United States Department of Agriculture
Animal and Plant Health Inspection Service

APHIS/USDA Petition 92-204-01 for Determination of Nonregulated Status for ZW-20 Squash

Environmental Assessment and Finding of No Significant Impact

December 1994

Finding of No Significant Impact

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture has conducted an environmental assessment prior to issuing a determination of the nonregulated status of a line of genetically engineered, virus resistant squash designated as ZW-20 squash. APHIS received a petition from the Upjohn Company/Asgrow Seed Company, regarding the status of the squash line ZW-20 as a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of Upjohn/Asgrow's petition and supporting documentation, as well as 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 the ZW-20 squash shall no longer be a regulated article.

Terry L. Medley, J.D.
Director
Biotechnology, Biologics, and Environmental Protection
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date: DEC 7 1994

Trade and company names are used in this publication solely to provide specific information. Mention of a trade or company name does not constitute a warranty or an endorsement by the U.S. Department of Agriculture to the exclusion of other products or organizations not mentioned.

Registrations of pesticides are under constant review by the U.S.Environmental Protection Agency (EPA). Only pesticides that bear the EPA registration number and carry the appropriate directions should be used.

List of Abbreviations

BWYV	Beet western yellows virus
CaMV	Cauliflower mosaic virus
CMV	Cucumber mosaic virus
CP	Coat protein
ELISA	Enzyme-linked immunoabsorbent assay
FLCP	Free-living *Cucurbita pepo*
mRNA	Messenger RNA
PRSV	Papaya ringspot virus (previously watermelon mosaic virus 1)
PVY	Potato virus Y
TMV	Tobacco mosaic virus
TVMV	Tobacco vein mottling virus
WMV2	Watermelon mosaic virus 2
ZYMV	Zucchini yellow mosaic virus

Table of Contents — Environmental Assessment

I. Summary 1

II. Background 3

A. Development of ZW-20 Squash 3

B. APHIS Regulatory Authority 3

III. Purpose and Need 4

IV. Alternatives 4

A. No Action 4

B. Determination That ZW-20 Squash is No Longer a Regulated Article 4

C. Determination That ZW-20 Squash is No Longer a Regulated Article Under Certain Geographic Limitations 5

V. Affected Environment and Potential Environmental Impacts 5

A. Potential for the Appearance of New Plant Viruses 5

B. Potential Impacts Based on Increased Weediness of ZW-20 Squash Relative to Traditionally Bred Squash 6

C. Potential Impacts on the Free-Living Relatives of Squash Arising From Pollination by ZW-20 Squash 6

D. Potential Impact on Nontarget Organisms, Including Beneficial Organisms Such as Bees and Earthworms 8

VI. Conclusion 9

VII. Literature Cited 10

IX. Agencies and Individuals Consulted 11

X. Preparers and Reviewers 11

XI. Agency Contact 12

Determination:

Response to the Upjohn Company/Asgrow Seed Company Petition for Determination of Nonregulated Status for ZW-20 Squash.

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 "ZW-20 squash." The developer of ZW-20 squash, the Upjohn Company/Asgrow Seed Company (Upjohn/Asgrow), petitioned APHIS requesting the determination on the regulated status of ZW-20, a plant that has been a regulated article under APHIS regulations. Under APHIS regulations, interstate movements and field tests of ZW-20 squash have required permits issued by APHIS. Upjohn/Asgrow has petitioned APHIS for a determination that ZW-20 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 ZW-20 squash has been developed to resist infection by two plant viruses that infect squashes. The genes conferring viral resistance in ZW-20 were introduced via genetic engineering techniques. These techniques enabled the developer to introduce into yellow crookneck squash viral coat protein genes from 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 ZW-20 squash plants to resist infection by ZYMV and WMV2. The genes were introduced into ZW-20 via a well-characterized procedure that utilizes the bacterium *Agrobacterium tumefaciens* to introduce genes into plant genomes.

From 1990 through 1993, APHIS has issued 14 permits to Upjohn/Asgrow to conduct 46 field tests in 10 States with ZW-20 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 ZW-20 squash. With respect to the unconfined growth of ZW-20 squash, APHIS has reached the following conclusions:

1. ZW-20 squash exhibits no plant pathogenic properties. Although plant pathogenic organisms were used in the development of ZW-20 squash, these squash plants are not infected, nor can they incite disease in other plants.

2. ZW-20 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 ZW-20 squash to resist infection by 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. ZW-20 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 ZW-20 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 ZW-20 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. ZW-20 squash should not cause damage to processed agricultural commodities.

5. ZW-20 should 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 ZW-20 squash would be no different than traditionally bred, virus resistant squash cultivars with respect to the appearance of new plant viruses.

6. ZW-20 squash 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 ZW-20 squash varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the ZW-20 squash lines already field tested, or those observed for squashes in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that ZW-20 squash will be just as safe to grow as virus resistant squash cultivars developed through traditional breeding practices. The cultivation of ZW-20 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 ZW-20 squash will no longer be a regulated article under the regulations in 7 CFR Part 340.

II. Background

A. Development of ZW-20 Squash

The ZW-20 squash has been developed to resist infection by two plant viruses that commonly infect squashes. The genes conferring viral resistance in ZW-20 were introduced via recombinant DNA (genetic engineering) techniques rather than conventional breeding techniques. The recombinant techniques enabled the developer to introduce two viral coat protein genes from plant viruses into the yellow crookneck squash variety YC77E. The genes were obtained from zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV2), two members of the potyvirus group of plant viruses. Incorporation of these genes into the squash to yield the ZW-20 plants does not cause plant disease, but rather enables the plants to resist infection by ZYMV and WMV2. The genes were introduced into ZW-20 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.

ZW-20 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 1993, APHIS has issued 14 permits for field tests of ZW-20 squash at 46 sites in 10 States. The field tests of ZW-20 have been conducted in controlled agricultural settings, under permit conditions that have stipulated physical and reproductive confinement of the ZW-20 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 Upjohn/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 ZW-20 squash as a regulated article under APHIS regulations. The developer of ZW-20 squash, Upjohn/Asgrow, submitted a petition to USDA/APHIS requesting that APHIS make a determination that ZW-20 squash shall no longer be considered a regulated article under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act of 1969 (NEPA) and the pursuant implementing regulations published by the Council on Environmental Quality (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274).

IV. Alternatives

A. No Action

Under the Federal "no action" alternative, APHIS would not come to a determination that ZW-20 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 ZW-20 squash. APHIS might choose this alternative if there were insufficient evidence to predict the lack of plant pest risk from unconfined cultivation of ZW-20 squash.

B. Determination That ZW-20 Squash is No Longer a Regulated Article

Under the Federal action to render a determination that ZW-20 squash is no longer a regulated article under the regulations at 7 CFR Part 340, ZW-20 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 ZW-20 squash or its progeny.

C. Determination That ZW-20 Squash Is No Longer a Regulated Article Under Certain Geographic Limitations

In this alternative, the geographic limitation would stipulate that ZW-20 squash could only be grown at sites that are not near populations of free-living *Cucurbita pepo* (FLCP). The intent of this condition would be to minimize the unintentional pollination of FLCP plants by locating ZW-20 squash fields outside the effective pollination distance for squash. Although this alternative was considered briefly, APHIS believes that such a determination is not warranted based upon the improbability of potential plant pest risks or impacts on the environment arising from the transfer of the virus resistance trait to FLCP.

V. Affected Environment and Potential Environmental Impacts

This EA addresses potential environmental impacts from a determination that ZW-20 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 ZW-20 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 ZW-20 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 ZW-20 squash, and another analysis that supports APHIS' conclusion that ZW-20 squash has no potential to pose plant pest risks.

A. Potential for the Appearance of New Plant Viruses

As mentioned above, ZW-20 squash was developed by engineering the viral coat protein genes of 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 ZW-20 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 (termed "transencapsidation"), which comprise the coat of the virus particle. It is theoretically

possible for new plant viruses to arise in the ZW-20 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 Issue 1 of the Determination document appended to this EA. After careful consideration of the physical and biological properties of ZYMV and WMV2, APHIS concluded that it is unlikely that new viruses will appear as a consequence of the widespread cultivation ZW-20 squash.

B. Potential Impacts Based on Increased Weediness of ZW-20 Squash Relative to Traditionally Bred Squash

APHIS evaluated whether the ZW-20 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 ZYMV and WMV2, is unlikely to make the ZW-20 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 ZW-20 squash becoming a weed (see the Determination, Issue 2).

No other attributes of ZW-20 squash suggest that it is any more "weedy" than squash cultivars that are the result of traditional breeding. The ZW-20 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 ZW-20 Squash

APHIS evaluated two potential impacts that ZW-20 squash might have on the free-living relatives of squash. First, that the traits from ZW-20 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 ZW-20 squash via pollen is possible to a limited number of squash relatives (see Appendix II of the Determination document for a detailed, technical discussion). In the United States, the squash relatives that might be successfully pollinated by ZW-20 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 Issue 4 of the Determination).

It is unlikely that offspring arising from natural crosses of FLCP and ZW-20 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, Issue 5). 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 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 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 Issue 3 of the Determination).

Based upon our analysis of the biology of cultivated squash and its relatives, APHIS concludes that the environmental impacts of cultivation of ZW-20 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 ZW-20 squash were to be cultivated in agricultural regions around centers of *C. pepo* diversity, there is no reason to expect impacts from ZW-20 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. The impact of cultivation of ZW-20 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 ZW-20 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 ZW-20 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 ZW-20 squash might indirectly harm plants or plant products (such as some agricultural commodities). APHIS considered the potential impact that ZW-20 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 ZW-20 squash will pose any deleterious effects or significant impacts on nontarget organisms, including beneficial organisms. The coat proteins expressed in ZW-20 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 ZW-20 squash (see Issue 1 of the Determination).

APHIS believes that ZW-20 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 ZW-20 squash would have any adverse effect on other organisms, including any species recognized as threatened or endangered in the United States. The release of ZW-20 squash from regulation should have no adverse impact on agricultural commodities.

VI. Conclusion

APHIS has evaluated information from the scientific literature as well as data submitted by Upjohn/Asgrow that characterize the ZW-20 squash. After careful analysis, APHIS has identified no significant impact to the environment from issuance of a determination that ZW-20 squash would no longer be a regulated article under APHIS regulations at 7 CFR Part 340.

APHIS has considered the foreseeable consequences of removing ZW-20 from its regulation and reached the following conclusions:

1. ZW-20 squash exhibits no plant pathogenic properties. Although plant pathogenic organisms were used in the development of ZW-20 squash, these squash plants are not infected, nor can they incite disease in other plants.

2. ZW-20 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 ZW-20 squash to resist infection by 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. ZW-20 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 ZW-20 squash to pollinate FLCP plants, the so-called "wild" relatives of cultivated squash. Although genes can move freely via pollen from ZW-20 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. ZW-20 squash should not cause damage to processed agricultural commodities.

5. ZW-20 should 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 ZW-20 squash would be no different than traditionally bred, virus resistant squash cultivars with respect to the appearance of new plant viruses.

6. ZW-20 squash is unlikely to harm other organisms, such as bees, that are beneficial to agriculture.

APHIS has also concluded that there is a reasonable certainty that new progeny ZW-20 squash varieties bred from these lines should not exhibit new plant pest properties, i.e., properties substantially different from any observed for the ZW-20 squash lines already field tested, or those observed for squashes in traditional breeding programs.

Therefore, after review of the available evidence, APHIS concludes that ZW-20 squash will be just as safe to grow as virus resistant squash cultivars developed through traditional breeding practices. The cultivation of ZW-20 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 ZW-20 squash will no longer be a regulated article under the regulations in 7 CFR Part 340.

VIII. 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.

IX. Agencies and Individuals Consulted

Dr. Gus deZoeten, Chairman, Department of Plant Pathology and Botany, Michigan State University
Dr. John Hammond, USDA-ARS
Drs. R. Provvidenti, and D. Gonsalves, Cornell University
Dr. Hugh D. Wilson, Texas A&M University
Environmental Protection Agency
Departments of Agriculture of the 50 States and Puerto Rico

X. Preparers and Reviewers

Biotechnology, Biologics, and Environmental Protection

Terry L. Medley, J.D., Director
John Payne, Ph.D., Associate Director

Biotechnology Permits

Arnold Foudin, Ph.D., Deputy Director
Subhash Gupta, Ph.D., Staff Biotechnologist
David S. Heron, Ph.D., Staff Biotechnologist (Chief Preparer)
Catherine Joyce, 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., Chief, Microorganisms Branch
Sally L. Van Wert, Ph.D., Staff Biotechnologist (former)
James L. White, Ph.D., Chief, Plants Branch (Chief Preparer)

Biotechnology Coordination and Technical Assistance

Michael A. Lidsky, J.D., LL.M., Deputy Director
L. Val Giddings, Ph.D., Chief, Science Policy Coord. Branch
Shirley P. Ingebritsen, M.A., Program Analyst
Michael Schechtman, Ph.D., Senior Microbiologist (Preparer)
Frank Y. Tang, Ph.D., J.D., Biotechnologist

Environmental Analysis and Documentation

Carl Bausch, J.D., Deputy Director

XI. Agency Contact

Ms. Kay Peterson, Regulatory Analyst
Biotechnology, Biologics, and Environmental Protection
USDA, APHIS
6505 Belcrest Road, FB-850
Hyattsville, MD 20782
Phone: (301) 436-7601
Fax: (301) 436-8669

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

Prepared by:

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

Table of Contents

I. Summary 1

II. Background 3

Regulatory Authority 3

Oversight by Other Federal Agencies 6

III. Response to Comments 7

The Taxonomy of *C. pepo* in the United States 11

Issue 1. Will the Introduction of ZYMV and WMV2 Coat Proteins Increase the Likelihood of Creating New Plant Viruses? 12

Issue 2. Could the Introduction of Two New Virus Resistance Genes Cause Squash to Become a Weed? 20

Issue 3. Will the Virus Resistance Genes Move to FLCP Plants, and Will This Have a Detrimental Impact? 22

Issue 4. Are FLCP Plants Serious Weeds? Will Virus-Resistant FLCP Plants Be More Difficult to Control Than Virus Susceptible Plants? 29

Issue 5. Will Hybrids Between ZW-20 Squash and FLCP Plants Persist in the Environment and Become Weeds? 30

Other Comments to the Draft EA/Determination 31

IV. Analysis of the Properties of ZW-20 Squash 33

The Introduced Genes, Their Products, and the Added Regulatory Sequences Controlling Their Expression Do Not Present a Plant Pest Risk in ZW-20 33

The ZW-20 Squash is No More Likely to Become a Weed Than a Virus-Resistant Plant Developed by Traditional Breeding Techniques. . 35

The ZW-20 Squash is Unlikely to Increase the Weediness Potential for Any Other Cultivated Plant or Native Wild Species With Which the Organism Can Interbreed 35

The ZW-20 Squash Should Not Cause Damage to Processed Agricultural Commodities 35

The ZW-20 Squash Should Not Increase the Likelihood of the Emergence of New Plant Viruses 35

The ZW-20 Squash Should Not be Harmful to Beneficial Organisms, Including Bees 36

IV. Conclusion 37

V. References 38

VI. Appendices 45

Appendix I. Distribution of WMV 2 and ZYMV, Their Plant Hosts and Insect and Leading Cucurbit Production States

Table 1. Prevalence of WMV2 and ZYMV by State

Table 2. Host Plants of WMV2

Table 3. Host Plants of ZYMV

Table 4. Select List of Aphids That Transmit ZYMV, WMV2, PRSV, and CMV

Table 5. Acreage of Cucurbit Crops In States Containing FLCP Plants

Table 6. Results From Survey Performed by Upjohn of FLCP Plants for Plant Viruses

Appendix II. Free-Living *Cucurbita pepo* in the United States. Viral Resistance, Gene Flow, and Risk Assessment. Report to USDA, Biotechnology, Biologics, and Environmental Protection, by Dr. Hugh Wilson

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 ZW-20 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 ZW-20 squashes or their progeny.

This determination by APHIS has been made in response to a petition received from Asgrow Seed Company, a subsidiary of the Upjohn Company, Inc., Kalamazoo, Michigan, dated July 13, 1992. The petition requested a determination from APHIS that the ZW-20 squash does not present a plant pest risk and is therefore not a regulated article. On September 4, 1992, APHIS announced receipt of the Upjohn/Asgrow petition in the Federal Register (57 FR 40632) and stated that the petition was available for public view. In that notice, APHIS also announced its intent to issue an interpretive ruling that the ZW-20 squash does not present a plant pest risk and would therefore no longer be considered a regulated article under its regulations. APHIS invited written comments on this proposed action, to be submitted on or before October 19, 1992. On March 22, 1993, APHIS published a second Federal Register notice (58 FR 15323) requesting additional information on eight issues raised by commenters to the first Federal Register notice. Briefly, the issues raised included the weediness potential of squash and its taxonomic relatives, the distribution of zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV2) in the United States, and the likelihood of creating new plant viruses. Concurrently, APHIS commissioned Dr. Hugh Wilson of Texas A&M University, an expert in cucurbit taxonomy and ecology, to prepare a report (see Appendix II) addressing issues raised by commenters to the first Federal Register notice. On May 23, 1994, APHIS published a notice in the Federal Register (59 FR 26619-26620) announcing the availability of an environmental assessment (EA) and preliminary finding of no significant impact (FONSI) for comment at a public meeting and for written comment during a 45-day comment period, which ended July 7, 1994. At the public meeting held June 21, 1994, only two individuals spoke, one in favor of the EA and FONSI, and one against.

The ZW-20 squash, as defined by its developer, the Asgrow Seed Company, is a squash line that is designed to resist infection by two plant viruses that frequently infect squash, namely ZYMV and WMV2. ZW-20 squash has been modified with genes that express the coat proteins of ZYMV and WMV2. Expression of these coat protein (CP) genes does not cause plant disease, but rather confers resistance to infection by 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 three plant pathogenic organisms: the bacterium *Agrobacterium tumefaciens*, cauliflower mosaic virus (CaMV), and cucumber mosaic virus (CMV). 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 two 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, 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. ZW-20 squash has been considered a "regulated article" for field testing under Part 340 of the regulations in part because ZW-20 squash has been engineered with CP genes derived from the plant pathogenic viruses ZYMV and WMV2. Field testing of the ZW-20 squash has been done under APHIS permits in 1990, 1991, 1992, and 1993, and is continuing in 1994. All field trials were performed essentially under conditions of reproductive confinement.

APHIS has determined that the ZW-20 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 ZW-20 squash or their progeny. (Importation of ZW-20 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 ZW-20 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 ZW-20 squash varieties bred from these lines will exhibit new plant pest properties, i.e., properties substantially different from any observed for the ZW-20 squash lines

already field tested, or those observed for squashes in traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 U.S.C. 4331 *et seq.*; 40 CFR 1500-1509; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An Environmental Assessment (EA) was prepared and a Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that ZW-20 squash is no longer a regulated article under its regulations at 7 CFR Part 340.

The body of this document consists of the following two parts: (1) background information, which provides the legal framework under which APHIS has regulated the field testing, interstate movement, and importation of ZW-20 squash, and a summary and response to comments provided to APHIS on its proposed action during the public comment periods; and (2) analysis of the key factors relevant to APHIS' decision that the ZW-20 squash does not present a plant pest risk.

II. Background

Regulatory Authority

APHIS regulations, 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 (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products.

Under § 340.0 of the regulations, a person is required to obtain a permit before introducing a regulated article. A genetically engineered organism is deemed a regulated article either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. Permission to conduct a field trial with an article regulated under 7 CFR Part 340 is granted when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

Before the introduction of a regulated article, a person is required under § 340.0 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria

impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under § 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

The FPPA gives USDA the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests or diseases. Some imported plant material must be grown under confined conditions after importation and certified as free of pests before it can be released from oversight by USDA.

An organism is not 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, 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 does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

Section 340.6 of the regulations was published on March 31, 1993 (58 FR 17044), after publication of the initial notice of receipt of this petition. It is our intent that the interpretive ruling under which this petition was submitted utilizes standards equivalent to those for the petitioning procedure subsequently adopted. These standards include the opportunity for public comment on the petition and a reasoned consideration of the relevant scientific information and the comments.

ZW-20 squash has been considered a "regulated article" for field testing under Part 340 of the regulations in part because the CP genes were from plant viruses and the vector system used to transfer the viral CP genes was derived from *A. tumefaciens,* all of which are on the list of organisms in the regulation and are widely recognized as plant pathogens. In addition, certain noncoding regulatory sequences were derived from plant pathogens, i.e., from CaMV, CMV, and *A. tumefaciens*.

APHIS believes it prudent to provide assurance before commercialization that organisms such as the ZW-20 squash, that is derived at least in part from plant pests, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that the ZW-20 squash is not a regulated article is based in part on evidence provided by Upjohn/Asgrow concerning the biological properties of the

ZW-20 squash and its similarity to other varieties of squash grown using standard agricultural practices for commercial sale or private use. Through the end of 1993, the ZW-20 squash has been field tested under 14 APHIS permits at 46 sites in 10 States.

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

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

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) 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. Evidence has been presented by Upjohn/Asgrow that bears on these topics. Upjohn/Asgrow has also presented data that ZW-20 may alter current methods used for the control of ZYMV and WMV2. In addition, because the Upjohn/Asgrow petition seeks a determination regarding new squash varieties containing the virus resistance genes, it should be established that there is no reason to believe that any new squash varieties bred from ZW-20 squash lines will exhibit plant pest properties substantially different from any observed for squash in traditional breeding programs or as seen in the development of the ZW-20 squash lines already field tested.

Oversight by Other Federal Agencies

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

The USDA Food Safety Inspection Service (FSIS) is responsible for regulation of genetically engineered meat and poultry products (59 FR 12582-83; 56 FR 67054-55). Food safety in the United States, for products other than meat and poultry, is assured by regulation under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 201 *et seq.*). The FDA's policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Regulatory oversight for the safety of any food or feed products derived from ZW-20 squash is under the jurisdiction of the FDA, shared with the EPA when pesticides are involved. The FDA has completed its consultation and has concluded that ZW-20 squash is just as safe to consume as any other squash variety. On November 2 and 3, 1994, the FDA Food Advisory Committee concurred with the process used by FDA to arrive at this position. Under the FFDCA, the EPA has responsibility for establishing tolerances or exemptions from the requirement of tolerance for pesticide residues on food or feeds, including viral CP.

III. Response to Comments

On September 4, 1992, APHIS announced receipt of the Upjohn/Asgrow petition in the Federal Register (57 FR 40632) and announced its intent to issue an interpretive ruling that the ZW-20 squash does not present a plant pest risk and would no longer be considered a regulated article under its regulations. During the 45-day comment period, APHIS received 17 comments regarding its proposed interpretive ruling in response to Upjohn/Asgrow's petition. Of the 17 comments, 7 were generally supportive of APHIS' proposed action and 10 expressed serious reservations or disapproval of it.

On March 22, 1993, APHIS published a second Federal Register notice (58 FR 15323) requesting additional information on eight issues raised by commenters to the first Federal Register notice. Concurrently, APHIS commissioned Dr. Hugh Wilson of Texas A&M University, an expert in cucurbit taxonomy and ecology, to prepare a report (see Appendix II) related to issues raised in comments to the first Federal Register notice. Of the 12 comments to the second notice, 10 (none of whom commented to the first Federal Register notice) were generally supportive of APHIS' action and two expressed serious reservations. (The latter two commenters had previously commented negatively in the first Federal Register notice.)

After the close of the official comment period, APHIS received letters from two commenters urging the agency not to approve this petition because they believed that the agency would not fulfill its requirements under the National Environmental Policy Act (NEPA). One of the letters reiterated issues previously communicated in comments to the two previous Federal Register notices. The second letter reiterated issues dealing with gene movement also previously communicated in the two Federal Register notices and expressed the opinion that if the agency decides to prepare an EA that reaches a FONSI, the availability of such documents must be announced in the Federal Register with the opportunity to comment during a minimum 30-day comment period. The letter also requested that APHIS consider holding a public hearing during the 30-day comment period because of the "considerable controversy surrounding the Upjohn/Asgrow's petition." The plant pest risk issues raised in these two letters are addressed in this determination. Besides this determination document, the agency has prepared an EA in compliance with NEPA that addresses the environmental issues regarding the widespread use of virus-resistant squash in agriculture.

Most of the supportive comments in response to both Federal Register notices were based on scientific data concerning the lack of plant pest risk presented by ZW-20 squash and its plant pest-derived components. Several commenters mentioned that the *A. tumefaciens* derived transformation system is well-characterized, has been used extensively, and does not present any plant pest risk. Several commenters, including State

agricultural officials, said that they think that the virus resistant squash plants would not become noxious weeds. Several plant breeders and State agricultural officials mentioned that the viruses ZYMV and WMV2 cause severe losses in squash production, and that a heritable form of viral resistance would be advantageous for producers and preferable to use of chemicals or other control strategies.

Four commenters (including plant virologists) discussed in detail why they believe that transencapsidation (i.e., the coating of one viral RNA partially or completely by the CP of another virus) and the possible appearance of new plant viruses by recombination between plant encoded viral CP and endemic plant viruses would not pose a plant pest risk significantly different than could occur in natural mixed viral infections. APHIS agrees with these comments.

Eight commenters noted that pest-resistant squash plants are used and have been used in commercial production without any significant increase in weediness of squash or its sexually-compatible relatives. One commenter (a plant breeder) noted that free-living *Cucurbita pepo* is limited not by infection by plant viruses but by dispersal of the seeds. The range of dispersal is limited by deposition of the buoyant fruits that are usually deposited between high water marks during spring flooding. APHIS agrees with these comments.

