamson, M. 1994. Community response to transgenic plant release: Prediction from British experience of invasive plants and feral crop plants. Molecular Ecology 3:75-79.

Whitaker, T. W., Robinson, R. W. 1986. Squash breeding. In: Breeding Vegetable Crops. Basset, M. J. (ed.). AVI Publishing, Westpoint, Connecticut.

Whitaker, T. W., Davis, G. W. 1962. Cucurbits, botany, cultivation and utilization. Interscience Publishers Inc. New York.

Wilson, H. 1993. Free-living *Cucurbita pepo* in the United States. Viral resistance, gene flow, and risk assessment. Report to USDA Biotechnology, Biologics and Environmental Protection. 24 pp.

Yeh, S.-D., Gonsalves, D. 1985. Translation of papaya ringspot virus RNA *in vitro:* detection of a possible polyprotein that is processed for capsid protein, cylindrical inclusion protein, and amorphous inclusion protein. Virology 143:260-271.

Yie, Y., Tien, P. 1993. Plant virus satellite RNAs and their role in engineering resistance to virus diseases. Seminars in Virology 4:363-368.

Zambryski, P. 1988. Basic processes underlying *Agrobacterium* mediated DNA transfer to plant cells. Annual Review of Genetics 22:1-30.

Zink, F. W., Duffus, J. E. 1972. Association of beet western yellows and lettuce mosaic viruses with internal rib necrosis of lettuce. Phytopathology 62:1141-144.