Of the 12 comments opposed to granting this petition, 6 said that the petition should be rejected because there is no strong Federal program to which all transgenic crops should be subjected to assess and minimize their risks. APHIS disagrees. The "Coordinated Framework for Regulation of Biotechnology" (51 FR 23302-50), developed by the Office of Science and Technology Policy (OSTP), establishes a clear system for product-based coordinated reviews of the products of agricultural biotechnology. Roles are set out for APHIS, FSIS, the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA), based on the existing legal authorities of the respective agencies for oversight over particular aspects of this economic sector. This system is entirely adequate to identify and address any significant potential risks that may be posed by any of the new products of agricultural biotechnology. One comment suggested that APHIS' authority to regulate these products under the FPPA is questionable. APHIS also disagrees with this comment. Our responsibilities under the Act to protect against the introduction or dissemination of plant pests provide broad authority over any products that may have potentially significant impacts on the environment, based on the broad definition of "plant pest" in the statute.

One commenter argued that if seeds of ZW-20 squash are to be exported, the U.S. Government should address the potential adverse impacts on transgenic squash in the other centers of genetic diversity for cucurbits, Mexico and Central America. APHIS disagrees with the apparent assumption of the commenters that this determination is likely to allow unregulated commerce across national borders of the seeds of ZW-20

squash. The comment is correct, however, in suggesting that this determination does not carry with it any foreign safety presumption, since our authority and our review only extend to the borders of the United States and its territories and possessions. APHIS is in frequent contact with agricultural officials from many foreign nations, including those with interest in the cultivation of genetically engineered squashes. We are actively involved with many countries to help them develop national scientific and regulatory frameworks that will enable them to make their own scientifically credible decisions about the safety of new crop varieties.

Most countries prohibit the unrestricted importation of seeds, and many have specific requirements to address any releases of transgenic plants in their environment. We believe that the issues raised about use of ZW-20 squash in Mexico can be analyzed by the same approach used in the determination. Many crucial scientific facts explained in this determination for the United States also apply in Mexico: (1) ZYMV and WMV2 are major problems in cucurbit production in Mexico (Delgadillo et al., 1988). (2) Many of the same aphid species in Mexico are also vectors in the southern United States (see Appendix I, Table 4, data from Perring et al., 1992). (3) The common free-living cucurbit *C. pepo* ssp. *fraterna* of Mexico and northern Central America is most likely susceptible to these viruses as are all other *C. pepo* subspecies. (4) *C. pepo* ssp. *fraterna* or ssp. *pepo* is not reported to be a serious weed in Mexico (Holm et al., 1979).

The three major plant pest risk issues raised by commenters to **both** Federal Register notices that had reservations or disapproved of APHIS' proposed action were: (1) Will the introductions of ZYMV and WMV2 CP increase the likelihood of new plant viruses (five comments)? (2) Could the introduction of two new virus resistance genes cause squash to become a weed (three comments)? (3) Will the virus resistance genes move to wild squash relatives and would this have a detrimental impact on these wild plants (four comments)?

On May 23, 1994, APHIS published a notice in the Federal Register (59 FR 26619-26620) announcing the availability of an environmental assessment (EA) and preliminary finding of no significant impact (FONSI) for comment at a public meeting and for written comment during a 45-day comment period, which ended July 7, 1994. At the public meeting held June 21, 1994, only two individuals spoke, one in favor of the EA and FONSI, and one against. Each of the speakers at the public meeting submitted written comments as well. During the remainder of the 45-day comment period, APHIS received an additional 52 written comments from individuals. A total of 29 comment letters disagreed with APHIS' proposal to approve the subject petition, whereas 23 comments supported the APHIS findings in the EA and FONSI. The affiliations of the persons that commented were: private individuals (18); universities (12); agricultural experiment stations (11); public policy/public interest groups (6); industry (2); associations (1); cooperative extension service (1); and a

Federal research laboratory (1). Approximately two-thirds of the respondents in both groups wrote relatively brief, general comments expressing their views about the petition and APHIS procedures for conducting an environmental analysis and making a final determination. The remaining one-third of the respondents, both in opposition to and in favor of approval of the petition, provided detailed, issue-specific comments on the EA and FONSI.

The five major plant pest risk issues raised by commenters in all three Federal Register notices that had reservations or disapproved of APHIS' proposed action were:

(1) Will the introduction of ZYMV and WMV2 CP increase the likelihood of appearance of new plant viruses?

(2) Could the introduction of two new virus resistance genes cause squash to become a weed?

(3) Will the virus resistance genes move to wild squash relatives and would this have a detrimental impact on these wild plants?

(4) Are free-living *C. pepo* (FLCP) plants serious weeds?

(5) Are hybrids between ZW-20 squash and FLCP plants weeds, and will they persist in the environment and become weeds?

APHIS addresses each of these questions following a brief description of the taxonomy of squash.

The Taxonomy of *C. pepo* in the United States

It is generally assumed that before the development of New World agriculture, *C. pepo* existed as a single, wild-growing entity. The common ancestor of all cucurbits was probably an annual, gourd-producing plant adapted to colonization of disturbed ground in riparian habitats. This free-living ancestor was apparently first used in New World agriculture approximately 10,000 years ago. Subsequent evolution under both human and natural selection and dispersal by humans has produced the structurally diverse gourds that are currently available in the United States. *C. pepo* spp. include pumpkins, acorn squashes, zucchini, and ornamental gourds.

During the 1980's there was a significant change in the taxonomic description of *Cucurbita* spp. The *C. pepo* lineage is believed to be composed of two subsets, formally identified as subspecies *ovifera* and *pepo*. Subspecies *pepo* includes only domesticated plants including all pumpkin cultivars, all marrow cultivars (zucchini, spaghetti, cocozelle, etc.), and some ornamental gourd types (orange ball and miniature ball) that usually have reddish pigments in the fruit. Subspecies *ovifera* contains three varieties. Variety *ovifera* is the domesticated line that contains ornamental gourds that are not included in ssp. *pepo* (spoon, bicolor) as well as acorn squash, crookneck squash, yellow squash, straightneck squash, and scallop squash. Free-living gourds that occur in Texas are designated as *C. pepo* ssp. *ovifera* var. *texana*, and those living in Illinois, Missouri, Arkansas, Oklahoma, and Louisiana are designated *C. pepo* ssp. *ovifera* var. *ozarkana*. The latter two varieties will be designated in this document as FLCP (free-living *Cucurbita pepo*). The term "free-living" means that the plants can survive without direct human intervention. All *C. pepo* subspecies are freely interbreeding, and thus genes introduced in one subspecies, given time and proximity of the two subspecies, will be transferred via pollen to other subspecies (Wilson, 1993). (For a more detailed analysis of the taxonomy of *Cucurbita* species, see the report by H. Wilson in Appendix II).

The parental line used in the development of ZW-20 is a yellow crookneck squash, *C. pepo* ssp. *ovifera* var. *ovifera* variety YC77E.

Each of the five issues mentioned in comments as relevant to potential plant pest risk for ZW-20 squash will now be addressed.

Issue 1. Will the Introduction of ZYMV and WMV2 Coat Proteins Increase the Likelihood of the Appearance of New Plant Viruses?

Conclusion: ZYMV and WMV2 and the aphids that vector the viruses are widely prevalent in the United States. Based on the known physical and biological properties of ZYMV and WMV2, the likelihood of the appearance of new plant viruses with novel biological properties through field cultivation of ZW-20 plants is no greater than in naturally occurring potyvirus-infected squash.

ZYMV and WMV2 are members of the potyvirus group, which is one of the largest groups of plant viruses. At least 40 different potyviruses are reported to be widely prevalent in at least 43 States. ZYMV produces a severe disease consisting of mosaic (patchwork of yellow chlorotic tissues and green uninfected tissues), yellowing, shoestringing, stunting, and fruit and seed deformations on zucchini squash, muskmelon, cucumber, and watermelon. WMV2 causes mosaic and mottle diseases of cantaloupe, pumpkin, squash, and watermelon. On a given cucurbit host, ZYMV usually causes more severe symptoms than WMV2. The complete nucleotide sequence of at least three potyviruses (Reichmann et al., 1992) and partial sequences of WMV2 and ZYMV have been published (Quemada et al., 1990).

ZYMV and/or WMV2 are reported to be widely prevalent in at least 34 States (see Appendix I, Table 1) including those where commercial squashes are grown and FLCP plants are found.

There are many known and potential plant hosts to provide ZYMV or WMV2 as a source of inoculum to infect *C. pepo*. WMV2 and ZYMV are apparently not seed transmitted in common virus-susceptible cucurbit crops (Purcifull et al., 1984). The source of these viruses for infection of commercial plantings must be other host plants. These plants may be either: perennials; other plants that transmit the virus in seed; or in warm climates where continuous cucurbit production occurs, other production fields. WMV2 is potentially harbored in many wild and cultivated crops through the winter months in the Imperial Valley of California. By contrast, all the known sources for ZYMV are in the Cucurbitaceae (Perring et al., 1992). The reservoirs for ZYMV are home garden plantings of squash or sponge gourd, or commercial plantings of melons or squash grown under plastic or in greenhouses. The overwintering host for ZYMV in Florida is the wild perennial cucurbit *Melothria pendula* (Adlerz et al., 1983), while the host in New York State has not been identified (Provvidenti et al., 1984). The source for WMV2 in Texas is probably *M. pendula* (Chala et al., 1986), and in Arizona the sources are mallow, sour clover, and sweet pea (Nelson and Tuttle, 1969). The

overwintering hosts of WMV2 in Florida (Adlerz, 1978) and Massachusetts (Komm and Agrios, 1978) have not been identified. For most areas in the United States where cucurbits are grown, the specific overwintering host(s) has not been identified; however, many potential hosts have been identified (see Appendix I, Tables 2 and 3, data from Perring et al., 1992). Because of the extreme susceptibility of the FLCP plants to these viruses (Provvidenti et al., 1978), it is a reasonable assumption that FLCP plants would not be significant reservoirs for these viruses. APHIS could not identify evidence that FLCP plants have ever been naturally infected by ZYMV or WMV2.

The three aphid species involved in the dissemination of ZYMV and WMV2 are widespread in the United States. The viral CP is not the primary determinant of aphid transmission of potyviruses. Most potyviruses are transmitted by many aphid species in a nonpersistent manner (see Appendix I, Table 4, data from Perring et al., 1992). The most important and widespread of these aphid vectors are *Myzus persicae, Aphis gossypii,* and *Macrosiphum euphorbiae* (CMI/AAB Description of Plant Viruses, 1988; Perring et al., 1992). The main features of nonpersistent 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).

There is evidence that two virus-coded proteins, a noncapsid protein (called helper component) and the CP, play key roles in potyvirus transmission and vector specificity. The way in which helper component makes aphid transmission possible has not been established. The most likely effect is that the protein makes it possible for the virus to attach to sites within the aphid in a way that allows it to be transmitted. Although helper component appears essential for aphid transmission of potyviruses, its presence does not guarantee transmission (Matthews, 1991). Modifications to the CP can result in loss of aphid transmissibility (Atreya et al., 1990).

Coat proteins are the most extensively characterized potyviral gene products (Reichmann et al., 1992). The primary function of CP is to encapsidate viral RNA. Other CP functions reported for other plant viruses include host response determinant and cell-to-cell movement, but these functions have not been identified for potyviruses (Reichmann et al., 1992).

Upjohn/Asgrow's approach for achieving viral resistance is based on observations that plants expressing a viral CP gene are often resistant to infection by the virus from which the CP was derived (Powell Abel et al., 1986). Most evidence suggests that expression of viral CP by a plant

interferes with one of the first steps in viral replication uncoating (removal of CP) from the incoming virus (for a review of this topic, see Register and Nelson, 1992).

ZW-20 does not exhibit stronger disease symptoms than its parental variety when infected with common cucurbit-infecting viruses. To determine the response of ZW-20 to infection with common cucurbit-infecting viruses, ZW-20 plants (and control plants) were inoculated with each tested virus. The symptoms that developed and the amount of CP produced were determined. The ZW-20 population used was segregating for resistance to WMV2 and ZYMV. The line used is fixed for one block of genes that provides moderate viral resistance and segregating for another block that confers strong resistance. All the nontransgenic control plants inoculated were susceptible to viral infection. ZW-20 plants were resistant to WMV2 infection and to ZYMV in ZW-20 plants containing the strong resistance gene block. ZW-20 plants containing the moderate viral resistance block showed milder symptoms than the nontransgenic controls (data reports for permit numbers 90-365-02 and 90-365-03).

The levels of ZYMV and WMV2 CP detected by ELISA (enzyme-linked immunoabsorbent assay) did increase when ZW-20 plants were challenged with ZYMV and WMV2 singly or in combination (data reports for permit numbers 90-365-02 and 90-365-03, tables IV and V) as compared to mock-inoculated ZW-20 plants. This increase may be due to limited replication of the viruses in ZW-20 plants, or that ZYMV and WMV2 CP produced by the plants are stabilized in the presence of replication of limited amounts of ZYMV and WMV2. Similar results have been reported for other potyviruses (Farnelli et al., 1992).

According to data provided by Upjohn/Asgrow, ZW-20 plants are as susceptible as the parental plants to CMV and papaya ringspot virus (PRSV), which are two of the most prevalent cucurbit viruses (data reports for permit numbers 90-365-02 and 90-365-03). As anticipated, the levels of ZYMV and WMV2 CP detected by ELISA did **not** increase when ZW-20 plants were inoculated with CMV (data reports for permit numbers 90-365-02 and 90-365-03, tables IV and V), as compared to uninoculated ZW-20 plants.

In contrast to the reported synergism between CMV and ZYMV infections in cucumber (Poolpol and Inouye, 1986), no synergism was detected in CMV-infected ZW-20 (data reports for permit numbers 90-365-02 and 90-365-03). The levels of ZYMV and WMV2 CP detected by ELISA **did** increase when ZW-20 plants were inoculated with PRSV, a closely related potyvirus that often infects cucurbits (data reports for permit numbers 90-365-02 and 90-365-03, tables IV and V), as compared to uninoculated ZW-20 plants. This increase may be due to the stabilization of two CP when the potyvirus PRSV is replicating. Similar results have been reported for other potyviruses (Farnelli et al., 1992). The levels of CP produced, in general, were still less than those detected in naturally

occurring plant infections. Infections of ZW-20 were no more severe than those of the parental variety.

Genomic masking in ZW-20 virus-infected plants should not increase the likelihood of the appearance of plant viruses with altered host specificities. When two viruses multiply together in the same tissue, some progeny particles may be formed that consist of the genome of one virus encased in a particle made partially or completely from the structural component of the other virus. Where the genome of one virus is encased in a protein shell made entirely of subunits of another virus (or strain), the phenomenon has been termed "genomic masking" or "transencapsidation." When the protein coat consists of a mixture of proteins from the two viruses, it has been termed "phenotypic mixing" (Matthews, 1991). This phenomenon occurs with potyviruses and is important in insect transmissibility of certain potyviral isolates (Bourdin and Lecoq, 1991). Since these three potyviruses (PRSV, WMV2, and ZYMV) are already endemic in the United States and infect many of the same crops (according to APHIS survey of endemic viruses, Appendix I, Table 1, and data reports for permit numbers 90-365-02 and 90-365-03); therefore, one would expect that significant amounts of masked virus particles are naturally present.

Genomic masking between CMV and potyviruses has not been reported and would not be expected because of the different architecture of the virions, icosahedral and flexuous (slightly curved) rods, respectively.

The likelihood of genomic masking is expected to be higher between another potyvirus and ZW-20-encoded CP. The most common potyvirus in squash (besides ZYMV and WMV2) is PRSV. There are two questions that need to be addressed in considering the likelihood and significance in any potential instance of genomic masking. First, is there a sufficient amount of CP produced by the transgenic plant to produce masked virus? Second, if a masked virus is produced, would it have any new biological properties, e.g., the ability to be transmitted by insect vectors, especially vectors different from those that transmit the parent virus?

The amount of CP produced is important in genomic masking. Often, in mixed infections where genomic masking occurs, there is an increase in the amount of the viral CP produced by one of the viruses. For example, co-replication of carmovirus-like ST9 RNA and the luteovirus beet western yellows virus (BWYV) results in a 10-fold increase in BWYV CP levels over luteoviral replication alone (Falk and Duffus 1984; Passmore et al., 1993). In addition, the CP produced by the ZW-20 plant under the direction of CaMV 35 S promoter is found in the same tissues (leaf mesophyll cells) (Benfey et al., 1990) that CP is found during natural infections (Matthews, 1991).

Generally, lower levels of ZYMV and WMV2 CP are produced by PRSV-infected ZW-20 (as detected by ELISA) than in PRSV-infected nontransgenic squash plants also infected with ZYMV or WMV-2 (data reports for

permit numbers 90-365-02 and 90-365-03, tables IV and V). Thus, less ZYMV and WMV2 CP are available to potentially produce masked virus, so that production of masked virus in ZW-20 should be less efficient than in nontransgenic squash infected with combinations of the three viruses.

Even if a masked virus were generated, it would **not** pose a risk to the environment because it would not have any significant new biological properties. If masked virus arose in PRSV-infected (or any other cucurbit-infecting potyvirus) ZW-20 plants, the **primary** determinant for aphid transmissibility is not CP, but rather a noncapsid protein, the helper component of PRSV (Reichmann et al., 1992; Matthews, 1991). Thus, the primary determinant for transmission would be derived from the potyvirus that would infect ZW-20 squash (i.e., the helper component of the infecting virus), not the plant-encoded CP.

If masked PRSV, containing CP of ZYMV and/or WMV2, 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* (see Appendix 1, Table 4; data from Perring et al., 1992). In fact, these are the most common aphid vectors of potyviruses in temperate regions of the United States. One comment to the draft EA/Determination noted the existence of gaps in the list of aphids that transmit four cucurbit viruses (see Appendix I, Table 4). The data presented was tabulated from scientific literature (CMI/AAB Description of Plant Viruses and Perring et al. 1992). APHIS again notes that information provided in Table 4 is from published reports. Not every researcher could or would test every aphid vector for its ability to transmit each of the four viruses. APHIS stands by its earlier conclusion that the most economically important aphid vectors of ZYMV, WMV2, PRSV, and CMV in the United States are three aphid species listed above.

If masked virus were produced, it could only be maintained in the population as long as the virus replicated in ZW-20 plants or plants infected with 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 ZYMV and/or WMV2 CP would not be available for the production of masked virus.

Generation of recombinant virus in ZW-20 virus-infected plants should not increase the likelihood of the appearance of viruses with novel attributes. Recombination is defined as the formation of new genetic combinations by the exchanging of genes. In this case, the result of the recombination event would be that the plant-encoded CP from either ZYMV or WMV2 would replace the CP in a cucurbit-infecting virus (most likely a potyvirus).

First, recombination has **not** been demonstrated within the potyvirus group (Lai, 1992) although recombination has been seen with **other** plant

viruses under high selection pressure (Bujarski and Kaesberg, 1986; Allison et al., 1990; Greene and Allison, 1994). In contrast, under **weak** selection pressure no recombination is seen (Angenent et al., 1989; Robinson et al., 1987; Falk and Bruening, 1994).

"New" potyviral strains or viruses apparently have not arisen via recombination. In the early 1980's, a new cucurbit-infecting potyvirus ZYMV appeared in Europe, the Middle East, and Northern Africa (Lecoq et al., 1981); and in Connecticut, New York, Florida, and California (Provvidenti and Gonsalves, 1984). Subsequent molecular analysis of the nucleotide sequence of ZYMV revealed that it apparently did not arise from recombination between "known" cucurbit-infecting potyviruses WMV2 and PRSV, but these three viruses probably evolved from a common progenitor (Quemada et al., 1990; Shukla and Ward, 1989). Recently, a necrotic strain of potato virus Y (PVY) has caused severe losses in tobacco plants. Nucleotide sequencing reveals that it differs from existing PVY strains by several bases that are distributed throughout the genome (Robaglia et al., 1989; Sudarsono, et al., 1993) and apparently did not arise from recombination gene cassettes. Many "new" viruses are only newly recognized because they impact forest, nursery, and crop plant production.

Why these three closely related potyviruses maintain their individual genome sequence may be a result of selection pressure in their overwintering hosts and their reliance on aphids for transmissibility. In temperate regions of the United States where cucurbit crops are only grown during the summer months, these viruses must survive most of the year in other plant hosts. In Florida the overwintering host for ZYMV is the wild perennial cucurbit *Melothria pendula* (Adlerz et al., 1983), and for PRSV it is *Momordica charantia* (Adlerz, 1972). The overwintering host(s) for WMV2 in Florida has not been identified, although the above two plants have been ruled out (Adlerz, 1978). Thus, since each virus has a different overwintering host, this may be an important source of selection pressure on these viruses in maintaining their individuality. The viruses must maintain genome that replicates efficiently in the many commercially grown cucurbit crop plants (squash, cantaloupe, watermelon, and cucumber) and the different overwintering hosts, **and** be able to be transmitted by the aphid vectors between these plants. Another factor in maintaining separate identities of these three viruses may be that they replicate in different subcellular sites and that other viral RNAs cannot permeate the replication complexes, thus reducing viral RNA recombination frequency.

Recombination and ZW-20. For recombination to occur, at least two different viruses or viral strains must replicate in the same plant. In many field grown plants multiple viral infections (up to six viruses in a single plant) have been detected (Abdalla et al., 1985; Falk and Bruening, 1994; data report 93-365-02 and 93-065-03, table VI). Most evidence suggests that recombination occurs at a higher frequency between viruses

and viral strains that share significant nucleotide homology (homologous recombination) than those that do not share nucleotide sequences (Lai, 1992). Thus, recombination is more likely to occur between the ZW-20 encoded CP mRNAs and potyviruses rather than other nonrelated viruses. The primary function of CP is to encapsidate viral RNA. Its other biological property is as a secondary determinant of aphid transmissibility. Because the aphids that are the most important vectors for transmitting these viruses are the same for the most widely prevalent potyviruses in cucurbits (see above), the recombinant virus would not have gained any new attributes.

Issue 1 was the subject of several comments: Two plant virologists (Drs. de Zoeten and Palukaitis) who commented in support of the Agency's FONSI have written publications on this general subject area that were used by other commenters to support their opposing views. None of the plant virologists who commented on the draft EA/Determination disagreed with the conclusions made by APHIS regarding this issue. APHIS concurs with the several comments that indicated that there may be certain crops and gene constructs in which the creation of new plant viruses that pose increased disease risk may be theoretically possible, but that ZW-20 squash is not one of those.

APHIS' conclusion that new viruses are no more likely to be produced through cultivation of ZW-20 squash than through cultivation of traditional varieties is based in part on the fact that the insect vectors of the relevant squash viruses are all the same and also in part on the fact that multiple infections by these viruses are common in commercial squash.

APHIS disagrees with one commenter who suggested that the data presented from a random sampling of cucurbits from grocery stores does not support the contention that multiple infections are common. In our analysis of the data, we had never concluded that all cucurbits are always infected with plant viruses every year. For a variety of reasons (e.g., reduced population of insect vectors), the frequency and severity of viral infections in a given location can vary from year to year. The survey showed that in a random sample of 35 fruits, 89 percent showed at least one viral infection, 55 percent were infected with at least two viruses, and 18 percent were infected with all four viruses tested. APHIS concludes that these viruses are widely prevalent in the environment and that most samples tested contained two or more viruses. Other reports confirming multiple viral infection of crop plants have been reported (Falk, 1994; Abdalla et al., 1985).

FLCP plants have survived since the arrival of the severe strain of ZYMV. APHIS would like to respond to a general concern about the occurrence of severe strains of viruses, given that ZYMV can kill FLCP plants when inoculated with human intervention. In his classic textbook *Plant Virology* (1991), Matthews discusses survival of severe strains.

"A virus that kills its host plant with a rapid developing systemic disease is much less likely to survive than one that causes only a mild disease that allows the host plant to survive and reproduce effectively. There is probably natural selection in the field against strains that cause rapid death of the host plant. Leafhoppers living on desert plants of the western United States are infective primarily with strains of beet curly top virus that cause mild symptoms in beet. Virulent strains of curly top virus kill certain desert plant species before they can allow a generation of leafhoppers to mature. Thus, the virulent strains in the desert tend to be self limiting. However, disease severity has a very different effect in beet plants. Sugar beet is a good host for the leafhopper if the plants are small and exposed to full sunlight. They are poor hosts when large, providing a lot of shade. For this reason severe strains of curly top virus facilitate their own spread in beet by producing small, stunted plants, which favor vector multiplication (Bennett, 1963). Leafhoppers overwintering near beet fields carry strains of higher virulence than hoppers found in the desert."

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 even severe 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.

APHIS believes that the recent history of the development of biological control agents illustrates the general (though not exclusive) trend that virulent viruses can evolve or be selected by the host to become less virulent strains. One classic example is myxoma (rabbitpox) in Australia and Great Britain. A severe strain of the virus was introduced into the country to control rabbits. From the initial very severe strain of virus (highly virulent with essentially no recovery of infected rabbits) researchers

found in the field progressively less and less virulent strains of the virus with faster and faster recovery rates of infected rabbits (Morse, 1993). There is indirect evidence of a similar trend with plant viruses that have been considered for use in biological control. APHIS could find no evidence of the successful use of plant viruses to control weeds (i.e., no registration of plant viruses as biological control agents by EPA and no recent requests to APHIS for testing of plant viruses as biological control agents (C. Divan, USDA, BBEP, personal communication)). This has frequently been attributed by plant virologists to difficulty in finding strains that kill the plant, attenuation of the severe strain of the virus to a less virulent strain (less effective), and failure to obtain high infection rate. There are no specific literature citations for this finding because such negative data traditionally have not been judged suitable for publication.

Issue 2. Could the Introduction of Two New Virus Resistance Genes Cause Squash to Become a Weed?

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."

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.

Several comments criticized APHIS' mention in the draft EA/Determination of Baker's list of characteristics of known weeds. Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). Baker (1965) listed 12 common weed attributes,

almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. 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. APHIS listed Baker's characteristics as a preamble to introducing Keeler's characterization (1989) of *C. maxima*, a close relative of yellow crookneck squash. Keeler's article listed the weedy characteristic of *C. maxima*. We believe that Keeler described several important growth characteristics of squashes. Although Baker's list has been criticized, no other universally acceptable characters have been defined by ecologists (Williamson, 1994) and in our view, there is no formulation that is clearly superior at this time.

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). 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 ZW-20 squash is likely to be grown mostly in areas that are currently under squash cultivation, i.e., in typical growing regions for the crop. Upjohn/Asgrow has reported that there are no major changes in seed germination, cucurbitin levels, seed set viability, susceptibility or resistance to pathogens or insects (except ZYMV and WMV2), and there are no differences in overwintering survivability between virus resistant transgenic squash and nontransgenic squash (data reports for permit numbers 90-365-02, 92-027-01, 90-365-03, and 93-053-02).

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 Upjohn/Asgrow (H. Quemada, personal communication).

There are no morphological or physiological characteristics of the ZW-20 squash that would entail the use of agricultural practices that vary from the traditional practices used today for the cultivation and propagation of squash except a reduced need for control of aphids that vector these viruses.

One commenter who took issue with our draft EA/Determination stated, ". . . there are examples where resistance to stress is suspected

(emphasis added) of playing a role", and three references were cited. One, Darmency and Gasquez (1990), discussed the movement of a herbicide tolerance gene into a known common weed, lamb's quarters, and another was a review article covering the same subject (Darmency, 1994). The third paper (Burdon et al., 1981) dealt with biological control of an exotic weed in Australia. Several commenters cited a reference by Williamson (1994). This paper, which discusses the major invasive plant pests in Great Britain and their traits, is not relevant to the discussion. The paper does not address the introduction of resistance genes into indigenous plants of Great Britain. All of the 14 weedy plants described in the paper were foreign introductions (one from Australia, 5 from Asia, and 4 each from Europe and the Americas).

Even successful multiple virus infection of weed populations does not guarantee their poor growth and reproductive patterns. Plants from non-agricultural ecosystems are known to be infected and act as significant reservoirs for many viruses. *Plantago* species may be one of the most important potential virus reservoirs. These plants are efficient and adaptable perennial weeds with a worldwide distribution. They are listed as common or serious weeds in 37 countries (Holm, 1979). They have been found infected naturally with at least 26 viruses from 19 groups and families (Hammond, 1982). Although *Plantago* sp. are often infected with many viruses, they are still successful and troublesome weeds. Of course, viruses are not the only pathogens and pests of these plants that exert stress on the plant.

Issue 3. Will the Virus Resistance Genes Move to FLCP Plants, and Will This Have a Detrimental Impact?

Conclusion: The survey of FLCP plants for the presence of plant viruses was adequate and scientifically based. Many plants can be infected with a plant virus with direct human intervention but are never naturally infected. A survey for the presence of viral infections is an appropriate, adequate, and **proven** means for determining whether a plant is a significant natural host for a particular virus. APHIS believes that other experimental approaches suggested by commenters to determine impact of movement of virus resistance genes to FLCP plants are flawed.

The major scientific controversy for this issue was APHIS' reliance on a survey of FLCP plants for the presence of several plant viruses as a basis for determining whether the virus resistance genes would provide selection advantage to the FLCP plants. Two types of experiments were proposed as alternatives to survey approach. We will discuss the scientific merits of the survey approach and the deficiencies in the other experimental approaches before our analysis of the impact.

a. The survey for the presence of plant viruses in FLCP plants— To determine whether FLCP plants are natural hosts for ZYMV or WMV2 Upjohn/Asgrow surveyed at APHIS' request natural stands of FLCP

plants in Arkansas, Louisiana, and Mississippi for the presence of ZYMV, PRSV, WMV2, CMV, squash mosaic virus, tomato ringspot virus, and tobacco ringspot virus. No FLCP plants were taken in Texas because the previously identified C. pepo ssp. ovifera var. texana stands could not be located and stands in Illinois were inundated by floods in 1993. Analysis of previous published data and anecdotal evidence strongly suggests that the FLCP plants are not infected by ZYMV and WMV2. The presence of viruses was assayed by double diffusion and ELISA tests. The plant samples were also visually inspected for symptoms, and plant extracts assayed for viruses on indicator plants (Chenopodium quinoa, Nicotiana benthamiana, cucumber, tobacco, and zucchini). Although a sample of FLCP plants from a single site showed symptoms consistent with viral infection, none of the above-listed viruses were detected in FLCP samples even when susceptible commercially-grown nontransgenic squash plants were growing less than 2,500 feet away (see Appendix I, Table 6).

The survey that was done to address the presence of these viruses in FLCP plants was performed by agricultural Extension agents and agricultural scientists who are trained to recognize and identify disease symptoms. These scientists were asked to survey the FLCP stands for plants showing symptoms of virus infection (e.g., necrosis, foliar mosaic, etc.) and collect representative plant tissue samples to conduct laboratory and greenhouse tests to confirm the presence of the viruses. The surveys were done in mid-July to late August 1993 to maximize the likelihood that infected FLCP plants would be present and detectable. APHIS wanted to ensure that commercial cucurbit production was well underway to increase the potential for viral inoculum from aphid populations in the commercial plantings of squash and other crops. Sampling later in the growing season also increases the likelihood of detecting FLCP plants that are infected with multiple viruses (Matthews, 1991). Provvidenti et al. (1978) have shown that infection of young FLCP plants with certain cucurbit viruses can yield severe symptoms, but if infection occurs when the *C. pepo* plants are older, the symptoms are less severe. This observation is true for many plant/virus combinations, i.e., that once seed production in a plant is initiated (therefore, the older the plant is) the less effect viral infections have on plant seed production and plant survival (Matthews, 1991). This is a trade-off for this sampling date. When a greater number of plants may potentially be infected, less severe symptoms may be observed.

The surveyors saw no clear evidence of viral infection in any FLCP plants. The only suggestion of any infection was slight chlorosis in a single plant. The vine-like nature of these plants renders it difficult to determine the number of FLCP plants growing in a given area, especially late in the growing season. However, because these viruses move systemically throughout the infected plants, sampling one or two leaves of a plant should be sufficient to detect the viruses in any size plant, assuming the initial infection started several weeks before the sampling date. (By this time the virus would have moved systemically throughout

the plant from initial site of infection). Because the surveyors did not see any viral symptoms on the FLCP plants, they took only a limited number of samples at each site. There is a low probability of viral infection in plants that do not exhibit symptoms. The asymptomatic samples were tested for the presence of seven different viruses that infect cucurbits, and none was detected.

Occasionally virus concentrations in field grown plants are too low to allow easy detection. To increase the likelihood of detecting virus if it were present, sap from sampled FLCP plants was used to inoculate other plants (including cultivated squash) that are highly susceptible to the seven viruses. None of these plants showed viral symptoms, nor could any viruses be detected by any of the serological tests performed.

b. The first proposed alternative experiment to the survey— Several commenters to our draft EA/Determination stated that counting the number of plants that exhibit symptoms of infection by a virus does not necessarily give an adequate indication of the frequency of the presence of the virus in those plants. They mentioned the nonviral example of cactus moth and prickly pear in Australia. The commenters noted that in this instance the moth populations are low and the host plant prickly pear populations are small. They pointed out that even though the number of cactus moths that one could count is low, the moth has been effective in reducing cactus populations. APHIS believes that the Australian scenario is not comparable to FLCP plants and cucurbit viruses for the following reasons. First, the FLCP plants are native to the United States. By contrast, all cacti are native to the Americas and are exotics in Australia. Cactus moth was successfully introduced to Australia as a biological control agent for prickly pear. Second, the cucurbit viruses and their aphid vectors populations are not low in the United States. In fact, the viruses are widely prevalent and aphid populations high.

Several commenters to our draft EA/Determination suggested additional experimental approaches that they believed appropriate and/or necessary in order to study the impact of movement of virus resistance genes to FLCP plants. In a report prepared by Dr. Hugh Wilson for APHIS, Dr. Wilson described a series of experiments (see Appendix II, p. 17-19) to decide if the introduction of virus resistance gene(s) into FLCP plants would increase the weediness potential of FLCP plants. Briefly, he suggested that the following plants be inoculated with ZYMV: ZW-20; nontransgenic yellow crookneck squash; FLCP plants; and F_1 and F_2 hybrids between ZW-20 and nontransgenic parent and FLCP plants. Wilson suggested that the experiments should be performed in a greenhouse because: (1) FLCP plants are "unwieldy" and (2) pollen carrying the virus resistance gene from transgenic plants should not be allowed to fertilize FLCP plants. As part of the experiments to determine the response of the FLCP carrying the virus resistance gene, Wilson suggests that the listed genotypes be inoculated with ZYMV in the greenhouse.

APHIS does not believe that this greenhouse experiment will yield reliable or useful information, because the response of plants to viral infection under artificial conditions cannot predict their resistance or susceptibility under natural conditions. APHIS believes that viral inoculation of plants under artificial conditions may give rise to conclusions that are not substantiated under natural conditions. It is not uncommon for a crop plant to be susceptible under controlled conditions to a widely prevalent virus yet are rarely infected by that virus under natural conditions. One of the best documented examples is that of CMV infection of tobacco (*Nicotiana tabacum*). Tobacco plants are readily infected by all widely prevalent strains of CMV under artificial conditions (Kaper and Waterworth, 1981). All commercial tobacco cultivars grown in North Carolina and Kentucky are susceptible to CMV under greenhouse conditions with human intervention (E. Wernsman, North Carolina State University, personal communication). CMV is widely prevalent in North Carolina and Kentucky (APHIS List of Widely Prevalent Viruses). CMV is transmitted under field conditions by the aphid vector *M. persicae,* which is common in tobacco fields and is the **same** vector that transmits the major tobacco potyviral diseases: potato virus Y, tobacco etch virus, and tobacco vein mottling virus (Shew and Lucas, 1991). **However, field grown tobacco plants are only rarely infected with CMV** (Shew and Lucas, 1991; E. Wernsman, North Carolina State Univ., personal communication). Thus, reliance on greenhouse data in this instance would have led to incorrect conclusions, i.e., one would have predicted that CMV would be a major problem of tobacco plants but it is not. This observation is not an isolated case. APHIS has identified other virus-plant combinations (Jones et al., 1991; Hooker, 1981) similar to CMV-tobacco scenario described above: the aphid-transmitted potyvirus, tobacco vein mottling virus infecting tomato (confirmed by T. Pirone, University of Kentucky, personal communication); the aphid transmitted luteovirus, potato leafroll virus infecting tomato; the aphid transmitted potyvirus, tobacco etch virus infecting potato; and the aphid transmitted potyvirus, tobacco vein mottling virus infecting potato (confirmed by T. German, University of Wisconsin, personal communication).

APHIS concludes: (1) there is sufficient scientific evidence that surveying of host plants for the presence or absence of a particular virus is adequate to determine if a particular virus will have a significant impact on those plants; and (2) this approach will provide more reliable information than a greenhouse experimentation-based one.

c. The second proposed alternative experiment to the survey— A second experiment described in two comments entails exclusion of, "a particular natural enemy . . . as **the** way to determine the effect of that natural enemy on a wild population". APHIS believes that after careful consideration exclusion of the "natural enemy" is not feasible. First, although the details of this experiment were not given by the commenters, we assume that the commenters' experiment involved placing insect-proof cages over FLCP plants or using pesticides to eliminate the aphid

vectors. Wilson noted the "unwieldy nature" of FLCP plants. Performing this experiment for an extended period under natural conditions would be difficult or impossible.

Second, what is the "natural" enemy? Aphids are the only important vectors for transmission of ZYMV and WMV2 under field conditions. However, the same aphids that vector these viruses are plant pests themselves and cause significant crop losses even when they do not vector viruses (Davidson and Lyon, 1987). Thus, placing insect-proof screens around plants would prevent not only virus-infected aphids but noninfected aphids from developing on the FLCP plants. Furthermore, screening or insecticide treatment to exclude the aphid vectors would also eliminate other insects, e.g., thrips, leafhoppers, beetles, vine borers, squash bugs, mites, and whiteflies, that are known pests of cucurbits (Davidson and Lyon, 1987). Several of these insects also vector serious viruses of cucurbits. For example, thrips transmit tomato spotted wilt virus; beetles transmit squash mosaic virus; and aphids transmit zucchini yellow fleck virus, CMV, and PRSV (Matthews, 1991); all of which would be eliminated.

Therefore, APHIS believes that the experiment described to exclude the "natural enemy" would not only eliminate the two viruses in question but many insect pests and the plant pathogens that they transmit. This type of experiment would not only eliminate selective pressure of aphids containing ZYMV or WMV2, but all aerial pests and the pathogens that they may vector. It would therefore potentially grossly overestimate any potential impact from the viruses in question. While APHIS believes that additional scientific research in these general directions could yield information about virus replication in FLCP plants and about total pest and disease pressure on FLCP plants, we believe that Upjohn/Asgrow's experiments are most appropriate for addressing the relevant concerns.

In conclusion, APHIS believes that the additional experiments proposed by commenters were neither appropriate nor necessary in order to study the impact of movement of virus resistance genes to FLCP plants.

The virus resistance genes in ZW-20 will be transferred via pollen to FLCP plants. APHIS assumes pollen from ZW-20 squash is likely to be carried by bees and successfully pollinate FLCP plants. Although Upjohn/Asgrow has presented data (data reports for permit numbers 89-300-01 and 90-365-03) showing that pollen movement declines rapidly at distances greater than 50 feet from a source plant, we assume that fertile hybrids between ZW-20 plants and FLCP plants will occur given a sufficient period of time and widespread use of ZW-20 squash in agricultural settings.

FLCP plants are susceptible to ZYMV and WMV2 infection but are not infected in the wild. 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). Recently, several accession lines of *C. pepo* that have genes for resistance to CMV and WMV2 have been identified by the USDA Germplasm Information Network (Kyle et al., 1993). Thus, earlier reports that no sources of resistance to potyviruses and cucumoviruses have been reported in *C. pepo* are incorrect (Provvidenti, 1990). However, the survey of FLCP plants failed to detect seven important cucurbit-infecting viruses (see Appendix, Table 6).

APHIS believes that further reviews of the botanical record with respect to FLCP plants in Arkansas supports our previous decision that FLCP plants have not been impacted by ZYMV or other 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) ZYMV and WMV2 are widely prevalent in cucurbit crops in many of the major honeydew melon-, cucumber-, pumpkin-, and squash-producing States (see Appendix I, Tables 1 and 5); (2) many potential weed and annual plants that are hosts of ZYMV and WMV2 are present in these States (see Appendix 1, Tables 2, 3, and 5); and (3) many aphid vectors that can transmit ZYMV and WMV2 (see Appendix I, Table 4, and data from 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 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 two cucurbit viruses' ZYMV and WMV2 have the cucurbit names "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 be *Melothria* yellow mosaic virus.

The absence of (poty)viral infection of FLCP 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. Therefore, if these viruses impacted FLCP populations, there should have been natural selection for resistance or symptomless infections. The virus survey of FLCP plants performed by Upjohn/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 (see Appendix I, Table 6).

The movement of the virus resistance genes from ZW-20 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 selective pressure to maintain the virus resistance genes in natural populations of FLCP plants should be minimal.

Issue 4. Are FLCP Plants Serious Weeds? Will Virus-Resistant FLCP Plants Be More Difficult to Control Than Virus Susceptible Plants?

Conclusion: FLCP plants are not serious weeds in unmanaged or agricultural ecosystems. No scientific or anecdotal evidence was presented in comments or uncovered in APHIS review establishing that FLCP plants are weeds in unmanaged ecosystems. 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 WMV2 and ZYMV from ZW-20 squash, the control of the virus-resistant wild plants in soybean or cotton fields should not be more difficult or require new measures than of their nonengineered counterparts. Incorporation of the ZYMV and WMV2 resistance genes into FLCP plants growing in cotton or soybean fields

would not make the control of these plants more difficult. Soybean and cotton crops are not affected by these two viruses (or PRSV and CMV) and no viruses are known that cause both squash diseases, in either of these crops (Matthews, 1991). ZYMV and WMV2 resistance genes will not confer any resistance to any soybean or cotton virus. Thus, even if the soybean or cotton plants were severely infected by a plant virus, the virus resistant FLCP plants would not have any selective advantage over their nonengineered counterparts with respect to viruses present in soybean or cotton plants. 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.

Issue 5. Will Hybrids Between ZW-20 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.

Several comments criticized APHIS' draft EA/Determination for not adequately addressing whether there might be a risk of a new weed pest arising after the pollination of FLCP plants by ZW-20 squash. A number of comments suggested that APHIS should require greater testing of actual hybrids to determine if there are any potential risks. APHIS agrees that the petition does not contain extensive data to address definitively the potential "weediness" of such hybrids under a large variety of environmental conditions. However, APHIS believes it is possible to reach some valid conclusions based on our current knowledge of FLCP plants in managed and unmanaged ecosystems.

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 ZW-20 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 phenotypically identical to ZW-20.

Upjohn/Asgrow has supplied to APHIS additional information of ongoing field tests with hybrid plants derived from controlled crosses of ZW-20 with FLCP plants. Based upon the limited observations in field tests

during the past 2 years, 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 (interim data report 93-041-01). Whereas these results cannot predict the behavior of any future hybrid progeny when FLCP plants are pollinated by ZW-20 plants, the evidence supports APHIS' contention that the introgression of the virus resistance from ZW-20 into FLCP plants does not appear to pose a risk of developing a weed pest.

By contrast, unlike squash and FLCP plants, there are several well-known interbreeding crop-weed complexes that are serious pests. One example is the shattercane that is derived from crosses of wild and cultivated sorghum. Shattercane is a serious pest in the United States and world-wide. Other hybrid complexes that are serious pests outside the United States include rice, barley, wheat, and corn (Harlan, 1992).

Other Comments to the Draft EA/Determination

One commenter asked APHIS to clarify its statement, "Genetically engineered crops are comparable to traditionally bred resistant varieties." This statement was meant to convey a limited meaning: that blockage of viral replication results in a similar phenotype whether produced by genetic engineering, traditional breeding, or whether the plant is tolerant via natural cross protection. It is true that the nature and mechanism of only one plant virus resistance gene (*N* gene for resistance to tobacco mosaic virus in tobacco) has been identified (Whitmam et al. 1994). However, it has been established that plants bred via traditional means do not achieve that resistance by means of expressing a gene identical to, or homologous, to any viral CP genes (Matthews, 1991). By contrasts, genetically engineered cross protection is likely mediated directly by CP, as is the phenomenon of natural cross protection. In the latter cross protection, the production of CP by a mild strain of virus has been suggested as the means by which the challenge virus replication is blocked. In fact, this hypothesis was one of the bases for the landmark research by Dr. Roger Beachy (Scripps Research Institute), that determined that expression of CP genes in plants results in resistance. Thus, when ZYMV or WMV2 infect a ZW-20 plant or another cross protected squash plant, replication is blocked. All the virus "sees" is that CP is present and does not "recognize" whether it was produced by another strain of the virus or the transgenic plant. APHIS acknowledges that other modes of action of cross protection have been suggested (Matthews, 1991). APHIS also notes one significant difference between traditional breeding and genetically engineered resistance, i.e., the presence of viral sequences in the plant. Issues dealing with that difference have already been addressed above.

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 ZW-20 is no greater than has been accepted without alarm in the past with no noted ill effects.

The use of ZW-20 squash provides an alternative method for the control of ZYMV and WMV2. Currently, chemical, physical, biological, and genetic methods are used to control these viruses. Attempts to reduce virus spread through vector control by insecticides are largely ineffective but are still used in limited situations. Although these insecticides can kill the aphids, the aphids transmit these viruses efficiently throughout the field before their death. Insecticides registered for control of aphids include diazinon, lannate, metasystox-R, phosdrin, and thiodan. Many insecticides are toxic to nontarget organisms. Many growers now use mineral oils (often supplemented with insecticidal soaps or insecticides) to control both aphid and non-aphid insect pests. These chemicals are effective, but the oil sprays need to be applied frequently, every 3 to 5 days, to effectively control aphids.

Physical measures, like reflective surfaces and sticky yellow sheets, can also diminish vector spread. These measures are expensive and cumbersome to use on a large scale.

Another approach for protecting plants from viral infection requires inoculation of the plants with an asymptomatic (mild) strain of the virus. Infection of a plant by the mild strain of a virus protects the plant from the effects of subsequent inoculation with another severe strain of the same virus (Matthews, 1991). This phenomenon is called cross protection. It has been field tested successfully to control ZYMV in Taiwan (Wang et al., 1991) and Hawaii (Cho et al., 1992). Cross protection has several disadvantages that have been summarized by Fulton (1986), but is not widely used in the United States for controlling viruses.

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 phenotypically identical to ZW-20 is commercially available**

from Harris Moran Seed Company. 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 Upjohn/Asgrow or Cornell University are, or shortly will be, on the market.

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 (see Appendix, Table 1). 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.

IV. Analysis of the Properties of ZW-20 Squash

To reach its determination that ZW-20 squash does not present a plant pest risk, APHIS has addressed not only issues raised in public comments, but also considered basic information on the biology of squash and data presented by Upjohn/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 ZW-20 squash.

The Introduced Genes, Their Products, and the Added Regulatory Sequences Controlling Their Expression Do Not Present a Plant Pest Risk in ZW-20

The ZW-20 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 ZW-20 squash line was produced using an *Agrobacterium*-meditated transformation protocol to transform yellow crookneck squash with genes designed to confer resistance to ZYMV and WMV2, two plant viruses that frequently infect squash. The genes that confer this resistance are derived from virus genes that encode the CP of ZYMV and WMV2. Expression of these CP genes in the squash does not cause plant disease, but rather confers resistance to infection by ZYMV and WMV2.

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 *Agrobacterium tumefaciens*, CaMV, and CMV. 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. In addition, the amino-terminus of the WMV2 coding region is fused to the 5' intergenic region and the first 48 nucleotides (N-terminus) of the CMV CP gene. 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. Because of the physical and biological properties of ZYMV and WMV2, the likelihood of creating new plant viruses with novel biological properties through field cultivation of ZW-20 plants is no greater than in naturally occurring potyvirus-infected squash.

During characterization of the performance of ZW-20 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 ZYMV and WMV2 infection. In APHIS' opinion, the components and processing characteristics of ZW-20 squashes reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity. The ZW-20 plants have no plant pest characteristics.

The ZW 20 Squash is No More Likely to Become a Weed Than a Virus-Resistant Plant Developed by Traditional Breeding Techniques

APHIS' analysis of this issue can been found in Section III, Response to Comments, Issue 2. Briefly, 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). Upjohn/Asgrow has reported that there are no major changes in seed germination, cucurbitin levels, seed set viability, susceptibility or resistance to pathogens or insects (except ZYMV and WMV2), and there are no differences in overwintering survivability between ZW-20 squash and non-transgenic squash. APHIS concludes that ZW 20 is unlikely to increase the weediness of yellow crookneck squash and is no more likely to become a weed than virus-resistant plants

developed by traditional breeding techniques.

The ZW 20 Squash is Unlikely to Increase the Weediness Potential for Any Other Cultivated Plant or Native Wild Species With Which the Organism Can Interbreed

APHIS' analysis of this issue can been found in Section III, Response to Comments, Issue 3, 4, and 5. Based on review of current data, FLCP plants are not serious weeds in unmanged or agricultural ecosystems. The virus resistance gene from ZW-20 plants will move via pollen to the FLCP plants. Since all evidence supports the conclusion that FLCP populations are **not** under significant environmental stress from viral infection, the selective pressure to maintain the virus resistance genes in natural populations of FLCP plants should be minimal. APHIS concludes that widespread cultivation of ZW 20 squash is unlikely to increase the weediness potential for any other squash or native wild species with which ZW 20 can interbreed.

The ZW-20 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 ZW 20 Squash Should Not Increase the Likelihood of the Emergence of New Plant Viruses

APHIS' analysis of this issue can been found in Section III, Response to Comments, Issue 1. APHIS concludes that based on the known physical and biological properties of ZYMV and WMV2 and data provided by

V. 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.

Adenijji, 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. 1972. *Momordica charantia* as a source of watermelon mosaic virus 1 for cucurbit crops in Palm Beach County, Florida. Plant Disease (Reporter) 56:563-564.

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.

Allison, R. F., Thompson, C., and Ahlquist, P. 1990. Regeneration of a functional RNA virus genome by recombination between deletion mutants, and requirement for cowpea chlorotic mottle virus 3a and coat protein genes for systemic movement. Proceedings of National Academy of Sciences (USA) 87:1820-1824.

Angenent, G. C., Postthumus, E., Brederose, F. Th., and Bol, J. F. 1989. Genome structure of tobacco rattle virus strain PLB: Further evidence on the occurrence of RNA recombination among tobraviruses. Virology 171:271-274.

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.

Bennett, C. W. 1963. Highly virulent strains of curly top virus in sugar beet in western United States. Journal American Society Sugar Beet Technology 12:515-520.

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.

Bujarski, J. J., and Kaesberg, P. 1986. Genetic recombination between RNA components of a multipartite plant virus. Nature (London) 321:528-531.

Burdon, J., Groves, R., and Cullen, J. 1981. The impact of biological control on the distribution and abundance of *Chondrilla juncea* in southeastern Australia. Journal of Applied Ecology 18:957-966.

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.

Cho, J. J., Ullman, D. E., Wheatley, E., Holly, J., and Gonsalves, D. 1992. Commercialization of ZYMV cross protection for zucchini production in Hawaii. Phytopathology 82:1023.

CMI/AAB Description of Plant Viruses. 1988. Association of Applied Biologists, Warwick, United Kingdom.

Darmency, H. 1994. The impact of hybrids between genetically modified crop plants and their related species: introgression and weediness. Molecular Ecology 3:37-40.

Darmency, H., and Gasquez, J. 1990. Appearance and spread of triazine resistance in common lambsquarter (*Chenopodium album*). Weed Technology 4:173-177.

Davidson, R. H., and Lyon, W. F. 1987. Insect Pests of Farm, Garden, and Orchard. John Wiley and Sons, New York. 640 pp.

de Wet, J. M. J., and Harlan, J. R. 1975. Weeds and domesticates: Evolution in the man-made habitat. Economic Botany 29:99-107.

Delgadillo, F., Garzon, J. A., and Vega, A. 1988. Detection of cucurbit viruses in Mexico. Phytopathology 78:626.

Falk, B. W. 1994. The specificity and significance of interactions between luteoviruses and associated RNAs. Annual meeting of the American Phytopathology Society.

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

Falk, B. W., and Duffus, J. E. 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa-pastoris*. Phytopathology 74:1224-1229.

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.

Fulton, R. W. 1986. Practices and precautions in the use of cross protection for plant virus disease control. Annual Review of Phytopathology 24:67-81.

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.

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. 1982. *Plantago* as a host of economically important viruses. Advances in Virus Research 64:667-676.

Harlan, J. R. 1992. Crops & Man. American Society of Agronomy, Madison, Wisconsin. 284 pp.

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.

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

Jones, J. B., Jones, J. P., Stall, R. E., and Zitter, T. A. 1991. Compendium of tomato diseases. American Society of Phytopathological Society, St. Paul, Minnesota. 73 pp.

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.

Komm, D. A., and Agrios, G. N. 1978. Incidence and epidemiology of viruses affecting cucurbit crops in Massachusetts. Plant Disease 62:746-750.

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

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

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.

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

McCammon, S. L., and Medley, T. L. 1990. Certification for the planned introduction of transgenic plants in the environment. *In*: The Molecular and Cellular Biology of the Potato, pp. 233-250. Vayda, M. E., and Park, W. D. (eds.). CAB International, Wallingford, United Kingdom.

Morse, S. S. 1993. Emerging Viruses. Oxford University Press. 317 pp.

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

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.

Nelson, M. R., and Tuttle, D. M. 1969. The epidemiology of cucumber mosaic and watermelon mosaic 2 of cantaloupes in an arid climate. Phytopathology 59:849-856.

Passmore, B. K., Sanger, M., Chin, L-H., Falk, B. W., and Bruening, G. 1993. Beet western yellows virus-associated RNA: An independently replicating RNA that stimulates virus accumulation. Proceedings of National Academy of Sciences (USA) 90:10168-10172.

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., 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.

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

Purcifull, D., Edwardson, J., Hiebert, E., and Gonsalves, D. 1984. Watermelon mosaic virus 2. CMI/AAB Description of Plant Viruses, number 293.

Quemada, H., Sieu, L. C., Siemieniak, D. R., and Gonsalves, D., and Slightom, J. L. 1990. Watermelon mosaic virus II and zucchini yellow mosaic virus: cloning of 3'-terminal regions, nucleotide sequences, and phylogenetic comparisons. Journal of General Virology 71:1451-1460.

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.

Reichmann, J. L., Lain, S., and Garcia, J. A. 1992. Highlights and prospects of potyvirus molecular biology. Journal of General Virology 73:1-16.

Robaglia, C., Durand-Tardif, M., Tronchet, M., Boudazin, G., Astier-Monifacier, S., and Casse-Delbart, F. 1989. Nucleotide sequence of potato virus Y (N strain) genomic RNA. Journal of General Virology 70:935-947.

Robinson, D. J., Hamilton, W.D.O., Harrison, B. D., and Baulcome, D. C. 1987. Two anomalous tobravirus isolates: Evidence for RNA recombination in nature. Journal of General Virology 68:2551-2561.

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

Shukla, D. D., and Ward, C. W. 1989. Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Archives of Virology 106:171-200.

Sudarsono, Woloshuk, S. L., Xiong, Z., Hellman, G. M., Wernsman, E. A., Wessinger, A. K., and Lommel, S. A. 1993. Nucleotide sequence of the capsid cistrons from six potato virus Y (PVY) isolates infecting tobacco. Archives of Virology 132:161-170.

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.

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

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.

Whitman, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., Baker, B. 1994. The product of tobacco mosaic virus resistance gene N: Similarity to Toll and the interleukin-1 receptor. Cell 78:1101-1115.

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.

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.

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

Appendices

Appendix I.
Distribution of WMV2 and ZYMV, Their Plant Hosts and Insect and Leading Cucurbit Production States

Table 1. Prevalence of WMV2, ZYMV, CMV and PRSV by State

Table 2. Host Plants of WMV2

Table 3. Host Plants of ZYMV

Table 4. Select list of aphids that transmit ZYMV, WMV2, PRSV, and CMV

Table 5. Acreage of cucurbit crops in States containing FLCP plants

Table 6. Results from survey performed by Upjohn/Asgrow of FLCP plants for plant viruses

Appendix II.
Free-Living Cucurbita pepo in the United States. Viral Resistance, Gene Flow, and Risk Assessment. Report to USDA, Biotechnology, Biologics, and Environmental Protection by Dr. Hugh Wilson

Table 1. Prevalence of WMV 2, ZYMV, CMV, and PRSV by State

State	WMV 2	ZYMV	CMV	PRSV
Alabama[1]	+[2]	+	+	+
Arkansas	+	+	+	+
Arizona	+	[3]	+	+
California	+	+	+	
Connecticut		+	+	
Delaware	+	+	+	+
Florida	+	+	+	+
Georgia	+	+	+	+
Hawaii		+		
Idaho		+		
Illinois		+	+	+
Iowa			+	
Kansas	+	+	+	+
Kentucky	+		+	
Louisiana	+	+	+	+
Maine		+	+	
Massachusetts	+		+	+
Maryland	+		+	+
Michigan	+	+	+	+
Minnesota	+	+	+	
Mississippi	+		+	
North Carolina	+		+	+
Nebraska	+		+	
New Jersey	+	+	+	+
New York	+	+	+	+
Ohio	+	+	+	+
Oklahoma	+	+	+	+
Oregon		+	+	
South Carolina	+	+	+	+
Tennessee	+	+	+	+
Texas	+	+	+	+
Vermont	+	+	+	+
Virginia	+	+	+	+
Washington	+		+	+
Wisconsin	+	+		+

[1] States listed in italic typeface contain FLCP populations.
[2] (+) = virus is widely prevalent in the State.

Table 2. Host Plants of WMV 2

Family Curcurbitaceae	
Citrullus lanatus (Thunb.) Matsum. & Nakai	Watermelon
Citrullus lanatus Var. citroides (L.H. Bailey) Mansf.	Citron
Cucumis africanus L.f.	
Cucumis anguna L.	West India gherkin
Cucumis dipsaceus C.G. Ehrenb. ex Spach.	Hedgehog gourd
Cucumis ticitolius A. Rich	
Cucumis heptadactylus Naud.	
Cucumis melo L.	Muskmelon
Cucumis metuliferus E.H. Mey ex Schrad.	African horned cucumber
Cucumis myriocarpus Naud.	Bitter apple
Cucumis sativus L.	cucumber
Cucumis zeyheri Sond.	
Cucurbita andreana Naud.	
Cucurbita ticifolia Bouche	Malabar gourd
Cucurbita gracilior L.H. Bailey	
Cucurbita lundelliana L.H. Bailey	
Cucurbita martinezii L.H. Bailey	
Cucurbita maxima Duch. ex Lam.	Winter squash
Cucurbita moschata (Duch. ex Lam.) Duch. ex Poir	Winter squash
Cucurbita okeechobeensis (Small) L.H. Bailey	
Cucurbita pepo L.	Squash
Cucurbita sorona L.H. Bailey	
Cylanthera brachystachya (Ser.) Cong.	
Echinopepon wrightii Cong.	Wild cucumber
Lagenaria sicerana (Mol.) Standl.	Calabash gourd
Sicyos angulatus L.	Star cucumber
Trichosanthes anguina L.	Snake gourd
Family Chenopodiaceae	
Chenopodium album L.	Lambsquarter
Chenopodium amaranticolor Coste & Reynier	
Chenopodium quinoa Willd.	Quinoa
Spinacia oleracea	Spinach

Table 2. Host Plants of WMV 2 (continued)

Family Compositae	
Dimorphotheca pluvalis (L.) Moench	Cape marigold
Senecio vulgans L.	Common groundsel
Family Cruciferae	
Capsella bursa-pastons (L.) Medic.	Shepherd's purse
Family Euphorbiaceae	
Euphorbia marginata Pursh	Snow-on-the-mountain
Family Hydrophyliaceae	
Phacelia congesta Hook	Bluecurls
Phacelia minor (Harv.) Thell. ex F. Zimm	Wild canterbury-bell
Phacelia tancetifolia Benth	Fiddleneck
Family Labiatae	
Lamium amplexicaule L.	Henbit
Family Leguminosae	
Cyamopsis tetragonolobus (L.) Taub.	Guar
Lathyrus odoratus L.	Sweet pea
Lupinus albus L.	Lupine
Macroptilium lathyroides (L.) Urb	Phasey bean
Melilotus indicas (L.) All	Sour clover
Phaseolus vulgans L.	Bean
Pisum sativum L.	Pea
Trifolium incamatum L.	Crimson clover
Trifolium sp.	Huban clover
Trigonella calliceras	
Trigonella comiculata	
Trigonella cretica	
Trigonella foenum-graecum L.	Fenugreek
Vicia narbonensis L.	Narbonne
Vicia sativa L.	Spring vetch

Table 2. Host Plants of WMV 2 (continued)

Family Malvaceae	
Gossypium hirsutum L.	Cotton
Lavatera trimestris L.	Tree mallow
Malva moschata L.	Musk flower
Malva parviflora L.	Cheeseweed, Mallow
Malva verticillata L.	Curled mallow
Family Plantaginaceae	
Plantago psyllium L.	Flax-seed plantain
Family Ranunculaceae	
Adonis aestivalis L.	Pheasant's-eye flower
Family Resedaceae	
Reseda odorata L.	White cut-leaved mignonette
Reseda luteloa L.	Dyer's rocket
Reseda odorata L.	Common mignonette
Family Scrophulariaceae	
Alonsoa lineans Ruiz & Pav.	Mask flower
Collinsia heterophylia Buist ex R.C. Grah.	Chinese houses
Torenia foumieri Linden ex E. Foum.	Bluewings
Family Solanaceae	
Ammi majus L.	Bishop's weed
Anthriscus cerefolium (L.) Hoffm.	Chervil
Nicotiana benthamiana	
Nicotiana clevelandii Gray Umbelliferae:	
Family Valerianaceae	
Valerianella locusta (L.) Betcke	Lamb's lettuce
Valerianella olitoria (L.) Betcke	Corn salad

Table 3. Host Plants of ZYMV

Family Cucurbitaceae	
Citruilus lanatus (Thunb.) Matsum & Nakai	Watermelon
Cucumis anguria L.	West India gerkin
Cucumis dipsaceus C.G. Ehrenb. ex Spach	Hedgehog gourd
Cucumis ticitolius A. Rich	
Cucumis heptadactylus Naud.	
Cucumis melo L.	Muskmelon
Cucumis metuliferus E.H. Mey ex Schrad.	African homed cucumber
Cucumis mynocarpus Naud.	Bitter apple
Cucumis sativus L.	Cucumber
Cucumis zeyheri Sond.	
Cucurbita andreana Naud.	
Cucurbita ticifolia Bouche	Malabar gourd
Cucurbita foetidissima H.B.K.	Buffalo gourd
Cucurbita gracilior L.H. Bailey	
Cucurbita lundelliana L.H. Bailey	
Cucurbita martinzii L.H. Bailey	
Cucurbita maxima Duch. ex Lam.	Winter squash
Cucurbita moschata (Duch. ex Lam.) Duch. ex Poir	Winter Squash
Cucurbita okeechobeensis (Small) L.H. Bailey	
Cucurbita palmata S. Wats.	Coyote gourd
Curcurbita pepo L.	Squash
Cucurbita sorona L.H. Bailey	
Echinopepon wrightii cong.	Wild cucumber
Luffa acutangula (L.) Roxb.	Chinese okra
Luffa aegyptiaca Mill.	Sponge gourd
Momordica charantia L.	Bitter melon
Trichosanthes anguina L.	Snake gourd
Family Labiatae	
Lamimum amplexicaule L.	Henbit

Table 3. Host Plants of ZYMV (continued)

Family Leguminosae	
Trigonella foenum-graceum L.	Fenugreek
Family Ranunculaceae	
Ranunculus sardous Crantz	Crowfoot
Family Scrophulanaceae	
Torenia foumien Linden ex E. Foum.	Bluewings

Table 4. Select List of Aphids That Transmit ZYMV, WMV 2, PRSV, and CMV

Scientific name	Common name	ZYMV	WMV 2	PRSV	CMV
Acyrthosiphon kondoi Shinji	Blue alfalfa aphid	yes	yes		
Acyrthosiphon pisum (Harris)	Pea aphid	yes	yes		yes
Aphis craccivora Koch	Cowpea aphid	yes	yes	yes	yes
Aphis fabae Scopoli	Bean aphid		yes	yes	yes
Aphis gossypii Glover	Melon/Cotton aphid	yes	yes	yes	yes
Aphis glycines Matsumura	Soybean aphid		yes		
Aphis illinoisensis Shimer	Grapevine aphid		yes		
Aphis middletonii (Thomas)	Erigeron root aphid	yes	yes		
Aphis neni Fonscolombe	Oleander aphid		yes		
Aphis nasturtii Kaltenbach	Buckthorn aphid		yes	yes	yes
Aphis sambuci L.	Elder aphid		yes		
Aphis spiraecola (citricola) Patch	Spirea aphid		yes		
Aphis spireacola Van der Goot	Spirea aphid	yes	yes	yes	
Aulacorthum circumflexum (Buckton)	Mottled aurm aphid		yes		
Aulacorthum magnoliae (Essig & Kuwana)			yes		
Aulacorthum nipponicum (Essig & Kuwana)			yes		
Aulacorthum solani (Kaltenbach)	Glasshouse potato aphid		yes	yes	
Brachycadus helichrysi (Kaltenbach)	Leaf-curling plum aphid		yes		yes
Brachycaudus cardui (L.)	Thistle aphid		yes		yes
Cavanella aegopodii (Scopoli)	Willow-carrot aphid		yes		

Scientific name	Common name	ZYMV	WMV 2	PRSV	CMV
Crypotomyzus ribis (L.)	Redcurrant blister aphid		yes		
Dysaphis crataegi (Kaltenbach)	Hawthorn-carrot aphid		yes		
Hyalopterus pruni (Geoffroy)	Mealy plum aphid		yes		yes
Lipaphis erysimi (Kaltenbach)	Turnip aphid	yes			
Macrosiphoniella sanbomi (Gillette)	Chrysanthemum aphid		yes		
Macrosiphum euphorbiae (Thomas)	Potato aphid	yes	yes	yes	yes
Myzus cerasi F.	Cherry blackfly		yes		
Myzus persicae (Sulzer)	Green peach aphid	yes	yes	yes	yes
Phorodon humuli (Schrank)	Hop aphid		yes		
Phorodon humuli japonensis Takahashi	Asian hop aphid		yes		
Rhodobium porosum (Sanderson)			yes		
Rhopalosiphum padi (L.)	Bird-cherry oat aphid		yes		yes
Semiaphis dauci (F.)			yes		
Toxoptera citricidus (Kirkaldy)	Tropical citrus aphid		yes		
Uroleucon formosanus (Takahashi)			yes		
Uroleucon (Urolmelan) *gobonis* (Matsumura)			yes		
Uroleucon sp.		yes	yes		

[1] Aphid listed is not a vector, or aphid was not tested as a potential vector.

Table 5. Acreage of Cucurbit Crops in States Containing FLCP*

State	Cucumber	Pumpkin	Honeydew Melons	Squash
Illinois	[1]	6442	n.r.[2]	572
Missouri	[1]	749	n.r.	252
Arkansas	[1]	181	n.r.	564
Texas	5500	1500	4700	4417
Louisiana	[1]	103	n.r.	392
Alabama	[1]	281	n.r.	725
Indiana	1600	2116	n.r.	466
Tennessee	n.r.	1086	n.r.	349
Oklahoma	[1]	369	n.r.	391
Kansas	n.r.	181	n.r.	564
Mississippi	[1]	261	n.r.	143

[1] Limited production reported in Agricultural Statistics (1992) but exact acreage not reported (approximately 1000 acres per State).
[2] No significant production reported in Agricultural Statistics (1992).

* Data from Wilson (1993) and Agricultural Statistics (1992).

Table 6. Results From Survey Performed by Upjohn* of FLCP Plants for Plant Viruses[1]

County or Parish Location	Visual Assess-ment	Double Diffusion	Host Indexing	ELISA	Electron Micro-scopy	Distance to Soybean	Distance to Squash
Faulkner AR	slight chlorosis	no virus	no virus	no virus	no virus	25 feet	N.D.
Washing-ton AR	no symptoms	no virus	no virus	no virus	no virus	0.25 mile	0.5 mile
Red River LA	no symptoms	no virus	no virus	no virus	no virus	within soybean field	0.5 mile
Red River LA	no symptoms	no virus	no virus	no virus	no virus	25 yards	>6 miles
Red River LA	powdery mildew	no virus	no virus	no virus	no virus	10 yards	>6 miles
Red River LA	no symptoms	no virus	no virus	no virus	no virus	0.1 mile	0.1 mile
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Warren MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Issaquena MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles
Issaquena MS	no symptoms	no virus	no virus	no virus	no virus	>2 miles	>2 miles

[1] Viruses tested for in double diffusion and ELISA tests were ZYMV, PRSV, WMV 2, cucumber mosaic, squash mosaic, tomato ringspot, and tobacco ringspot viruses. For host indexing studies, extracts from FLCP plants were inoculated to *Chenopodium quinoa*, *Nicotiana benthamiana*, cucumber, tobacco and zucchini.

* Data reprinted from correspondence dated October 5 and 18, 1993.

Appendix II

Free-Living *Cucurbita pepo* in the United States.
Viral Resistance, Gene Flow, and Risk Assessment.
Report to USDA, Biotechnology, Bioloigics, and
Environmental Protection by Dr. Hugh Wilson

Free-living *Cucurbita pepo* in the United States

Viral Resistance, Gene Flow, and Risk Assessment

A report prepared for:

USDA APHIS BBEP Biotechnology Coord
Federal Building, rm. 852A
6505 Belcrest Road
Hyattsville, MD 20782
Order# 43-6395-3-C4203

By:

Dr. Hugh D. Wilson
Department of Biology
Texas A&M University
College Station, TX 77843
12 Jul 1993

Introduction: This report brings together information relating to free-living gourd populations of the United States, their association with domesticated squash and pumpkin populations growing under cultivation, possible genetic contact between free-living and domesticated populations, and possible consequences if this contact involved a transgenic domesticated line that carries viral resistance. Specific questions include:

- ❑ **What is known about the geographic distribution of *Cucurbita texana*, especially with respect to the areas where cucurbits are cultivated commercially?**
- ❑ **Is *Cucurbita texana* a weed? Is it a common weed or a serious weed in any localities? What weedy properties does it possess?**
- ❑ **Is *Cucurbita texana* difficult to control? How is it controlled?**
- ❑ **What is known about the viability, fertility, and ecological fitness of hybrids arising from crosses of *Cucurbita texana* and *Cucurbita pepo*? What impacts on *Cucurbita texana* might be expected from gene flow from commercial plantings of cucurbits?**
- ❑ **How would you design an experiment if you wanted to determine whether virus resistant *texana-pepo* hybrids were more 'weedy' than the parental species? Would gene copy number, homozygosity, or heterozygosity of the virus resistance gene influence the design or interpretation of results?**

The report begins with a review of recent information relating to *Cucurbita* taxonomy and evolution, agricultural history of the eastern United States, and biological definition of the plants under consideration. This overview of recent research, published either in the past five years or in press, provides a rational foundation and biological context for consideration of the specific questions listed above.

What is *Cucurbita texana*?

Cucurbita texana is a free-living system of populations that fall within the primary gene pool of *Cucurbita pepo*, a species that also includes fully domesticated elements and other free-living population systems.

The category 'species' is used here in the biological sense, i.e., to denote those population systems that share a common ancestor and lack full reproductive isolation. The term 'free-living' is assigned to plant populations that are able to survive, without direct human assistance, over the long term in competition with the native flora. This is a general ecological category that includes plants that colonize open, disturbed, prime habitat that is either under human control (weedy populations) or natural disturbed areas such as river banks and sand bars (wild populations). Domesticated populations show structural features that have resulted from human selection and, usually, an inability to maintain long term populations without human assistance.

A rational approach to any problem involving free-living *C. pepo* of the United States requires definition of the biological elements involved as well as the relative position of these elements within the broader biological context of the species. This involves two fundamental aspects of plant systematics: classification and phylogeny.

Classification: The biological connection between *C. texana* and *C. pepo* was not appreciated when the plant was first 'discovered' by Western Science. The first documented collections of the plant were taken from South Texas in 1835 by J. L. Berlandier, a botanist working for the U.S.-Mexico Boundary Commission. G. H. A. Scheele, examining collections of the plant made by F. J. Lindheimer along the upper Guadalupe River in 1845, decided that these specimens represented a genus of the Cucurbitaceae new to science, and provided the name *Tristemon texanum* in 1848. Asa Gray later studied the Lindheimer collections from Texas and noted that the 'Texas Gourd' was quite similar to forms of the domesticated ornamental gourds, then known as *Cucurbita pepo* var. *ovifera*. In an effort to insure that the classification system reflected this similarity, Gray changed the name from *Tristemon texanum* to *Cucurbita texana* in 1850. While the 'Texas Gourd' remained formally classified as a distinct species of *Cucurbita* until 1988, students of the genus recognized a close affinity between this free-living taxon and *C. pepo*. Debate centered on the position of *C. texana* as either a native element of the flora and possible progenitor of the domesticate, or a derivative, feral 'escape' from pre-historic cultivation of ornamental gourd-like plants that originated in Mexico (Bailey 1929;1943;Erwin, 1938). This debate has continued to the present (Heiser, 1985), although data generated in the 1980s has placed the problem of 'Texas Gourd' classification as one component of a broader problem that involves free-living types from Mexico and the eastern U.S.

Cucurbita fraterna, a putative relative of *C. pepo* was rediscovered at several sites in northeastern Mexico during the early 1980s (Nee, 1990). Subsequent comparative analyses indicate that *C. fraterna* will hybridize with *C. pepo* (Nee, 1990) and, in terms of both allozyme genetic identities (Wilson, 1989;Decker-Walters et al., 1993) and shared chloroplast DNA restriction site mutations (Wilson et al., 1992) placement of this taxon as a free-living element of *C. pepo* by Andres (1987) is generally accepted. Similar criteria, cross-compatibility and high indices of genetic similarity, were used by Decker (1985, 1986, 1988) to formally place the Texas Gourd as an intraspecific element of *C. pepo*.

In addition to justifying the placement of two free-living elements within *C. pepo*, recent studies of molecular genetics have also revealed a fundamental pattern of intraspecific variation, and the classification has been changed to reflect that perspective. As indicated by congruent patterns of variation among both from nuclear (Decker, 1985;Decker and Wilson, 1987;Wilson, 1989;1990) and plastid (Wilson, et al., 1992) molecular markers, the *C. pepo* lineage is composed of two fundamental elements, formally identified as subspecies *ovifera* and *pepo*. These, however, do not represent free-living vs. domesticated lineages. Subspecies *pepo* includes only domesticated elements. These include all Mexican landraces examined to date, all 'pumpkin' cultivars, all 'marrow' (zucchini, cocozelle, etc.) cultivars, and a subset of the ornamental gourd types (orange ball, miniature ball, warty hardhead) that usually carry reddish pigment in the fruit. Subspecies *ovifera*, on the other hand, contains a mixed group

that is organized into three varieties. Variety *ovifera* is the domesticated element of the subspecies. It contains domesticated ornamental gourd types that are not included in ssp. *pepo* (spoon, egg, bicolor, crown of thorns, pear, etc.), as well as cultivars of acorn squash, crookneck squash, straightneck squash, and scallop or 'paddy-pan' squash. As most recently defined (Decker-Walters, et al., 1993), free-living gourds that occur in Texas are classified as *C. pepo* ssp. *ovifera* var. *texana*. Similar, free-living plants that occur in the U.S. beyond Texas (Illinois, Missouri, Arkansas, Oklahoma, and Louisiana) are placed in *C. pepo* ssp. *ovifera* var. *ozarkana*.

A summary of *C. pepo* classification, both 'traditional' (based primarily on plant structure) and 'current' (based on crossability and genetic structure), is presented here as Fig. 1. The 'current' alignment stands as a working hypothesis and a functional foundation for on-going studies of the species. While there is general agreement on fundamental aspects of this classification system, i.e., a basic, two-parted pattern of differentiation of the species indicated by the "Lineage Division" of Fig. 1, and conspecific placement of free-living and domesticated elements, other aspects of the classification are debated and under test at this point in time. However, primary areas of disagreement among those working with the *C. pepo* 'problem' do not center on the pattern of genetic differentiation that has produced the entities defined as subspecies and varieties in Fig. 1. These have been relatively well defined by a series of comparative studies that show considerable congruence. The 'Texas Gourd' has not been treated as a distinct species by specialists since the formal name change in 1988 (Decker, 1988;Decker-Walters, 1990;Decker-Walters et al., 1990;Decker-Walters et al., 1993;Harlan, 1992;Heiser, 1989;Smith, 1992;Cowan and Smith, 1993;Wilson, 1989;1990). There are, however, several competing hypotheses regarding phylogenetic linkage between both free-living and domesticated elements of *C. pepo*. As indicated below, these different notions relate to eastern North America as a possible center of New World agricultural origin and genetic differentiation under human selection. This, in turn, produces different perspectives on the biological/historical significance of free-living gourd populations currently inhabiting eastern North America.

Phylogeny: As indicated by the structural difference between an 'egg' gourd cultivar and a world record pumpkin that might weigh several hundred pounds, *C. pepo* shows remarkable polymorphisms in plant structure. It also shows a worldwide pattern of distribution within a wide range of habitats, both cultivated and natural. The extant pattern of distribution and variation represents the end points of a lineage or 'family tree' that evolved through time. Phylogenetic analysis is an attempt to define this evolutionary history.

It is generally assumed that, prior to the development of New World agriculture, *C. pepo* existed as a single, wild-growing entity. This is based on comparative analyses of crossing relationships (Whitaker and Bemis, 1964;1975;1976), pollen vectors (Hurd et al., 1971), and chloroplast mutations (Wilson et al., 1992) among species of *Cucurbita*. All elements of the species, as depicted in Fig. 1, therefore share a common ancestor, i.e. the species is monophyletic. The common ancestor of extant members of the *C. pepo* lineage was probably an annual, gourd-producing plant adapted to colonization of disturbed ground in riverine habitats. The archaeological record indicates that this free-living ancestral type

initially became involved as a fundamental element of the New World agricultural revolution in the highlands of Oaxaca, Mexico more than 10,000 years ago. (Smith, 1986). Subsequent evolution under both human and natural selection, coupled with human-mediated and 'natural' means of dispersal, has produced the remarkable suite of structural forms that now comprise the species and now show a global distribution.

The archaeological record shows domesticated *C. pepo* in central (Tehuacan) and northeastern (Tamaulipas) Mexico at about 7,000 years ago (McClung de Tapia, 1992). The plant is found among the first archaeological indications of agriculture in the northern desert borderlands of the Mexican agricultural center (southwestern U.S.) by about 3,000 years before present (Minnis, 1992). This sequence of dates establishes a relatively uncomplicated picture of origin and diffusion, with the *fraterna* types from northeastern Mexico or *texana* types from Texas representing extant descendants of the ancestral progenitor type of *C. pepo* (Nee, 1990). It appears, however, that this is not the full picture of domestication and differentiation of *C. pepo*.

Centers of origin are usually centers of genetic diversity. However, in terms of molecular and structural differentiation, landraces now under cultivation in traditional milpas throughout Mexico are remarkably uniform (Decker, 1985;Wilson, 1990). The produce section of most U.S. supermarkets carries a more diverse sample of *C. pepo* than could be obtained from a full sample of modern field collections taken from throughout Mexico. While native *C. pepo* cultivar diversity in Mexico could have been extirpated during European colonization, it is also quite possible that one arm of the *C. pepo* lineage - diverse elements associated with ssp. *ovifera* (Fig 1) - evolved under human selection in the eastern North America. (Whitaker and Carter, 1946;Decker, 1988;Smith, 1992;Cowan and Smith, 1993).

Recent archaeological work has demonstrated that *C. pepo* was present in the eastern U.S by 7,000 years ago. It is difficult to determine with certainty if these early remains represent a domesucated form (Smith, 1987), although there appears to be a consensus that fully domesucated types were present in eastern North America at least 3,000 years ago (Heiser, 1989;Smith, 1992). Perspectives on the phylogenetic significance of *C. pepo* in eastern North America are reviewed by Smith (1992- chapter 4), Cowan and Smith (1993), Decker-Walters et al. (1993), and Asch and Sidell (1992). Data relating to this issue are a complex mix of biochemical genetics, archaeology, history, plant population biology, and personal inclinations. Assessment of the situation and interpretation of the data are difficult for the non-specialist as well as those actively engaged in the issue. Of various phylogenetic scenarios presented by Smith (1992), two are presented here (redrawn and modified) to depict opposing hypotheses. The first (diagram A) firmly roots the wild progenitor of *C. pepo* domesucates in Mexico, with human-mediated dispersal from Mexico into eastern North America whereas the second (diagram B) places the ancestral wild type with a broader distribution that includes eastern North America, and subsequent independent domestication in both Mexico and eastern North America.

Depictions of *C. pepo* evolution present as panels A and B in Fig. 2 are consistent with available data, both botanical and archaeological. In terms of gaining a better understanding

of the genetic structure and phylogenetic history of *C. pepo*, the difference between these two notions is critical. However, for the purposes of this report, both scenarios contain common elements that speak directly to the issue of free-living gourds of the eastern U.S. and risk assessment:

- many free-living *C. pepo* populations of the eastern U.S. do not appear to be recent (historical) escapes from cultivation (see Asch and Sidell, 1992 for an opposing view). They appear to represent an ancient lineage that carries a genetic legacy of long-term human selection.

- extant free-living *C. pepo* populations of the eastern U.S. appear to be the result of long-term genetic interactions that have involved both domesticated and free-living entities.

Consideration of both classification and phylogeny reveals that the biological entity defined by the name '*C. texana*' constitutes only a portion of a broader system of free-living *C. pepo* populations that inhabit eastern North America. Plants carrying this name are not biologically distinct or reproductively isolated from other elements of *C. pepo*, both domesticated and free-living. The U.S. populations show levels of genetic differentiation that allow classification as *C. pepo* ssp. *ovifera* var. *texana* (Texas) and *C. pepo* ssp. *ovifera* var. *ozarkana* (Illinois, Missouri, Arkansas, Oklahoma, and Louisiana). Populations from other areas of the U.S. distribution are difficult to classify, possibly because of hybridization or other connections to domesticated elements of the species (Asch and Sidell, 1992;Decker-Walters et al., 1993). Since the focus of this report is not restricted to the traditional '*texana*' types that are limited to Texas, plants under consideration here will be referred to as free-living *C. pepo* of the U.S (excluding the Mexican ssp. *fraterna*), or FLCP.

What is known about the geographic distribution of FLCP, especially with respect to the areas where cucurbits are cultivated commercially?

Geographic Distribution of FLCP:

Data relating to plant distributions are housed in herbaria, collections of plant specimens that are maintained by private and public institutions throughout the world. While this is a unique and extremely valuable resource, interpretation of herbarium data requires sensitivity to possible bias. Some plants are collected more often than others. Herbarium collections often house a relatively full assemblage of rare plants that are of interest to the field botanist and relatively easy to collect and process. Thus, genera and species of orchids are usually well represented in herbarium collections. On the other hand, common weeds that are difficult to identify, possibly not part of the native flora ('escaped' garden plant), and carrying awkward structures that are difficult to manage in a plant press (large, fragile flowers and gourds), are often neglected by the field botanist and, as a result, not present in herbarium collections. Given this potential bias, it is reasonable to assume the FLCP is under-collected and therefore under-represented in herbarium collections. The herbarium sample is also biased geographically in that areas near centers of botanical activity (universities, botanical

gardens) are more intensively sampled. The distribution data presented here must be interpreted with these factors in mind.

The first effort to map the distribution of FLCP was made by Andres (1983). Subsequent work in this area has been reviewed by Smith (1992). The distribution presented here (Fig. 5) is based on data compiled by Asch and Sidell, (1992), Smith (1992) and Cowan and Smith (1993). This has been expanded by inclusion of data from Andres (1983), unpublished collection data from research collections at Texas A&M, and data taken from a request for information sent to 77 herbaria and 35 weed specialists from the eastern U.S. as part of the development of this report. While most of the county records depicted in Fig. 5 are vouchered by herbarium specimens, some represent sightings of the plant made by field botanists or weed specialists. The nature and source of these records are listed in Appendix I, which is an extension of Smith's table 4.1 (1992-chapter 4).

The distribution depicted in Fig. 5 represents records of established, free-living populations. Reports that might represent recent escapes from cultivation were not mapped, although these were included in Appendix I.

Documented FLCP populations are recorded for 125 counties in 12 states. Given dates of collection (Appendix I), it is reasonable to assume that, with the possible exception of the extreme West Texas cluster (area 'A', fig. 5), this is a reasonably accurate depiction of the general range of distribution of extant populations. Clusters of county records from south-central Texas (Texas A&M), northwestern Arkansas (University of Arkansas), south-central Alabama (University of Alabama), and western Missouri (Missouri Botanical Garden) probably reflect institutional-proximity collection bias. This probably also explains relatively large areas in the central portion of the range of distribution, such as northern Alabama/Mississippi and western Tennessee, that lack FLCP records. Thus, a more dense pattern of county records would be expected from a fully accurate FLCP distribution map. This would, however, probably correspond to patterns of drainage in that the entire range of distribution is associated with river systems that lead from the central U.S. to the Gulf of Mexico. This corresponds to the floodplain or 'riverine' ecological adaptations of FLCP.

The southeastern limit of distribution depicted in Fig. 5 is problematic. Duncan and Kartesz (1981) list *C. pepo* var. *ovifera* as present on the Georgia coastal plain, and there are records for *C. pepo* growing wild in the Carolinas (Asch and Sidell, 1992). However, contacts with a full complement of field botanists at the University of Georgia, floristic workers from northern Florida, and agricultural experiment station weed specialists from several areas of Georgia (S. Brown, pers. com.) produced no FLCP records for the states of Georgia and Florida. Northern limits are also uncertain. A fully accurate map might include more county records from Indiana and southeastern Kansas, and possibly some records for Ohio.

As indicated above, the genetic structure of FLCP within this range of distribution is complex and ill-defined at this point in time. It appears, however, that plants showing the classic 'wild type' morphology of free-living *Cucurbita* species, as depicted by L. H. Bailey (Fig. 3) can only be found along streams that lead to the Gulf of Mexico in Texas and the

Ozark highlands of Arkansas, Missouri, and Oklahoma (areas 'B' in Fig. 5). While other populations are comparable in terms of vegetative morphology, patterns of variation in fruit color, shape, and the absence of bitterness, suggest genetic linkage - either ancient (Cowan and Smith, 1993) or recent (Asch and Sidell, 1992) - with domesticated forms of the species.

Geographic Distribution of Domesticated *C. pepo* within the FLCP range of distribution:

An accurate mapping of domesticated *C. pepo* populations during a given growing season would probably include nearly every county within the 12 state FLCP distribution presented in Fig. 5. Domesticated *C. pepo*, both ssp. *pepo* (zucchini) and ssp. *ovifera* (crookneck, acorn) are common elements of the U.S. home garden. With the exception of the trans-pecos and Edwards Plateau areas of western Texas, it is reasonable to assume that home gardens are common throughout the range and also that many of these support cultivated populations of *C. pepo* domesticates. While this aspect is difficult to quantify, a Gallop Poll census contracted by the U.S. Gardening Association (B. Butterfield, pers. com.) indicates that 40% of all households in the mid-western U.S. maintained a home garden during the 1991-1992 growing season. Of these 9,300,000 gardens, 18.3% grow 'summer squash' (mostly 'yellow crookneck' and 'zucchini'), 9.8% have 'winter squash' (mostly 'acorn squash') as part of the garden, and 13.5% cultivate 'pumpkins'. These data indicate that possible weed/crop hybridization events in the central U.S., with only 'summer squash' as the putative domesticated pollen source, involve 1,701,900 local, cultivated populations that are distributed throughout the FLCP range of distribution. The entire FLCP range is within the range of distribution of specialized pollen vectors, 'squash bee' species of the genera *Xenoglossa* and *Peponapis* (Hurd and Linsley, 1964). These large, solitary bees can transfer pollen between domesticated and free-living *Cucurbita* populations that are separated by at least 1300 m (Kirkpatrick and Wilson, 1988). Density of home gardens within the 12 state FLCP range is probably highest in fertile lands of floodplains and valleys; the habitat of FLCP Thus, in terms of both population density and ecology, crop/weed genetic interaction within the FLCP range is most likely to involve domesticated elements inhabiting home gardens. If only 1% of home gardens carrying 'summer squash' fall within 'bee range' of a FLCP population, then over 17,000 sites would be expected to show some type of crop/weed genetic interaction during a given growing season.

In terms of crop-to-weed gene flow, large monocultures of domesticates that are typical of commercial plantings constitute a significant element in that these present a massive pollen source. The national census of agricultural production, produced by the U.S. Census Bureau, tracks national commercial production of 'squash' and 'pumpkins'. Data listed under these names are mostly linked to plants assignable to *C. pepo* cultivars. While commercial production of other taxa, such as *C. argyrosperma* (cushaw squash), *C. moschata* (butternut squash), and *C. maxima* (large pumpkins), are included in these figures, it is assumed here that their contribution is minimal.

The most recent data for 1987 indicate that 58,198 acres of squash were produced at 7,763 farms in the U.S. National production of pumpkins at 6,921 farms was drawn from 40,652 acres in 1987. Total squash production within the 10 'core' (excluding Kansas and

Indiana) state FLCP range of distribution was, in terms of acres harvested, 13.7% of the national total. Commercial pumpkin crops were taken from 11,189 production acres within the FLCP range of distribution, which was 27.5% of the national total. A ranking of commercial production by state for both squash and pumpkin acreage combined, as a percentage of the national total, is presented in Fig. 6. This figure is based on data taken from the 1987 agricultural census that are present here as Appendix II. States within the 'core' FLCP range of distribution (Fig. 5) are indicated by an arrow in Fig. 6 and bold font in Appendix II.

As indicated by these figures, significant levels of commercial *C. pepo* production occur in Illinois (#3), Texas, (#5), and Tennessee (#20). The southern center, Texas, features high levels of squash production (ssp. *ovifera* var. *ovifera*) whereas there is a shift to pumpkin production (ssp. *pepo*) at the northern portion of the FLCP range of distribution. Examination of state census data by county reveals that 41% of Illinois pumpkin production is centered along the upper Illinois River bottoms in Tazewell County (Fig. 5) and the state center for squash production (49% of the state total) is adjacent Mclean County. Of the 15 Illinois counties known to carry populations of FLCP, 3 are listed by census data as squash producers (8% of the state total acreage) and 4 report a total of 186 acres of commercial pumpkin production (3% of the state total). Squash production in Texas is centered along the lower Rio Grande Valley in Cameron County with 25% of total harvested acres. The set of 33 counties with documented records of FLCP populations in Texas includes 5 that report squash harvest of 97 acres (2% of the State total) to the census.

Is FLCP a weed? Is it a common weed or a serious weed in any localities? What weedy properties does it possess?

The ecological niche of FLCP is centered in open, disturbed, fertile ground. It is an annual plant that becomes established each Spring as a seedling that germinates in loose soil at an open, sunny site. The seedling develops into a clambering vine that, through the use of tendrils, uses other plants as support to gain access to the sun. The size of the plant is limited by available nutrients and moisture. As elements of the native, riverine flora of North America, FLCP are adapted to climb tall trees of the native floodplain forests and gather light from the forest canopy. They are also able to form roots at each stem node (point of leaf attachment) if the node is in contact with moist soil. This allows horizontal converge of open areas (sandbars, river banks). Since new shoots can also be produced at each node, individual plants are able to access light through both ground and canopy cover. Thus, under favorable conditions, individual FLCP plants can be massive. Large, yellow, unisexual, bee-pollinated flowers, identical to those produced by domesticated squash (Fig. 4), are initially produced in late Spring. Flower production continues until the first frost. Large plants have the potential to produce 25-50 fruits, each containing 100-200 seeds. Seed dispersal is accomplished by the buoyant fruits which are typically deposited at the high water level during spring floods, often in fertile accumulations of 'drift' deposits along the river margin. Thus, FLCP is distributed along drainage systems and fertile floodplains within its range. As indicated above, these are also ecological centers of agricultural activity.

The suite of structural and ecological factors expressed by FLCP are also manifested by other elements of the Cucurbitaceae that have been introduced into the North American flora and survive today as aggressive, economically important weeds. These include the 'Bur-Gherkin' (*Cucumis anguria*), the weed form of Muskmelon or 'Dudaim melon' (*Cucumis melo* var. *dudaim*), and the weed form of Watermelon or 'Citron' (*Citrullus vulgaris* var. *citroides*). While these plants rarely achieve 'top 10' ranking on State listings of noxious weeds, local infestations can represent a significant agricultural problem. This is also the case for FLCP.

Population structure for FLCP in what appear to be 'native' areas within its range of distribution (Fig. 5 - 'B') is expressed by Correll and Johnston (1970) as "rare but abundant where found." High population densities within the native area could reflect annual situations where minimal Spring flooding has left fruits in place. However, this writer has observed a large, relatively permanent (10 years) population at Lake Sommerville, an impoundment of Yegua Creek in Burleson County, Texas. This population is apparently maintained and increased by water level fluctuations associated with the reservoir. These function to 'float' fruits into areas surrounding the impoundment, such as pastures and fields, that are not typical habitat. Subsequent colonization of these areas allows expansion of the population into areas where they could present a problem for the local human population, i.e., a transition to the 'weedy' condition as defined by the Weed Science Society of America. Thus, it would appear that the plant can take advantage of human-mediated habitat changes to 'invade' open ground beyond the drainage course, maximize population size, and thereby impact agricultural plant populations of the floodplain.

This response does not appear to present a significant agricultural problem within the native subareas (Fig. 5 - 'B') of FLCP distribution. It has, however, produced significant weed populations in Arkansas, Louisiana, Mississippi, Illinois, and Kentucky (Asch and Sidell, 1992). The plant is listed, as *C. texana*, as a weedy contributor to current crop losses in Arkansas (Bridges, 1992) and has been ranked among the top 10 weeds in that state for some time (McCormick, 1977). Large weed populations have been reported (Harrison et al., 1977;Oliver et al., 1983) from counties along the Arkansas and Red River drainages (Fig. 5 - 'C'). Ford Baldwin (pers. comm.) "knows of it along 100-km segment of Arkansas Valley, Clarksville to Conway, not south of Little Rock; and Red River, southwest Arkansas. Mostly occurs on very sandy, very well-drained overflow areas; very tenacious where established, but doesn't easily spread. A weed of soybean and rice" (Asch and Sidell, 1992). The plant is described as a "troublesome weed in field crops of the Arkansas and Red River bottomlands in Arkansas" with a "potential to spread to other areas within the Mississippi Delta" by Oliver et al., 1983). Reports from Mississippi and Louisiana suggest that this has occurred. Large, weedy populations of FLCP have been reported from soybean and cotton fields along the lower Yazoo drainage in Mississippi (D. Asch, C. Elmore, pers. com.;Asch and Sidell, 1992), and the Mississippi bottoms in northeastern Louisiana (D. Reynolds, pers. comm.;Smith, 1992). A small, local instance has been reported along the Red River floodplain just North of Shreveport in northwestern Louisiana (Smith, 1992). Asch and Sidell (1992) cite D. Sanders (pers. comm.); "A weed problem of cotton in north half of state; Red River and Mississippi River valleys. Not rare, but localized; an intense problem where it is established" and R.

Rogers (pers. comm.) as indicating the FLCP is a weed problem "from 11 northeast parishes of Louisiana."

While the weedy populations of Arkansas are evidently a perennial problem, reports from Mississippii and Louisiana are based on 'outbreaks' that are evidently linked to dispersal from sporadic flooding events and associated fruit dispersal into cultivated fields (C. Elmore, D. Reynolds, pers. comm.). Infested fields are "difficult to harvest and may have reduced yields" that result from "uncontrolled early-season or late-season gourds which entwine soybean plants and eventually cause soybean lodging" (Oliver et al., 1983). This also appears to be significant for FLCP populations in Illinois. Asch and Sidell (1992), citing communications with Kaskaskia Island (Randolph County) farmers R. Bartels and C. Jokerst, indicate, "Bartels recalls seeing plant on his farm as long ago as 1953 or 1954 when he was 12 or 13 years old. Plant became a serious field weed on island after it was widely dispersed by 1973 flood. Also spread by soybean combines. Bartels collected and destroyed 60,000 lbs. of gourds from a 140 acre field in 1987. Bartels has seen only round or pear forms; none long-necked or warty."

Is FLCP difficult to control? How is it controlled?

Asch and Sidell (1992), citing personal communication with G. Brown regarding FLCP as a weed in Kentucky, indicate that the plant is "prevalent in Union Co. (West Kentucky), but only in Ohio River Bottoms. A soybean-field weed, but also in corn if atrazine herbicide not used." G. Kapusta, also responding to questions from Asch and Sidell (1992) indicates that FLCP can be easily controlled by Atrazine in corn fields but, in Illinois, it is mostly a weed of soybeans due to wide rows and open habitat.

Control of FLCP in soybean fields can be provided by preemergence application of metribuzin, metribuzin plus alachlor, and oxadiazon, although success depends of soil and climate conditions (Oliver et al., 1983). Adequate postemergence control can be obtained by treatments of acufluorfen, oxyfluorfen, and metribuzin plus 2,4-DB if applied at an early soybean growth stage and repeated (Oliver et al., 1983). However, effective chemical control in soybean fields has been complicated by the need for repeated applications due to "continual emergence of Texas Gourd under favorable conditions throughout the growing season" (Weideman and Templeton, 1988). Also, the mix of herbicide agents used for FLCP control in soybeans fields is not applicable for use in cotton fields (D. Sanders, pers. comm.). Tests by Oliver et al. (1983) indicate that "none of the herbicides evaluated provided a consistent level of Texas gourd control over all years and locations." Consistent control is difficult "since preemergence herbicides such as metribuzin are rainfall-dependent and postemergence herbicides must be applied by the V2 stage of soybean growth and repeated at the V4 stage or as needed." The literature provides no informatioin regarding mechanical control of FLCP weed populations. Continuous seed germination and a capability to root at the nodes probably minimize the efficacy of mechanical control. Thus, efforts are underway to perfect a biological control that targets FLCP specifically using 'mycoherbicides' (Yu and Templeton, 1983;Boyette et al., 1984;Yu et al., 1988). These efforts demonstrate that FLCP is a problem weed that can be difficult to control with traditional methods.

What is known about the viability, fertility, and ecological fitness of hybrids arising from crosses of FLCP and domesticated types of *Cucurbita pepo*? What impacts on FLCP might be expected from gene flow from commercial plantings of cucurbits?

The nature of FLCP/domesticate hybrids and their progeny

Initial connections between FLCP and domesticated *C. pepo* were established when it became evident the F_1 hybrids between the two types were readily produced and fully fertile (Whitaker and Bemis, 1964). Eventual recognition that FLCP and domesticated forms constitute a conspecific unit resulted from demonstration of normal mendelian genetic behavior of the F_1 hybrid (Kirkpartick et al., 1985) and experimental proof that crop/weed hybridization can be expected to result if crop and weed populations are growing in proximity (a radius of at least 1300 meters) and within the distributional range of specialized 'squash' bees (Kirkpartick and Wilson, 1988), which occur throughout the FLCP range of distribution. This perspective has been supported by the discovery of first-generation gene flow from cultivars to FLCP, as indicated by concordant heterozygosity at multiple isozyme loci among progeny taken from FLCP populations (Decker and Wilson, 1987; Decker-Walters et al., 1993). Allozyme frequency distributions and distinctive patterns of variation in fruit structure, color, and bitterness within populations of FLCP clearly indicate that past hybridization events have resulted in permanent gene transfer, or introgression, between domesticates and FLCP in the eastern U.S. (Decker and Wilson, 1987; Decker-Walters et al., 1993;Smith, 1992;Wilson, 1989;1990).

Given current theory regarding genetic relationships among crop/weed systems as they relate to crop plant evolution (Harlan, 1992), this pattern of sporadic crop/weed genetic interaction is to be expected. It provides a unique foundation for "mixing the gene pool" (Harlan, 1965;1969) and injecting high levels of genetic variation that are needed to produce the types of extreme structural adaptations that characterize domesticated plants. However, as indicated by "wild carrots" (Queen Ann's Lace-*Daucus carota*) in the northeastern United States, "wild cucumbers" (Bur-Gherkin, Dudaim Melon-*Cucumis* spp.) in the southeast, and "wild oats" (*Avena fatua*) in the plains and West, this pattern of gene exchange can function to increase adaptive fitness of the non-domesticated participant.

Relative fitness of *C. pepo* crop/weed hybrids and F_2 progeny has not been examined. However, Kirkpatrick (1983), in an effort to define general linkage relationships between structural and molecular characters, conducted a detailed quantitative study of crop/weed F_1 hybrids, parental types, and F_2 progeny generated by self-pollinating the F_1. The hybrids were developed using ssp. *ovifera* var. *texana* from Fayette County, Texas as the staminate parent and a zucchini cultivar (ssp. *pepo*) as the pistillate parent. The following discussion is based on data extracted from that study.

The F_1 hybrids generated from this study were clearly heterotic, i.e., more robust and vigorous than either parent. This is reflected by data depicted in Fig. 7. The *texana* parent was a typical vining plant with relatively small fruit and leaves, whereas the zucchinis were 'bush' types with suppressed lateral shoots, larger fruits and larger leaves. The F_1 plants combined features of both parents, retaining the longer, more numerous lateral shoots of the *texana* parent and the larger fruits and leaves of the zucchini parent. Expression of the 'wild type' features was weighted, mainly because these are generally genetic dominants in the Mendelian sense (Robinson et al. 1976). Thus, a combination of heterosis resulting from a relatively 'wide' intraspecific cross and simple genetic dominance produces a crop/weed F_1 that is essentially greater than the sum of its parts. One would assume that F_1 plants would, at the very least, compete well with typical FLCP in a 'weedy' agricultural habitat and, more likely, show greater fitness in terms of progeny production. This, however, has not been tested. One would also expect a reduced heterotic response from a crop/weed F_1 plant if the crop parent was more closely related to FLCP, i.e., an element of ssp. *ovifera* var. *ovifera* such as crookneck or acorn squash. This has not been tested.

The F_1 hybrid is an important, but ephemeral, element of the crop/weed introgressive process. More important is the nature of the F_2 generation. This would derive from either self pollination of the F_1 or back crossing from parental types. Given the breeding system of FLCP and a probable 'home garden' pollen source, the generation immediately following a crop/weed hybridization event would most likely result from self-pollination of the F_1. As indicated by the 'F_2' panel in Fig. 7 self pollination of the *texana*/zucchini F_1 produces an F_2 progeny that shows a broad pattern of variation that reflects the results of "mixing the gene pool."

A more detailed perspective on the nature of variation among F_2 plants is presented in Fig. 8. Analysis of this F_2 family was conducted to test the notion that differentiation between crop and weed forms of *C. pepo* is based on 'supergenes' or tightly linked gene clusters. This involved measurement of 14 characters, representing both vegetative and floral morphology, from 272 field-grown plants. The pattern of variation among measured plants was examined by principal component analysis, a procedure that reduces a complex pattern of variation to 'factors' which allow visualization via two-dimensional plots such as Fig. 8. The diffuse pattern of data points present in Fig. 8, each representing a single plant, indicates that genetic segregation is mostly independent, i.e., loci that carry differentiated crop/weed alleles are probably dispersed throughout the genome and not localized in linkage groups or centered on specific chromosomes.

The data presented in Fig. 8 roughly define that nature of progeny that might be produced by crop/weed hybrids generated under 'natural' field conditions. It is clear that F_1 hybrids (Fig. 8 - 'F') and their progeny (Fig. 8. - 'circle') tend to express the genetic constitution of the FLCP parent (Fig. 8 - 'T'). Adaptations characteristic of the domesticate are typically the product of homozygous, recessive genetic expression (Robinson et al., 1976). Thus, F_1 hybrids ('F') cluster with the FLCP parents ('T') in the ordination (Factor 1) provided by the suite of variables that separate domesticate ('P') from weed ('T'). Variables influencing Factor 1 are mostly vegetative (shoot and leaf characters). Ordination of samples along Factor

2, which is weighted by floral characters, places the F_1 plants ('F') in a unique position, i.e., the variables to not separate parental crop ('P') and weed ('T') plants along the axis of Factor 2. This unique phenetic placement of the F_1 plants is concordant with data presented in Fig. 7 as an indication that first generation hybrids constitute, in terms of phenotypic expression, a unique entity that transcends either parent. Weighted variables for Factor 2 include measurements of reproductive structures. Higher values along the axis of Factor 2 signify larger staminal columns, both anther and filament. Thus, in terms of general reproductive potential, F_1 plants and a subset of their progeny can be expected to offer more pollen to pollination vectors than either parent. Consequently, over the long term history of 'hybrid swarm' populations in nature, plants expressing a genetic 'mix' of crop and weed genomes will probably show a slight reproductive edge which would function maximize introgressive gene flow and recombination.

The pattern of variation among F_2 plants depicted in Fig. 8 does not reflect hybrid intermediacy. Structural variation ordinated by both Factors is clearly skewed toward the FLCP condition in both the F_1 plants ('F') and their progeny ('circle'). This pattern, probably also a function of 'wild type' genetic dominance, demonstrates the tendency of both first generation and segregating progeny to move toward the FLCP phenotype. This suggests that the frequency of plants that are ill-adapted for existence in the wild, i.e., those that resemble the domesticated parent, will be minimal in a hybrid swarm population. Conversely, the percentage of plants in a given hybrid swarm that either resemble the FLCP parent, or carry unique features associated with the FLCP condition that might enhance fitness in the wild, is maximized. The pattern of variation among both F_1 and F_2 plants in Fig. 8 therefore suggests that the products of 'natural' hybridization events between domesticated and free-living *C. pepo* are genetically 'pre-adapted' for life in the wild or weedy habitat.

It should be noted that the data generated by Kirkpatrick (1983) were not produced to approach the problem of hybrid fitness, and the analyses were not conducted with this aspect of the crop/weed interface in mind. However, the data do reflect phenotypic variation as indicated by measurements of both vegetative and floral characters. If plant size is an element of fitness, then the patterns of variation depicted in Figs. 7 and 8 are relevant to the question of hybrid fitness.

Possible impacts from crop-to-weed gene flow

Given the demonstrated potential for crop/weed hybridization within the FLCP range of distribution, as well as the documentation of both first generation and introgressant plants in extant FLCP populations within this range, it is reasonable to assume that genetic contact between domesticated and free-living *C. pepo* has occurred within the FLCP range, and that this has resulted in the production of self-perpetuating, introgressed population systems. Given the agricultural history of eastern North America (Harlan, 1992;MacNeish, 1992;Watson, 1993), and the possible role of *C. pepo* (Smith, 1992;Cowan and Smith, 1993), it is quite possible that some of these populations are extant manifestations of ancient crop/weed interactions in the area (Wilson, 1990).

This set of circumstances suggests that the introduction of new *C. pepo* strains into the FLCP range of distribution (Fig. 5) will genetically impact FLCP populations in that those genetic features that mark the strain as 'new' will be 'captured' by FLCP populations via crop/weed hybridization events. All available evidence indicates that this has happened in the past, it will happen during the growing season of 1993, and there is no reason to believe that it will not happen in the future.

If crop/weed genetic interaction has occurred within the *C. pepo* complex of domesticated and free-living forms throughout the 3,000 year history of human agricultural activity in the eastern U.S., then why be concerned about the possible involvement of transgenic strains? All available evidence, both archaeological and botanical, indicates that new, domesticated elements of the *C. pepo* complex have been sequentially introduced into the agricultural system of eastern North America over the past 3,000 to 7,000 years (Smith, 1992). However, these introductions have not carried genetic material that is that has been obtained from phyletic lineages that are not part of the *C. pepo* complex. Thus, the source of transgenes, and unknown interactions between these unique genetic elements and the *C. pepo* genome, represent, within the biological and historical context of *C. pepo*, an unknown and untested factor. The process of injecting a foreign genetic element, a functional gene that has no precedent within the phylogenetic history of a complex crop/weed system such as *C. pepo*, constitutes a biological risk. The dynamics of this risk, in terms of level and nature of impacts, are difficult - if not impossible - to predict. Specific negative impacts, if any, cannot be determined with any accuracy. However, the historical record of both intentional and accidental human genetic manipulations and subsequent impacts on natural populations (most recently the 'Africanized' honey bee) suggests caution.

A cautious approach is reinforced in this instance by the *action* of the unique genes carried by the a transgenic *C. pepo* strain. Calgene's flavr-savr tomato, for instance, poses minimal risk in that action of the gene has no obvious selective relevance to free-living elements of the tomato primary gene pool of Central and South America. However, any genetically transmissible trait that provides enhanced fitness in the wild is cause for concern. This is based on the simple fact that any selective advantage that might be 'captured' from a transgenic domesticated line by FLCP could alter existing ecological and genetic balances in such a way that individual recipient plants, and FLCP progeny that carry the advantage, would expand their numbers and their range of distribution. A transgene-mediated range extension would have negative impacts in two critical areas of the human agricultural enterprise:

- crop losses due to FLCP infestation could increase

- potentially valuable genetic diversity associated with an economically important crop plant could be diminished or exturpated through displacement of extant FLCP populations by plants representing the relatively narrow genetic base of the transgene-equipped, 'expanding' lineage

Engineered resistance to either predators (Bt-toxin) or disease (multi-viral resistance) that impact both domesticated and free-living population systems represent the type of genetic trait that could produce these changes in population structure.

Watermelon mosaic virus-2 (WMV-2) and zucchini yellow mosaic virus (ZYMV) are among the most important viruses transmitted by aphids infecting squash (Provvidenti, 1990). The viruses and their aphid vectors occur throughout the FLCP range of distribution. ZYMV has assumed great economic importance since its first recognition in 1981. It is one of the most destructive viruses occurring in squash, where it produces foliage mosaic, severe malformation, and plant stunting (Provvidenti, 1990). Symptoms of WMV-2 are less severe mosaics, chlorotic rings, and superficial changes in leaf structure, although expression varies among Cucurbit species and viral strain involved (Provvidenti, 1990).

It is important to keep in mind, for the purposes of this discussion, that interactions between plant viruses and host plant taxa are long-term, phylogenetic phenomena. This represents a basic host/parasite, co-evolving relationship that existed in the native flora long before the origin of domesticated plant species. It is therefore reasonable to assume that impacts on domesticated populations that are brought about by viral parasitism are fully comparable to similar impacts on populations of free-living relatives of the crop that exist in the native flora. This fundamental reality provides a rational justification for efforts, which are often successful, to find virus resistance among wild relatives of a given crop.

If extant FLCP populations carried resistance to important cucurbit viruses, such as WMV-2 and ZYMV, then the presence of engineered resistance in transgenic domesticated lines would pose no obvious problem in that increased fitness would not result from crop/weed introgression. This, however, is not the case. Genetic resistance to ZYMV and WMV-2 is not present in the *C. pepo* primary gene pool. This is clearly demonstrated by the fact that breeders have not been able to produce resistant *C. pepo* cultivars using traditional methods.

FLCP, a sample of var. *texana* from DeWitt County, Texas, was included in a suite of 20 free-living and domesticated taxa that were screened for viral resistance by Provvidenti et al. (1978). Plants were subjected to virus attack by inoculation of six virus strains in the greenhouse, and exposure to native viruses in the field. The FLCP sample showed resistance to one, Tobacco Ringspot Virus, of the six viral strains, as did most *Cucurbita* taxa tested. While resistance to several viral strains was present in several wild *Cucurbita* species tested, FLCP showed a pattern similar to that of the domesticated *C. pepo* used in the screen; full and often extreme susceptibility to viral attack.

If multi-viral resistance entered FLCP populations by hybridization/introgression events involving a transgenic line, it is reasonable to assume that this would provide a selective advantage in that extant negative selective pressures produced by viral parasitism would be released. Fitness of those FLCP plants carrying transgenic resistance would increase and, if viral disease constituted a significant negative selective factor in the natural habitat, this could lead to increases in population size and range of distribution for resistant FLCP

lineages. Clearly, FLCP populations have demonstrated an ability to expand and become weed problems within the agroecosystem. A relatively small shift in the selective forces that act on these populations could amplify this tendency.

While data presented here point toward the clear presence of risk in two areas - increased weedyness and loss of crop plant biodiversity - the nature of risk, in terms of magnitude and dimensions, is difficult to access. Efforts to simplify the problem by experimental determination of levels of 'fitness' or 'selective pressure' and units of selection (gene vs. genome) are complicated by the multifaceted nature of the problem. The potential participants, FLCP and *C. pepo* domesticates, are not uniform entities in terms of genetic structure. Different results might obtain from different points of genetic contact (ssp. *pepo* vs. ssp. *ovifera*, var. *texana* vs. var. *ozarkana*), either natural or experimental. Possible selective arenas include both 'natural' (undisturbed) and human (cultivated fields), as well as third 'weedy' realm that extends, more or less as a continuum of human vs. non-human selective pressures, between these extremes. While Kirkpatrick's study (1983) provides a rough picture of what might be expected to result from the combination of two differentiated *C. pepo* genomes (free-living vs. domesticated), the selective dynamics of heterosis and hybrid recombination are unknown. When considered against this diverse comparative background, assessment of 'fitness' or selective advantage that might be associated with a given transgene would be extremely difficult, even if the foreign genetic material was expressed cleanly (no pleiotrophy or epistasis) and without any genetic or recombinational 'load' (non-coding elements).

Given this set of circumstances, and that fact that these circumstances will exist when transgenic cultivars of *Helianthus annuus* are released into the U.S. and transgenic *Zea mays* is released into Mexico, one must consider the impact of this transgenic squash as a cultural precedent. If future agricultural development is to be insured, those responsible for environmental protection as it relates the development of biotechnology should - at the very least - move to insure that extant germplasm resources representing crop plant biodiversity in the United State (at a minimum) are located and salvaged as viable accessions as soon as possible. This would serve as a hedge against possible 'worse case' genetic interactions and also provide a foundation for eventual assessment of real risk over the long term.

How would you design an experiment if you wanted to determine whether virus resistant FLCP-domesticate hybrids were more 'weedy' than the parental species? Would gene copy number, homozygosity, or heterozygosity of the virus resistance gene influence the design or interpretation of results?

Experimental assessment of the possibility for enhanced 'weedyness' in virus-resistant FLCP is complicated by two factors: 1) containment, and 2) the large, unwieldy nature of FLCP and FLCP/domesticate hybrids. These factors work to negate the use of field studies based on open pollinated plants that are grown to reproductive maturity. Both problems would be eliminated by an experimental design that is based on greenhouse-grown plants and an assumption that variation in vegetative growth, or production of biomass, roughly corresponds to 'fitness' in the wild. This assumption is probably valid in that:

- All available data indicate no loss of fertility or seed-set in FLCP/domesticate hybrids and progeny
- Solitary flowers are produced at nodes. As the number of nodes increases with overall plant size, the number of flowers will increase
- The number of potential fruit increases with increases in the number of flowers.

Thus, an assumption of a direct relationship between seed production (reproductive fitness) and plant size (vegetative fitness) is reasonable.

Such an experiment could involve the following test groups:

- Domesticate A (ssp. *pepo*)
- Domesticate B (ssp. *ovifera*-non-transgenic cultivar - 'Domesticate C' without resistance)
- Domesticate C (transgenic, viral resistant)
- FLCP A (*texana* type)
- FLCP B (*ozarkana* type)
- First Generation Hybrids(10) involving crossing between Domesticates A-C and FLCP A and B as the pistillate parent (the most likely direction of gene flow under field conditions)
- F_2 progenies resulting from self-pollination of the 10 F_1s

These 25 families would be grown as single plants in 10 " pots under uniform greenhouse conditions with the following variables (5 plants from each family per variable):

1. control
2. virus inoculation (first priority should be the most virulent virus, the zucchini yellow mosaic virus [ZYMV])
3. competition with soybean
4. virus inoculation and competition with soybean

The experiment would require 500 10" pots. It would be replicated at least 4 times during the growing season. Consequently, to minimize the effect of varying photoperiod, light intensity, etc. encountered by different replicate plantings, data would be expressed as

percentage biomass produced relative to the control. The results would provide a reasonable picture of relative vigor in vegetative growth under the varying experimental conditions. This, as indicated above, should directly correspond to relative reproductive potential among the different test groups.

Literature Cited

Andres, T. C. 1983. A biosystematic Study of the *Cucurbita pepo/C. texana* complex. Manuscript of unpublished and uncompleted M.S. thesis. Texas A&M University.

_____. 1987. *Cucurbita fraterna*, the closest wild relative and progenitor of *C. pepo*. Cucurbit Genetics Cooperative Report 10:69-71.

Asch, David. Personal Communication. Office of the Iowa State Archaeologist. 319-335-1122.

Asch, D. L. and N. A. Sidell. 1992. Archeobotany. Pages 177-263 in C. R. Stafford (ed.), *Early Woodland Occupations at the Ambrose Flick Site in the Northern Sny Bottom of West-Central Illinois*. Center for American Archaeology (Research Series 10), Kampsville, Illinois.

Bailey, L. H. 1929. The domesticated *Cucurbitas*. Gentes Herb. 2:62-115.

_____. 1943. Species of *Cucurbita*. Gentes Herbarum 6: 267-322.

_____. 1951. *Manual of Cultivated Plants*. Macmillian, New York.

Baldwin, F. Personal Communication cited by Asch and Sidell, 1992. State Weed Science Specialist, Coop. Ext. Serv., University of Arkansas, Little Rock.

Bovette, G., E. Templeton, and L. R. Oliver. 1984. Texas Gourd (*Cucurbita texana*) control. Weed Science 32:649-655.

Bridges, D. C. 1992. *Crop Losses due to Weeds in Canada and the United States*. D. C. Bridges (ed). Weed Science Society of America, 309 West Clark Street, Champaign, IL. 403 pp

Brown, G. Personal Communication cited by Asch and Sidell, 1992. USDA Ext. Agent, Union Co., Kentucky.

Brown, S. M. Personal Communication. University of Georgia Cooperative Extension Service, Tifton.

Butterfield, B. Personal Communication. National Gardening Association. 802-863-1308.

Childs, D. Personal Communition cited by Asch and Sidell, 1992. Extension Weed Specialist, Department of Botany and Plant Pathology, Purdue University.

Correll, D. S. and M. C. Johnston. 1970. *Manual of the Vascular Plants of Texas*. University of Texas, Dallas.

Cowan, C. W. and B. D. Smith. 1993. New perspectives on a wild gourd in Eastern North America. Ethnobiology (in press).

Decker, D. S. 1985. Numerical analysis of allozyme variation in *Cucurbita pepo* L. Econ. Bot. 39: 300-309.

_____. 1986. A biosystematic study of *Cucurbita pepo*. Ph.D. dissertation, Texas A&M University, College Station.

_____. 1988. Origin(s), evolution, and systematics of *Cucurbita pepo* (*Cucurbitaceae*). Econ. Bot. 42: 4-15.

Decker, D. S., and H. D. Wilson. 1987. Allozyme variation in the *Cucurbita pepo* complex: *C. pepo* var. *ovifera* vs. *C. texana*. Syst. Bot. 12: 263-273.

Decker-Walters, D. S. 1990. Evidence for multiple domestications of *Cucurbita pepo*. Pages 96-101 in D. M. Bates, R. W. Robinson, and C. Jeffrey, eds. *Biology and Utilization of the Cucurbitaceae*. Cornell University Press, Ithaca, New York.

Decker-Walters, D. S., T. W. Walters, and U. Posluszny. 1990. Genealogy and gene flow among annual domesticated species of *Cucurbita*. Can. Jour. Bot. 68:782-789.

Decker-Walters, D. S., T. W. Walters, C.W. Cowan, and B. D. Smith. 1993. Isozymic characterization of wild populations of *Cucurbita pepo*. Ethnobiology (in press).

Duncan, W. H. and J. T. Kartesz. 1981. *Vascular flora of Georgia: an annotated checklist*. University of Georgia Press, Athens.

Elmore, C. D. Personal Communication. USDA Weed Science Laboratory, Stoneville, Mississippi. 601-686-5215

Erwin, A. T. 1938. An interesting Texas cucurbit. Iowa State College Journal of Science 12:253-255.

Handel, W. Personal Communication. Botanist, Illinois Natural History Survey.

Harlan, J. R. 1992. *Crops and Man*. American Society of Agronomy (2nd ed.). Madison, WI.

_____. 1965. The possible role of weed races in the evolution of cultivated plants. Euphytica 14: 173-176.

_____. 1969. Evolutionary dynamics of plant domestication. Japan J. Genetics 44: 337-343.

Harrison, S., L. R. Oliver, and D. Bell. 1977. Control of Texas gourd in soybeans. Proc. South. Weed Sci. Soc. 30:46

Heiser, C. B., Jr. 1985. *Of plants and people*. University of Oklahoma Press, Norman.

Heiser, C. B., Jr. 1989. Domestication of the Cucurbitaceae: *Cucurbita* and *Lagenaria*. Pages 471-81 in D. Harris and G. Hillman, eds. *Foraging and Farming*. Unwin Hyman, London.

Hurd, D. P. and E. G. Linsley. 1964. The squash and gourd bees - genera Peponapis Robertson and Xenoglossa Smith - inhabiting America North of Mexico (Hymenoptera: Apoides). Hilgardia 35:375-477.

Hurd, D. P., E. G. Linsley, and T. W. Whitaker. 1971. Squash and gourd bees (*Peponapis*, *Xenoglossa*) and the origin of the cultivated *Cucurbita*. Evolution 25: 218-234.

Kapusta, G. Personal Communication cited by Asch and Sidell, 1992. Weed Scientist, Department of Plant and Soil Science, Southern Illinois University, Carbondale.

Kerr, H. Personal Communication cited by Asch and Sidell, 1992. Agronomy, University of Missouri, Columbia.

Kirkpatrick, K. J. 1983. The relationship between isozyme phenotype and morphological variation in *Cucurbita*. M.S. Thesis, Texas A&M University, College Station.

Kirkpatrick, K. J., D. S. Decker and H. D Wilson. 1985. Allozyme differentiation in the *Cucurbita pepo* complex: *C. pepo* var. medullosa vs. *C. texana*. Econ Bot. 39: 289-299.

Kirkpatrick, K. J. and H. D. Wilson. 1988. Interspecific gene flow in *Cucurbita*: *C. texana* vs. *C. pepo*. Amer. J. Bot. 75: 517-525.

MacNeish, R. S. 1992. *The origins of agriculture and settled life*. University of Oklahoma Press, Norman.

McClung de Tapia, Emily. 1992. The origins of Agriculture in Mesoamerica and Central America. Pages 143-171 in C. W. Cowan and P. J. Watson, eds. *The Origins of Agriculture*. Smithsonian Institution Press, Washington.

McCormick, L. L. (compiler). 1977. Weed survey - southern States. Southern Weed Science Society, Research Report 30:184-215.

Meijer, W. Personal Communication. Curator of the Herbarium, School of Biological Sciences, University of Kentucky, Lexington.

Minnis, P. E. 1992. Earliest plant cultivation in the desert borderlands of North America. Pages 121-141 in C. W. Cowan and P. J. Watson, eds. *The Origins of Agriculture*. Smithsonian Institution Press, Washington.

Mohlenbrock, R. H. 1978. *The Illustrated Flora of Illinois. Flowering Plants: hollies to loasas*. Southern Illinois University Press, Carbondale.

Nee, M. 1990. The domestication of *Cucurbita* (*Cucurbitaceae*). Economic Botany 44(3):56-68.

Oliver, L. R., S. A. Harrison, and M. McClelland. 1983. Germination of Texas Gourd (*Cucurbita texana*) and its control in soybeans (*Glycine max*). Weed Science 31:700-706.

Provvidenti, R. 1990. Viral diseases and genetic sources of resistance in *Cucurbita* species. Pages 427-435 in D. M. Bates, R. W. Robinson, and C. Jeffrey, eds. *Biology and Utilization of the Cucurbitaceae*. Cornell University Press, Ithaca, New York.

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

Quemada, H. Personal Communication. Vegetable Biotechnology, The Upjohn Company.

Reynolds, D. Personal Communication. Northeastern Louisiana Research Station. 318-766-3769

Robinson, R. W., H. M. Munger, T. W. Whitaker, adn G. W. Bohm. 1976. Genes of the *Cucurbitaceae*. Hortscience 11:554-568.

Rogers, R. L. Personal Communication. Resident Director, Agricultural Experiment Station, NE Res. Branch, St. Joseph. Cited by Asch and Sidell, 1992.

Sanders, D. Personal Communication. Weed Science, Louisiana State University. 504-388-6195, also cited by Asch and Sidell, 1992.

Smith, B. D. 1987. The independent domestication of indigenous seed-bearing plants in eastern North America. Pages 1-47 in W. Keegan, ed. *Emergent Horticultural Economies of the Eastern Woodlands*. Center for Archaeological Investigations, Occasional Paper No. 7. Southern Illinois University, Carbondale.

_____. 1992. *Rivers of Change - Essays on Early Agriculture in Eastern North America*. Smithsonian Institution Press, Washington.

Smith, C. E., Jr. 1986. Preceramic plant remains from Guila Naquitz. Pages 265-274 in K. V. Flannery, ed. *Guila Naquitz, Archaic Foraging and Early Agriculture in Oaxaca, Mexico*. Academic Press, New York.

Thomas, D. Personal Communication. Herbarium Curator, Biology, Northeast Louisiana State University - 318-342-3108..

Watson, P. J. 1993. Review of MacNeish, 1992. Science 260:704-705.

Weidemann, G. J. and G. E. Templeton. 1988. Efficacy and soil persistance of *Fusarium solani* f. sp. *Cucurbitae* for control of Texas Gourd (*Cucurbita texana*). Plant Disease 72:36-38.

Whitaker, T. W., and W. P. Bemis. 1964. Evolution of the genus *Cucurbita*. Evolution 18: 553-559.

_____. 1975. Origin and evolution of the cultivated *Cucurbita*. Bull. Torrey Bot. Club 102: 362-368.

_____. 1976. Cucurbits. Pages 64-69 in N. W. Simmonds, ed. *Evolution of Crop Plants*. Longman, London.

Whitaker, T. W., and G. F. Carter. 1946. Critical notes on the origin and domestication of the cultivated species of *Cucurbita*. Amer. J. Bot. 33: 10-15.

Wilson, H. D. 1989. Discordant patterns of allozyme and morphological variation in Mexican *Cucurbita*. Syst. Bot. 14: 612-623.

_____ 1990. Gene flow in squash species. Bioscience 40: 449-455.

Wilson, H. D., J. Doebley, and M. Duvall. 1992. Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae). Theor. Appl. Genet. 84: 859-865.

Yu, S. M. and G. E. Templeton. 1983. The relationship of trifluralin to collor rot of Texas gourd caused by *Fusarium solani* f. sp. *Cucurbitae* (Abstr.). Phytopathology 73:823.

Yu, S. M., Ge. E. Templeton and D. C. Wolf. 1988. Trifluralin concentration and the growth of *Fusarium solani* f. sp. *Cucurbitae* in liquid medium and soil. Soil Biol. Biochem. 20:607-612.

Figure 1. Classification:

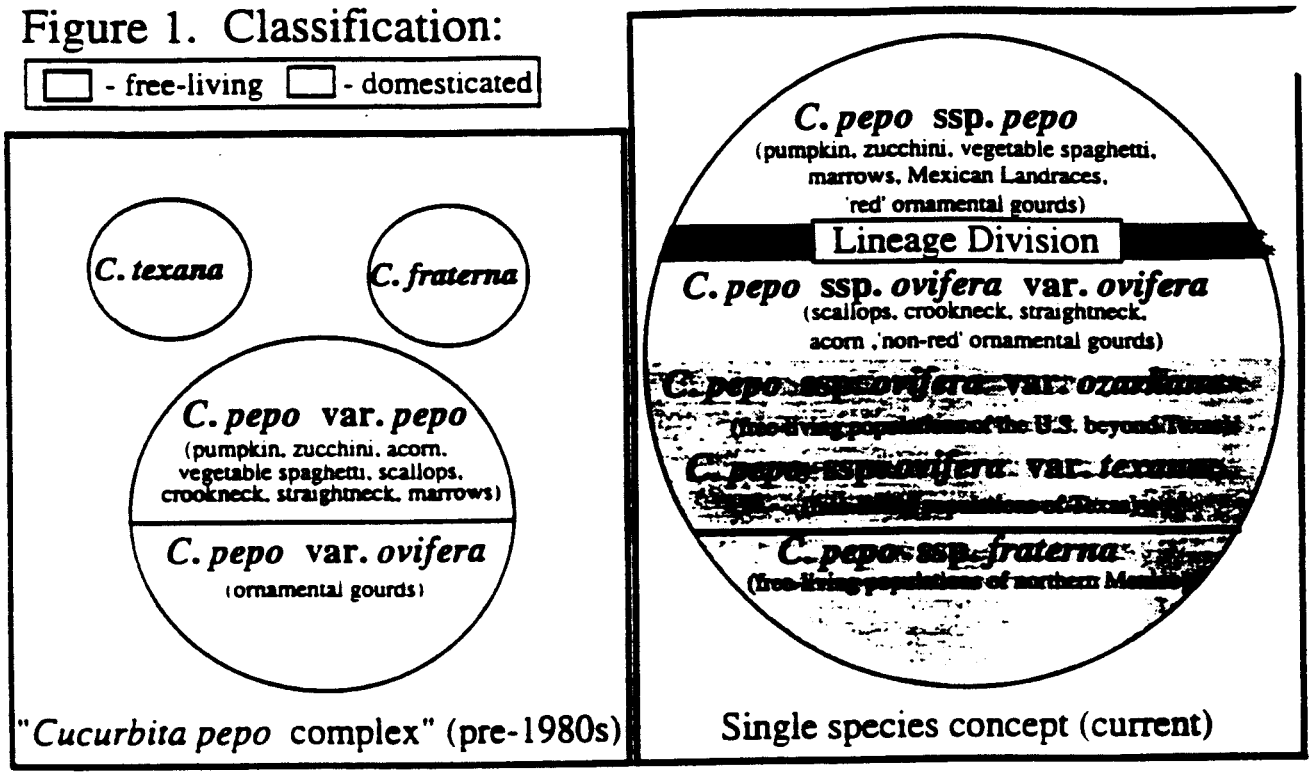
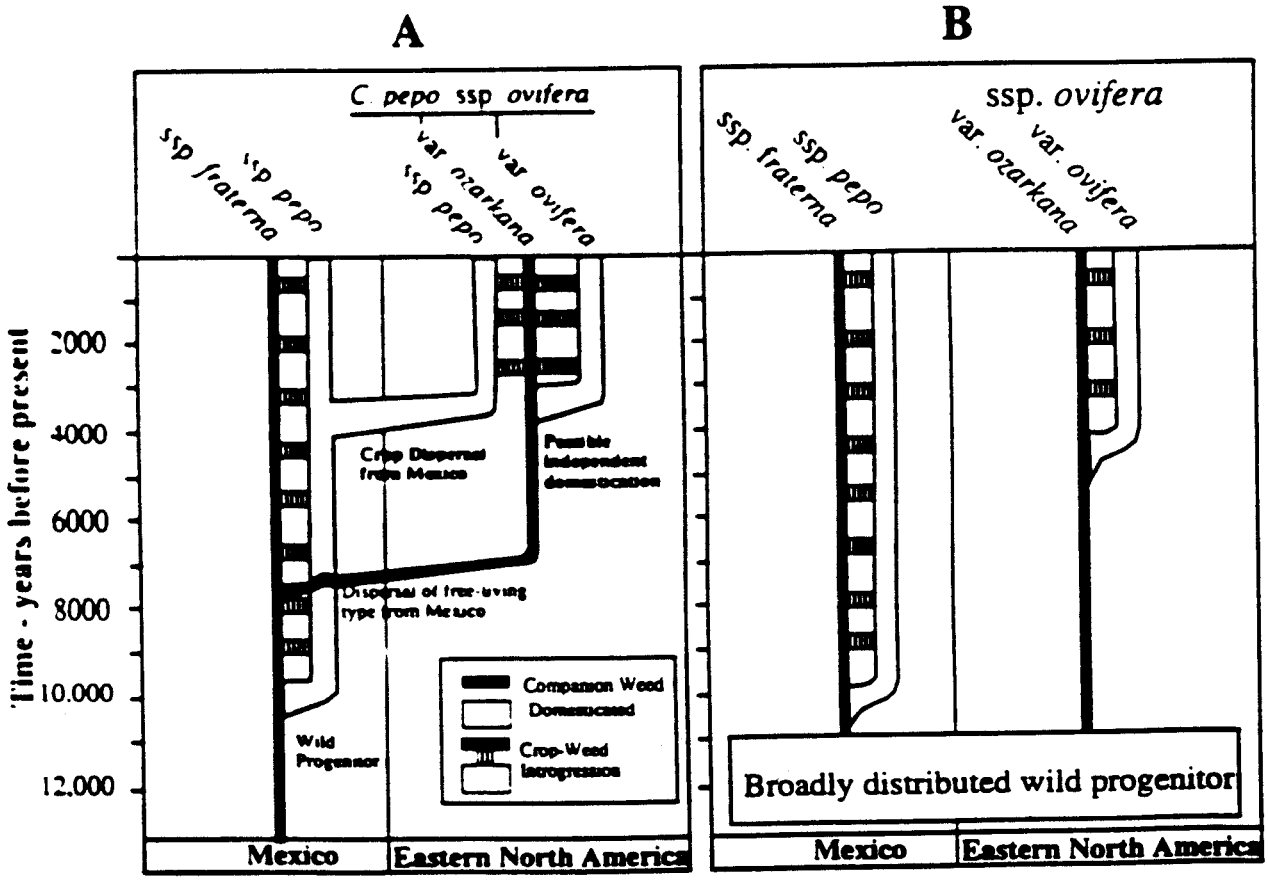


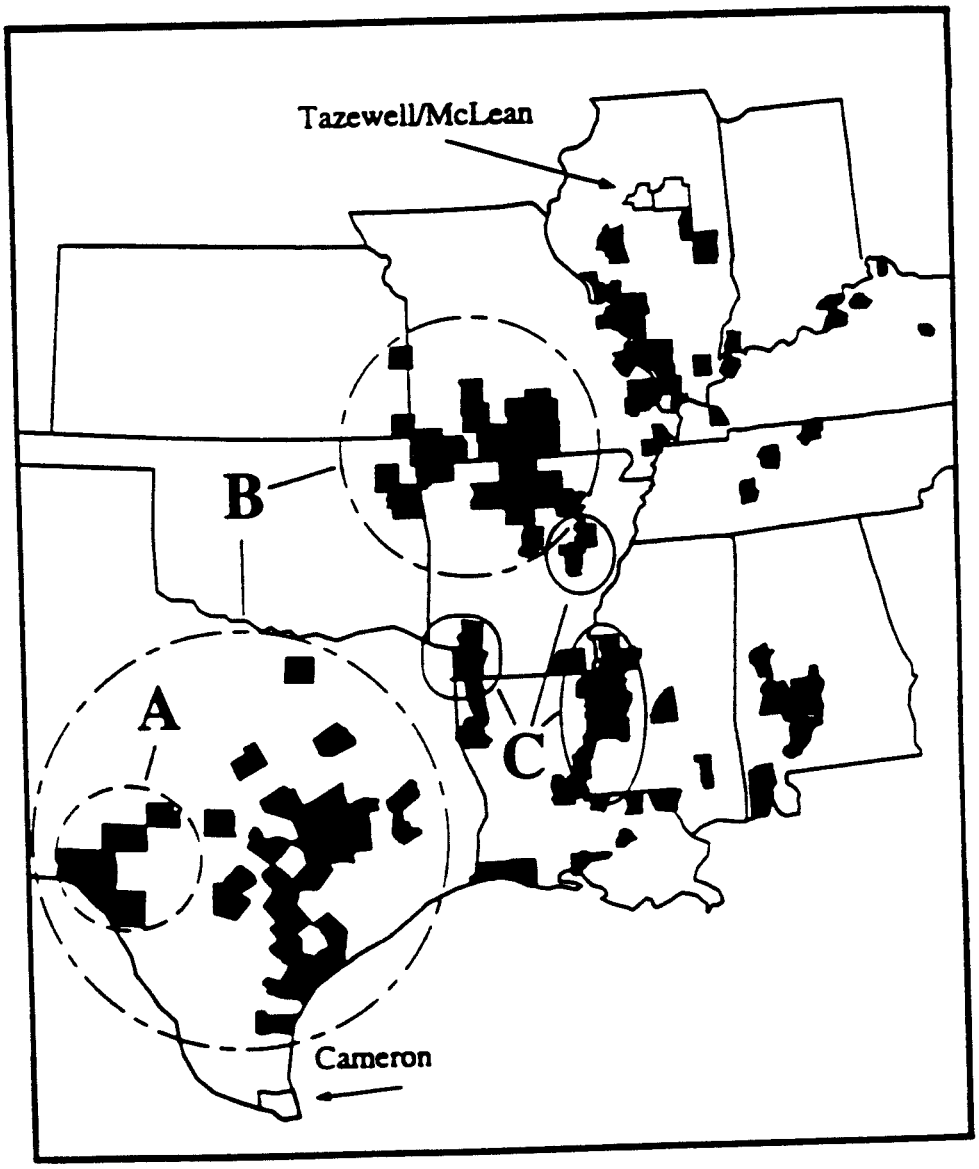
Figure 2. Origins and Dispersal - Theory:



Re-drawn and modified from Smith, 1992

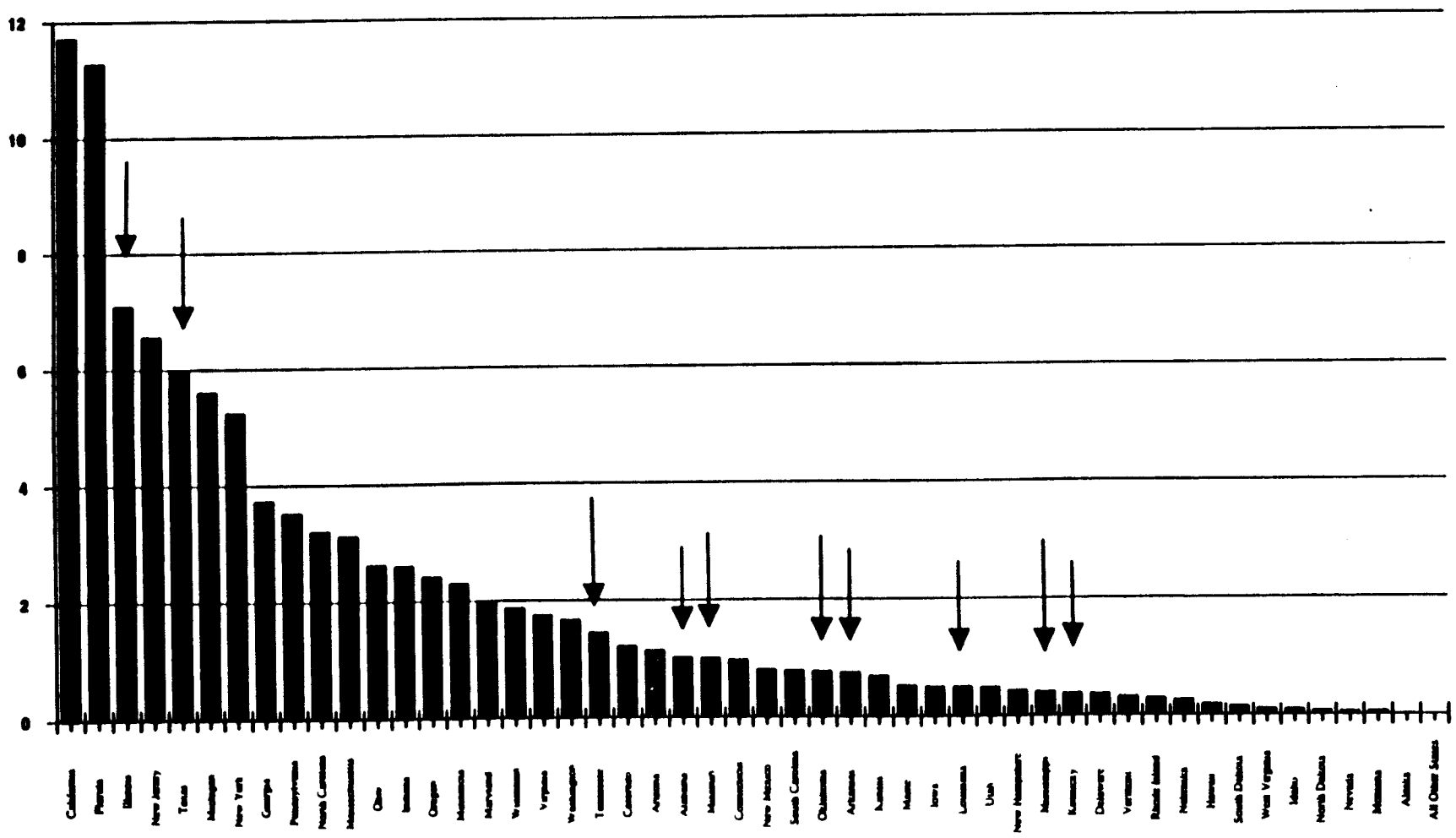
Figure 5. Free-Living *Cucurbita pepo*

Distribution in the United States



Counties shaded carry FLCP record, outlined counties are centers of commercial production. Data based on maps present in Heiser, 1989;Nee, 1990;Asch and Sidell, 1992;Smith, 1992, Smith and Cowan, 1993 with recent additions. See Appendix I for mapped records.

Figure 6. Squash/pumpkin production by state as a percentage of the national total



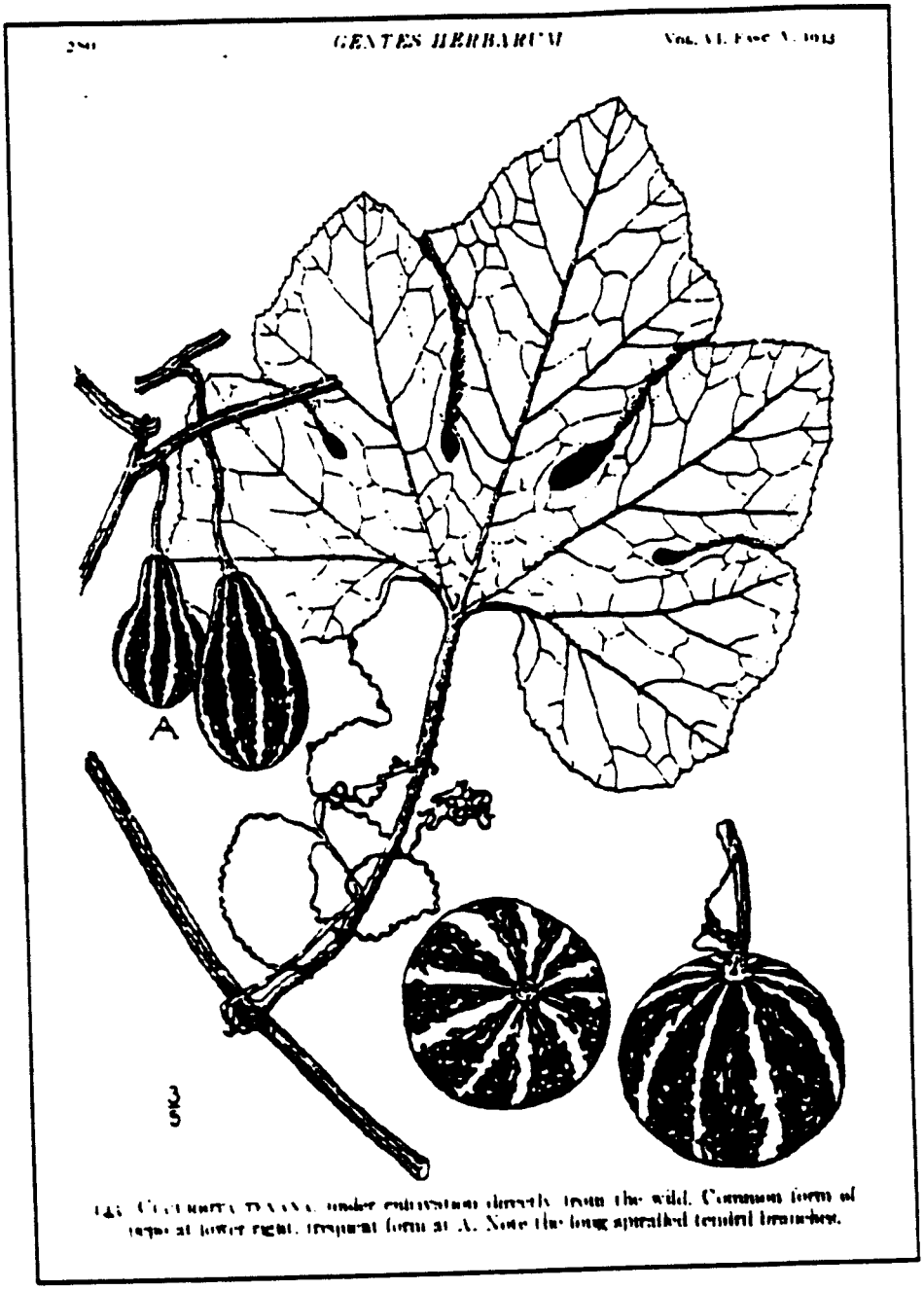


Figure 3. ***Cucurbita pepo*** **ssp.** ***ovifera*** **var.** ***texana***
(electronic re-draw from Bailey, 1943)

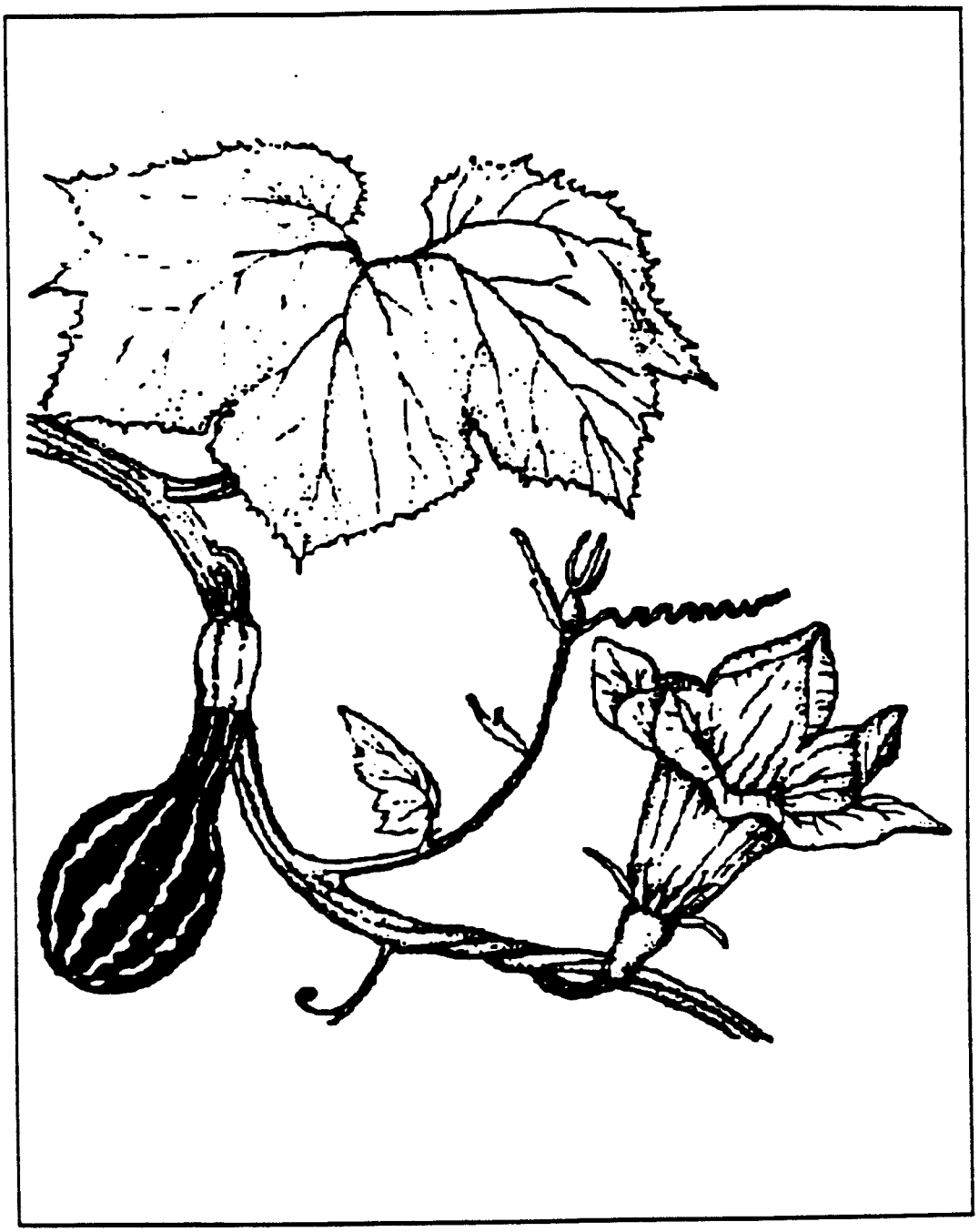
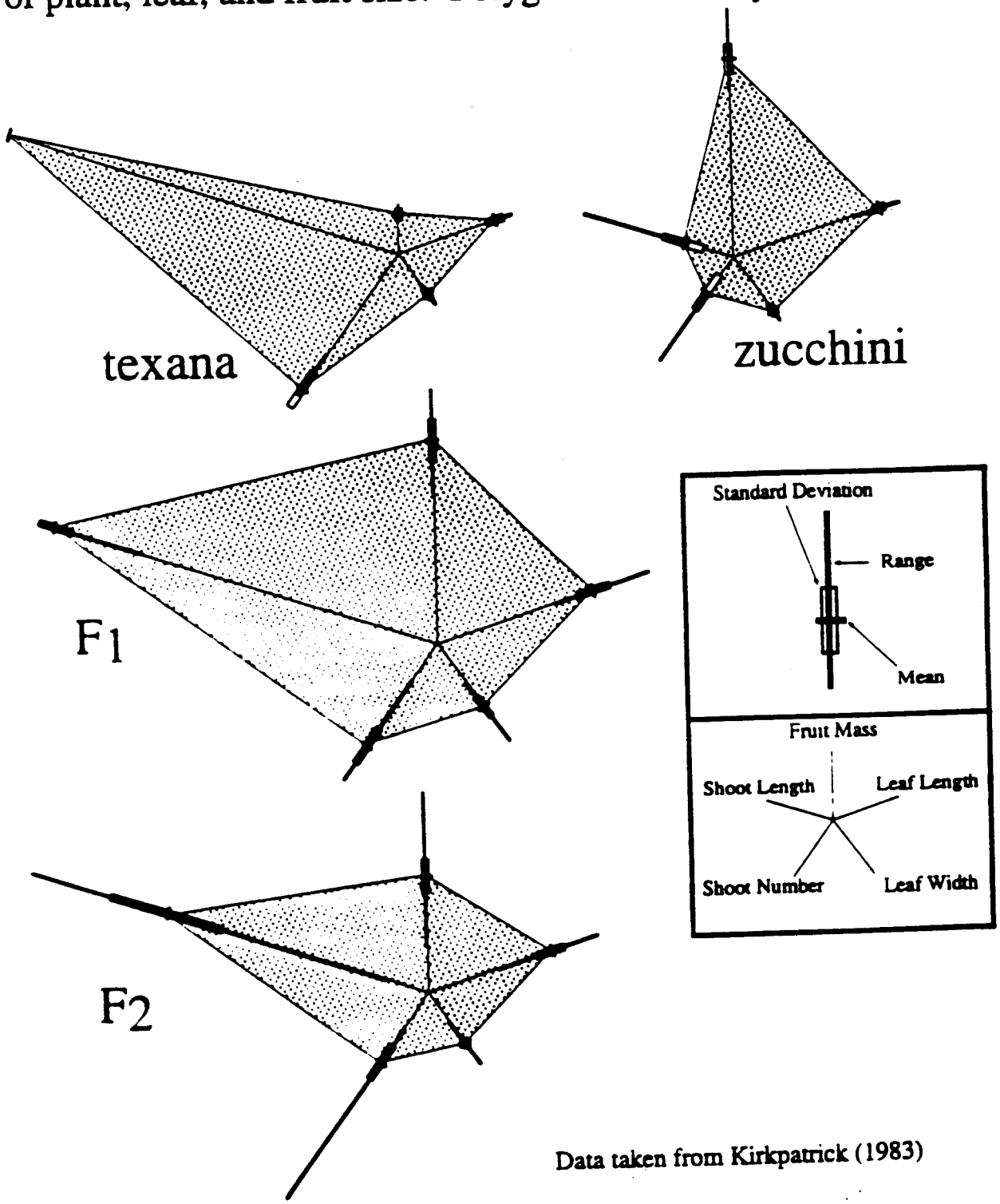


Figure 4. ***Cucurbita pepo*** **ssp.** ***ovifera*** **var.** ***ovifera***

'Bicolor spoon' type of 'ornamental gourd' - others include 'crown of thorns', 'egg', and 'ball' in various colors and fruit surface types

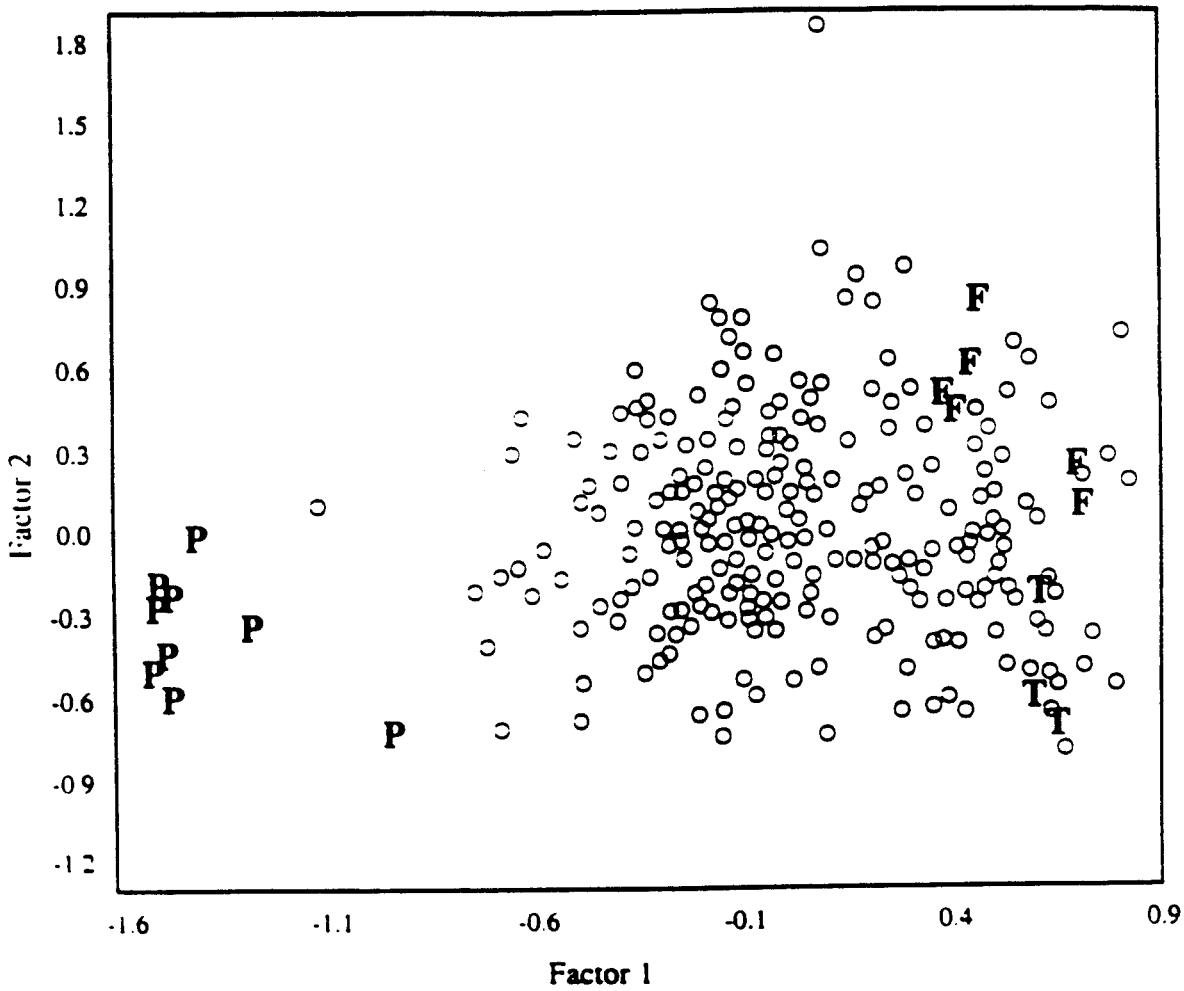
(electronic re-draw from Bailey, 1951)

Figure 7. Structural variation among free-living, domesticated and hybrid plants. Polygonal graphs depict relative patterns and levels of variation among parental and hybrid classes in terms of plant, leaf, and fruit size. Polygons defined by mean values.



Data taken from Kirkpatrick (1983)

Figure 8: Pattern of structural variation among plants of a 'hybrid swarm' sample.



Principal Component Analysis -1st two factors

P = domesticated parent (ssp. *pepo* - 'zucchini')

T = free-living parent (FLCP - ssp. *ovifera* var. *texana*)

F = F_1 plants (T x P [pistillate])

○ = F_2 progeny (from selfed F_1)

Analysis involving 14 floral and vegetative characters and 272 plants from Kirkpatrick (1983)

Appendix I. Distribution data (herbarium records, observations) for Free-Living *Cucurbita pepo* in the United States (Fig. 5).

State	County	Map[1]	Primary Reference[2]	Secondary Reference[3]	Herbarium[4]	Collector[5]	Year[6]
Alabama	Dallas	YES	Smith 1992			C. Sheldon	1988
Alabama	Greene	YES	Smith, 1992		University of Alabama	M. Roberts (5665)	1982
Alabama	Marengo	YES	Smith 1992	Decker 1986	Texas A&M (TAMU)	E. Shelton	1980
Alabama	Mobile	YES	Smith 1992		University of Alabama	R. Deramus	1966
Alabama	Monroe	YES	Smith 1992			C. Shelton	1988
Alabama	Wilcox	YES	Smith 1992		University of Alabama	R. Haynes	1978
Alabama	Wilcox	YES	Smith 1992			C. Shelton	1988
Alabama	Wilcox	YES	This study		Texas A&M (TAMU)	E. Shelton	1980
Arkansas	Ashley	YES	This study	D Thomas	Northeast Louisiana University	R.D.Thomas (93138)	1988
Arkansas	Ashley	YES	Smith 1988	Smith 1992			
Arkansas	Baxter	YES	This study		University of Arkansas	M. Hoffman	1990
Arkansas	Benton	YES	Smith 1992	Smith 1992		D. Dickson	1990
Arkansas	Benton	YES	Smith 1992	Smith 1992		E. McCollum	1990
Arkansas	Benton	YES	Smith 1992	Smith 1992	University of Arkansas	D. Oliver	1975
Arkansas	Faulkner	YES	Smith 1992	Smith 1988	University of Arkansas	S. Harrison	1976
Arkansas	Hempstead	YES	Smith 1992	Smith 1988	Northeast Louisiana University	R. D. Thomas (14858)	1969
Arkansas	Independence	YES	This Study	D Thomas			
Arkansas	Independence	YES	Smith 1992	Smith 1988		Smith and Cowan	1990
Arkansas	Independence	YES	Smith 1992	Smith 1992	Northeast Louisiana University	R.D.Thomas (24521C)	1971
Arkansas	Izard	YES	This Study	D Thomas			
Arkansas	Izard	YES	Smith 1992	Smith 1988		Smith and Cowan	1990
Arkansas	Izard	YES	Smith 1992	Smith 1992			
Arkansas	Lafayette	YES	Smith 1992	Smith 1988	University of Arkansas	B. L. Lipscomb	1975
Arkansas	Marion	YES	Smith 1992	Smith 1988	Texas A&M (TAMU)	B. Hinterthuer	1981
Arkansas	Marion	YES	This study		Texas A&M (TAMU)	J. Rettig	1990
Arkansas	Marion	YES	This study				
Arkansas	Miller	YES	Smith-1992	Smith 1988	Texas A&M (TAMU)	H. Wilson (3644)	1980
Arkansas	Newton	YES	Smith-1992	Decker-1986	University of Arkansas	D. Oliver	1975
Arkansas	Prairie	YES	Smith-1992	Smith-1988	University of Arkansas	B. Hinterthuer	1975
Arkansas	Searcy	YES	Smith-1992	Smith 1988		Smith and Cowan	1990
Arkansas	Searcy	YES	Smith-1992	Smith 1992	Texas A&M (TAMU)	B. Hinterthuer	1981
Arkansas	Searcy	YES	This study		Northeast Louisiana University	Lawson (833)	1972
Arkansas	Stone	YES	This study	D Thomas			
Arkansas	Stone	YES	Smith-1992	Smith-1988	University of Arkansas		
Arkansas	Van Buren	YES	This study			Smith and Cowan	1990
Arkansas	Woodruff	YES	Smith-1992	Smith 1992	Harvard University		
Connecticut	New Haven	NO	This study		University of Georgia		
Florida	Leon	NO	This study		Illinois Natural History Surv	R. A. Evers (104208)	1970
Illinois	Calhoun	YES	This study				
Illinois	Cass	YES	Smith-1992	Mohlenbrock & Ladd, 197			
Illinois	Coles	YES	Smith-1992	Mohlenbrock & Ladd, 197	Field Museum of Natural Hist	C. Buhl (1644.1)	1934
Illinois	Cook	NO	This study		Illinois	G. Jones	1966
Illinois	Douglas	YES	Smith, 1992		Florida	G. Jones	1966
Illinois	Douglas	YES	Smith, 1992		University of Illinois		
Illinois	Douglas	YES	This study				
Illinois	Jackson	YES	Smith, 1992	Mohlenbrock & Ladd, 197	Texas A&M (TAMU)	G. Fritz	1990
Illinois	Jersey	YES	Smith, 1992		Illinois Natural History Surv	R. A. Evers (112031)	1973
Illinois	Jersey	YES	This study		Illinois Natural History Surv	R. A. Evers (51969)	1956
Illinois	Lawrence	NO	This study				

Appendix I. Continued

State	County	Map[1]	Primary Reference[2]	Secondary Reference[3]	Herbarium[4]	Collector[5]	Year[6]
Illinois	Madison	YES	Smith, 1992	Mohlenbrock & Ladd, 197			
Illinois	Morgan	YES	Mohlenbrock, 1978	Asch and Asch, 1992			
Illinois	Perry	YES	Asch & Sidell, 1992	H. Kerr, pers. comm.	Observation		
Illinois	Piatt	YES	Mohlenbrock, 1978	Asch and Asch, 1992			
Illinois	Pulaski	YES	This study		Illinois Natural History Surv	R. A. Evers (19966)	1949
Illinois	Randolph	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3651)	1981
Illinois	Saline	YES	This study	W. Handel	Observation		1992
Illinois	St. Clair	YES	This study		Harvard University		
Illinois	St. Clair	YES	Smith, 1992		Missouri Botanical Garden	H. Eggert	1875
Illinois	St. Clair	YES	Smith, 1992		University of Texas	H. Eggert	1876
Illinois	St. Clair	YES	Smith, 1992		Harvard University	H. Eggert	1892
Illinois	St. Clair	YES	Smith, 1992		Missouri Botanical Garden	H. Eggert	1893
Illinois	St. Clair	YES	Smith, 1992		Missouri Botanical Garden	H. Eggert	1901
Illinois	St. Clair?	YES	Smith, 1992		Illinois	W. Welsch	1862
Illinois	Union	YES	Smith, 1992	Mohlenbrock & Ladd, 197			
Indiana	Clark	YES	Asch & Sidell, 1992	D. Childs		Observation	
Indiana	Porter	NO	This study		Field Museum of Natural Hist	D. C. Peattie (2055)	1925
Indiana	Posey	YES	Asch & Sidell, 1992	D. Childs		Observation	
Kansas	Cherokee	YES	Asch & Sidell, 1992	Brooks, R.	Univeristy of Kansas	R. Brooks	1978
Kansas	Linn	YES	This study		University of Kansas	S. Stephens (88764)	1975
Kentucky	Henry	YES	This study	W. Meijer	University of Kentucky	Gentry (811)	1962
Kentucky	Jefferson	YES	This study	W. Meijer	Observation		
Kentucky	Kenton	YES	This study	R. Kral	Vanderbilt University	P. Applegarth (411)	
Kentucky	Marshall	YES	This study	R. Kral	Vanderbilt University	Athey (s.n.)	
Kentucky	Powell	YES	Smith, 1992	Smith, 1992		W. Booth	1990
Kentucky	Union	YES	Asch & Sidell, 1992	Brown, G.	Observation		
Louisiana	Ascension	YES	This study	T. Wendt	Louisiana State University		
Louisiana	Avoyelles	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (420)	1966
Louisiana	Bossier	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (69354)	1979
Louisiana	Bossier	YES	Smith, 1992	Smith, 1992		L. Baker	1990
Louisiana	Caldwell	NO	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (s.n.)	1988
Louisiana	Cameron	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (130014)	1992
Louisiana	Concordia	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (106793)	1988
Louisiana	DeSoto	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (61220	1978
Louisiana	East Carroll	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (74283)	
Louisiana	Iberia	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (102570)	1987
Louisiana	Madison	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (54538)	1977
Louisiana	Madison	YES	This study		Field Museum of Natural Hist	R. Dale Thomas	1979
Louisiana	St. Helena	YES	Smith, 1992	Smith, 1992	Louisiana State University	C. Allen	1971
Louisiana	Tensas	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (86784)	1983
Louisiana	Tensas	YES	Smith, 1992	Smith, 1992	Texas A&M (TAMU)	G. Fritz	1990
Louisiana	W. Feliciana	YES	Smith, 1992	Smith, 1992	Louisiana State University	A. Martin	1972
Louisiana	Washington	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (85329)	1983
Louisiana	West Carroll	YES	This study	D. Thomas	Northeast Louisiana University	R.D. Thomas (60139)	1978
Massachusets	Middlesex	NO	This study		Harvard University		
Mississippi	Claiborne	YES	Smith, 1992	Smith, 1992	University of Tennessee	K. Rogers	1978
Mississippi	Forrest	YES	Smith, 1992	Smith, 1992	University of Tennessee	K. Rogers	1971
Mississippi	Humphries	YES	Asch & Sidell, 1992	C. D. Elmore	Observation		
Mississippi	Issaquena	YES	Asch & Sidell, 1992	C. D. Elmore	Observation		
Mississippi	Rankin	YES	Smith, 1992	Smith, 1992	University of Georgia	S. Jones	1970
Mississippi	Sharkey	YES	Asch & Sidell, 1992	C. D. Elmore	Observation		

Appendix I. Continued

State	County	Map[1]	Primary Reference[2]	Secondary Reference[3]	Herbarium[4]	Collector[5]	Year[6]
Mississippi	Warren	YES	Asch & Sidell, 1992	C. D. Elmore	Observation		
Mississippi	Washington	YES	This study	C. D. Elmore	Observation-weed outbreak		1979
Missouri	Barry	YES	Smith, 1992	Steyermark, 1963	University of Missouri	J. Steyermark	1955
Missouri	Barry	YES	Thus study	Kennedy	University of Missouri		
Missouri	Bollinger	YES	Thus study	Bornstein	Southeast Missouri State Univ		
Missouri	Butler	NO	Thus study	Bornstein	Southeast Missouri State Univ		
Missouri	Cape Girardeau	YES	Thus study	Bornstein	Southeast Missouri State Univ		
Missouri	Cape Girardeau	YES	Thus study	Bornstein	Southeast Missouri State Univ		
Missouri	Christian	YES	Smith, 1992	Smith, 1992			1990
Missouri	Douglas	YES	Smith, 1992	Steyermark, 1963	University of Georgia	J. Steyermark	1957
Missouri	Greene	YES	Smith, 1992	Smith, 1992		Smith and Cowan	
Missouri	Howell	YES	Smith, 1992	Steyermark, 1963			
Missouri	McDonald	YES	Smith, 1992	Steyermark, 1963			
Missouri	McDonald	YES	This Study		Southern Illinois University		
Missouri	New Madrid	YES	Asch & Sidell, 1992	H. Kerr	Observation	Observation	
Missouri	Newton	YES	Smith, 1992	Steyermark, 1963		Smith and Cowan	1990
Missouri	Ozark	YES	Smith, 1992	Smith, 1992		Smith and Cowan	1990
Missouri	Polk	YES	Smith, 1992	Smith, 1992	Field Museum of Natural Hist	M. K. Solecki (808)	1980
Missouri	St. Genevieve	YES	This study		Missouri Botanical Garden	G. Engelmann	1846
Missouri	St. Louis	YES	Smith, 1992		Missouri Botanical Garden	G. Engelmann	1846
Missouri	St. Louis	YES	Smith, 1992		Missouri Botanical Garden	Muehlenbach	1961
Missouri	St. Louis	YES	Smith, 1992		Missouri Botanical Garden	Muehlenbach	1964
Missouri	St. Louis	YES	Smith, 1992		Missouri Botanical Garden	Muehlenbach	1972
Missouri	St. Louis	YES	Smith, 1992				1990
Missouri	Taney	YES	Smith, 1992	Smith, 1992	Harvard University	J. Steyermark	1956
Missouri	Texas	YES	Smith, 1992	Steyermark, 1963	University of Missouri		
Missouri	Texas	YES	Thus study	Kennedy		Smith and Cowan	1990
Missouri	Wright	YES	Smith, 1992	Smith, 1992	Harvard University		
New Hampshire	Coos	NO	This study		New York State Museum		1960
New York	Chemung	NO	Thus study	Sheviak	New York State Museum		1950
New York	Erie	NO	Thus study	Sheviak	New York State Museum		1950
New York	Madison	NO	Thus study	Sheviak	New York State Museum		1960
New York	Oswego	NO	Thus study	Sheviak	New York State Museum		1960
New York	Rockland	NO	Thus study	Sheviak	New York State Museum		1950
New York	Saratoga	NO	Thus study	Sheviak	New York State Museum		1960
New York	Saratoga	NO	Thus study	Sheviak	New York State Museum		1950
New York	Schenectady	NO	Thus study	Sheviak	New York State Museum		1960
New York	Steuben	NO	Thus study	Sheviak	New York State Museum		1960
New York	Suffolk	NO	Thus study	Sheviak	New York State Museum		1960
New York	Yates	NO	Thus study	Sheviak	Ohio State University		1982
Ohio	Lawrence	NO	This study	Furlow	Ohio University		1938
Ohio	Licking	NO	This study	Cantino		M. Hoffman	1990
Oklahoma	Adair	YES	Smith, 1992	Smith, 1992		B. Meyer	1990
Oklahoma	Cherokee	YES	Smith, 1992	Smith, 1992		D. Dickson	1990
Oklahoma	Mayes	YES	Smith, 1992	Smith, 1992	Pennsylvania State University	W. F. Westerfeld	1960
Pennsylvania	Centre	NO	This study		Harvard University		
Pennsylvania	Chester	NO	This study		Field Museum of Natural Hist	J. K. Small	1897
Pennsylvania	Lancaster	NO	This study		Pennsylvania State University	R. L. Schaeffer	1957
Pennsylvania	Lehigh	NO	This study		Pennsylvania State University	W. F. Westerfeld	1955
Pennsylvania	Mifflin	NO	This study		Pennsylvania State University	R. L. Schaeffer	1942
Pennsylvania	Northampton	NO	This study				

Appendix I. Continued

State	County	Map[1]	Primary Reference[2]	Secondary Reference[3]	Herbarium[4]	Collector[5]	Year[6]
South Carolina	Calhoun	NO	This study	J. Nelson	University of South Carolina	J. Nelson (6711)	1988
Tennessee	Dickson	YES	This study	R. Kral	Observation		
Tennessee	Knox	NO	This study	Furlow	Ohio State University		1891
Tennessee	Perry	YES	This study	R. Kral	Observation	R. Kral	
Tennessee	Sumner	YES	This study	R. Kral	Vanderbilt University	R. Kral (56134)	1975
Texas	Aransas	YES	This study		Harvard University		
Texas	Aransas	YES	This study	Andres, 1983	Southern Methodist University	V. L. Cory (51158)	1945
Texas	Aransas	YES	This study	Rabeler	University of Michigan		
Texas	Bell	YES	Smith, 1992	Mahler, 1988	Southern Methodist University	S. Wolf (1294)	1929
Texas	Bexar	YES	This study	Cantino	Ohio University		1982
Texas	Brazos	YES	Smith, 1992		University of Texas	D. S. Correll	
Texas	Brazos	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3632)	1980
Texas	Burleson	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3196)	1978
Texas	Burleson	YES	This study		Texas A&M (TAMU)	H. Wilson	1983
Texas	Caldwell	YES	This study	Cantino	Ohio University		1982
Texas	Calhoun	YES	Smith, 1992		University of Texas	Hartman and Smith	
Texas	Comal	YES	Smith, 1992		University of Texas	Lindheimer (820,21)	1849
Texas	Comal	YES	This study		Field Museum of Natural Hist	Lindheimer	1849
Texas	Comal	YES	This study	Andres, 1983	Harvard University	Lindheimer (472)	1845
Texas	Denton	YES	This study	Andres, 1983	Howard Payne University	P. Cox (14)	1974
Texas	Dewitt	YES	This study		Harvard University		
Texas	DeWitt	YES	Smith, 1992		University of Texas	D. S. Correll	1962
Texas	DeWitt	YES	Smith, 1992	Andres, 1983	Southwest Louisiana State Univ	D. Correll (26173)	1962
Texas	DeWitt	YES	Smith, 1992	Andres, 1983	University of Texas	Tharp (46347)	1929
Texas	DeWitt	YES	This study	Rabeler	University of Michigan		
Texas	Fayette	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3170)	1978
Texas	Goliad	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	S. Hill (6111)	1977
Texas	Gonzales	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3173)	1977
Texas	Grimes	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3643)	1980
Texas	Hamilton	YES	This study	Andres, 1983	Howard Payne University	J. Stanford (3697)	1979
Texas	Hamilton	YES	This study		Texas A&M (TAMU)	H. Wilson (5796)	1987
Texas	Jackson	YES	This study	Andres, 1983	Harvard University	B. Tharp (275)	1941
Texas	Kinney	YES	This study		Harvard University		
Texas	Kinney	YES	This study	Andres, 1983	Texas A&M (TAES)	C. B. Smith (33483)	1939
Texas	Kleberg	YES	This study	Andres, 1983	Harvard University	J. Berlandier (3028)	1835
Texas	Lee	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3636)	1980
Texas	Llano	YES	This study	Andres, 1983	U.S. National Herbarium	E. Palmer (10292)	1916
Texas	Llano	YES	This study	Andres, 1983	Texas A&M (TAMU)	T. Walters (1309)	1980
Texas	Madison	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3657)	1981
Texas	Menard	YES	This study	Andres, 1983	Harvard University	V. Cory (9777, 78)	1934
Texas	Menard	YES	This study	Andres, 1983	Bailey Hortorium-Cornell	V. Cory (24726)	1937
Texas	Menard	YES	This study	Andres, 1983	Southern Methodist University	V. Cory (52893)	1947
Texas	Milam	YES	This study		Texas A&M (TAMU)	H. Wilson (3650)	1980
Texas	Navarro	YES	This study	Andres, 1983	Tarrent County Water District	G. Ajilvsgi (7609)	1980
Texas	Refugio	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	S. Hill	1977
Texas	Robertson	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	T. Starbuck (2538)	1982
Texas	Robertson	YES	This study	Andres, 1983	Harvard University	B. Tharp (47558)	1941
Texas	San Jacinto	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	B. Ertter	1983
Texas	San Patricio	YES	Smith, 1992	Jones, 1975			
Texas	San Patricio	YES	This report		Texas A&M (TAMU)	S. Hill (5847)	1977
Texas	Sutton	YES	Smith, 1992		Univeristy of Texas	Reed	

Appendix I. Continued

State	County	Map[1]	Primary Reference[2]	Secondary Reference[3]	Herbarium[4]	Collector[5]	Year[6]
Texas	Sutton	YES	This study	Andres, 1981	New York Botanical Garden	C. Wright	1849
Texas	Sutton	YES	This study	Andres, 1981	Texas A&M (TAES)	V. L. Cory (29795)	1938
Texas	Sutton	YES	This study	Andres, 1981	Southern Methodist University	H. R. Reed	1947
Texas	Sutton	YES	This study	Rabeler	University of Michigan		
Texas	Travis	YES	Smith, 1992	Erwin, 1938	Observation	A. T. Erwin	1938
Texas	Travis	YES	Smith, 1992	Andres, 1981	Univeristy of Texas	F. Barkley (13335)	1943
Texas	Travis	YES	Smith, 1992	Andres, 1981	Univeristy of Texas	Tharp	1941
Texas	Travis	YES	Smith, 1992	Andres, 1981	Univeristy of Texas	Strandtmann	1940
Texas	Travis	YES	This study	Andres, 1981	Field Museum of Natural Hist	B. Tharp (43Q021)	1942
Texas	Travis	YES	This study	Andres, 1981	Harvard University	B. Tharp	1929
Texas	Trinity	YES	This study	Andres, 1981	Sam Houston State University	C. McLeod (s.n.)	1978
Texas	Val Verde	YES	This study		Field Museum of Natural Hist	Hugh Cutler	1937
Texas	Washington	YES	Smith, 1992	Decker, 1986	Texas A&M (TAMU)	H. Wilson (3637)	1980

1 County record included in Fig. 5. Collections or observations that were not identified as either *C. texana* or *C. pepo* var. *ovifera* or were possibly recent 'escapes' from cultivation that are not established as free-living populations were not mapped in Fig. 5.

2 Primary source of data.

3 Secondary source of data, either cited by the primary source or incorporated into the data set for this study.

4 Herbarium holding voucher specimens for records that are known to have been vouchered by a plant collection from the county.

5 Collector of the plant specimen that serves as a voucher for the county record

6 Year collection was made.

Appendix II - Harvested Acres for Sale - U.S. Agricultural Census - 1987

STATE	PUMPKIN	SQUASH	TOTAL	PERCENT
California	3998	7586	11584	11.72
Florida	299	10855	11154	11.28
Illinois	**6442**	**572**	**7014**	**7.10**
New Jersey	2165	4328	6493	6.57
Texas	**1500**	**4417**	**5917**	**5.99**
Michigan	2145	3386	5531	5.60
New York	3108	2073	5181	5.24
Georgia	112	3572	3684	3.73
Pennsylvania	2608	870	3478	3.52
North Carolina	691	2482	3173	3.21
Massachusetts	1096	1995	3091	3.13
Ohio	1743	855	2598	2.63
Indiana	2116	466	2582	2.61
Oregon	332	2060	2392	2.42
Minnesota	1413	857	2270	2.30
Maryland	862	1076	1938	1.96
Wisconsin	1050	795	1845	1.87
Virginia	951	774	1725	1.75
Washington	508	1143	1651	1.67
Tennessee	**1086**	**349**	**1435**	**1.45**
Colorado	527	675	1202	1.22
Arizona	311	812	1123	1.14
Alabama	**281**	**725**	**1006**	**1.02**
Missouri	**749**	**252**	**1001**	**1.01**
Connecticut	581	376	957	0.97
New Mexico	273	517	790	0.80
South Carolina	68	707	775	0.78
Oklahoma	**369**	**391**	**760**	**0.77**
Arkansas	**181**	**564**	**745**	**0.75**
Kansas	548	136	684	0.69
Maine	211	299	510	0.52
Iowa	337	158	495	0.50
Louisiana	**103**	**392**	**495**	**0.50**
Utah	282	205	487	0.49
New Hampshire	218	206	424	0.43
Mississippi	**261**	**143**	**404**	**0.41**
Kentucky	**217**	**156**	**373**	**0.38**
Delaware	120	247	367	0.37
Vermont	154	153	307	0.31
Rhode Island	143	143	286	0.29
Nebraska	168	85	253	0.26

Hawaii	25	156	181	0.18
South Dakota	81	58	139	0.14
West Virginia	84	10	94	0.10
Idaho	55	36	91	0.09
North Dakota	31	27	58	0.06
Nevada	20	33	53	0.05
Montana	29	21	50	0.05
Alaska	0	6	6	0.01
All Other States	1	0	1	0.00
Total*	40653	58200	98853	100.00

*State totals do not sum exactly to national totals published by the census