Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area

Risk Management Analysis

United States
Department
of Agriculture

Animal and
Plant Health
Inspection Service

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Executive Summary

This document analyzes the potential of fresh commercially packed citrus fruit and associated packing material to serve as a pathway for the introduction of *Xanthomonas axonopodis* pv. *citri* (Xac) into new areas. It also identifies and evaluates options for regulating the interstate movement of fresh citrus fruit with the goal of reducing the potential for Xac introduction and spread. This document extends the application of an earlier APHIS pest risk analysis entitled "*Evaluation of asymptomatic citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease* (USDA 2006) to all commercially packed citrus fruit. That analysis concluded that asymptomatic, commercially produced citrus fruit that has been treated with disinfectant dips and subjected to other mitigations is not epidemiologically significant[1] as a pathway for the introduction of citrus canker disease.

The current evaluation reviewed available evidence regarding the biology and epidemiology of Xac and the management of citrus canker disease and determined that the introduction of Xac through the movement of commercially packed fresh citrus fruit is unlikely because:

- fresh citrus fruit is produced and harvested using techniques that reduce the prevalence of Xac-infected fruit;
- citrus fruit is commercially packed using techniques that reduce the prevalence of infected or contaminated fruit including disinfectant treatment that devitalizes epiphytic contamination;
- for a successful Xac infection that results in disease outbreaks an unlikely sequence of epidemiological events would have to occur;
- reports of citrus canker disease outbreaks linked to fresh fruit are absent; and
- large quantities of fresh citrus fruit shipped from regions with Xac have not resulted in any known outbreaks of citrus canker disease.

But the evidence is not currently sufficient to conclude that fresh citrus fruit produced in a Xac infested grove absolutely cannot serve as a pathway for the introduction of Xac into new areas. Furthermore, it is not possible to design an operationally feasible system that ensures only uninfected fruit moves from quarantined areas. Resource constraints and other practical considerations make it difficult to implement a grove-centered regulatory systems approach in Florida that ensures full compliance with the conclusions of the evaluation described above so this analysis evaluates several packinghouse-centered risk management options for the interstate movement of fresh commercially packed citrus fruit from regions with citrus canker disease to regions without the disease:

Option 1 Allow unrestricted distribution of all types and varieties of *commercially*

[1] The term "epidemiologically significant" refers to minimum conditions required for successful Xac infection.

packed citrus fruit to all U.S. States[2].

Option 2 — Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States, subject to packinghouse treatment with APHIS-approved disinfectant and APHIS inspection of finished[3] fruit for citrus canker disease symptoms.

Option 3 — Allow distribution of all types and varieties of *commercially packed* citrus fruit (except tangerines) in U.S. States except U.S. commercial citrus producing States[4]. Allow distribution of *commercially packed* tangerines to all U.S. States including commercial citrus-producing States. Require packinghouse treatment of all such citrus fruit with APHIS-approved disinfectant and APHIS inspection of finished fruit (all types and varieties) for citrus canker disease symptoms.

Option 4 — Allow distribution of all types and varieties of *commercially packed* citrus fruit in U.S. States except U.S. commercial citrus-producing States and require packinghouse treatment of citrus fruit with APHIS-approved disinfectant and APHIS inspection of finished fruit.

Option 5 — Leave the current regulations for the interstate movement of citrus fruit from citrus canker disease quarantined areas in place and unchanged.

Each option was considered within the context of available scientific evidence. Option 1 would allow unrestricted distribution of all types and varieties of commercially packed citrus fruit to all U.S. States. But, given that the available evidence suggests fresh citrus fruit is an unlikely pathway but is not currently sufficient to unequivocally conclude that fresh citrus fruit cannot serve as a pathway for the introduction of Xac into new areas, unrestricted movement of citrus fruit from quarantine areas was determined not to be scientifically justified. Consequently, the more restrictive Options 2, 3, 4 and 5 were evaluated and Option 1 was no longer considered.

To assist in evaluating Options 2, 3 and 4, we prepared a quantitative model (Appendix 1) based on Florida production and shipping data to determine the efficacy of three levels of phytosanitary inspection in ensuring that symptomatic fruit does not enter U.S. commercial citrus-producing States. The three inspection levels were determined by preliminary estimates of United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Citrus Health Response Program staff of inspection levels that might be operationally feasible. The three inspection levels evaluated were 500 fruit per lot; 1,000 fruit per lot; and 2,000 fruit per lot. Statistically, inspection of 500 fruit, 1,000 fruit or 2,000 fruit per lot will ensure, with 95 percent confidence, that the proportion of undetected symptomatic fruit in a cleared lot is no more than 0.75, 0.38 and 0.19 percent, respectively.

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[2] For clarity, the term “State” is defined here as any of the 50 U.S. States or U.S. Commonwealths, Trusts and Territories

[3] Fruit that has completed the packinghouse washing, disinfection, grading and inspection processes.

[4] American Samoa; Arizona; California; Florida; Guam; Hawaii; Louisiana; Northern Mariana Islands; Puerto Rico; Texas; and the Virgin Islands of the United States

The outputs of the quantitative model were probability distributions. The model determined, with 95 percent confidence, that the total number of citrus fruit shipped from Florida to five citrus-producing States (AZ, CA, HI, LA and TX) over a single shipping season would be 181,283,744 or less if unlimited distribution is permitted. The model determined, with 95 percent confidence, that the number of Xac-symptomatic fruit reaching those five States in a single shipping season would be: 633,152 or less at the 1,000 fruit inspection levels; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level. The model further determined with 95 percent confidence that the number of symptomatic fruit reaching citrus-producing areas within those States in a single shipping season would be: 2,135 or less at the 1,000 fruit inspectional level; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level. An inspection level that achieves a detection rate of 0.38 percent with 95 percent confidence was adopted because it is operationally feasible with small adjustments to the current phytosanitary inspection process in Florida. For the majority of lots, this would amount to inspection of about 1000 fruit per lot.

The potential for symptomatic fruit to reach citrus producing States coupled with the aforementioned uncertainty regarding fruit as a pathway led to the determination that additional mitigations were required.

Option 3 would allow the shipment of tangerines to all U.S. States. This option was evaluated in response to a proposal that tangerines have considerably less susceptibility to Xac and therefore are less likely to introduce Xac to previously free regions. Tangerines are grouped in the species *Citrus reticulata* which is widely regarded as less susceptible to citrus canker disease than other commercially grown *Citrus* species (Civerolo 1984). But many of the “tangerine” varieties grown in Florida are hybrids of *C. reticulata* with other more susceptible *Citrus* species (Morton 1987). Tangerines are not immune to Xac as APHIS records indicate that during the 2005-2006 growing season grove surveys; Xac was detected on 274 samples from tangerine, tangor and tangelo groves. APHIS pest interception data indicate that between 1985 and 2006, Xac was intercepted 632 times on *C. reticulata* fruit.

The level of susceptibility is expressed as a continuum across “tangerine” varieties rather than as a discrete immunity for all varieties. This creates a regulatory problem when an overlap occurs in the level of susceptibility expressed by, for example, a more susceptible tangerine variety and a more resistant non-tangerine citrus variety. Sufficient evidence does not exist to exclude tangerines from regulations applicable to all other Florida citrus varieties.

Option 4 prohibits distribution of all types and varieties of citrus fruit, including tangerines, to citrus-producing States. Option 4 includes all the requirements of Option 3 and further mitigates the risk of Xac introduction by prohibiting the distribution of all types and varieties of citrus fruit, including tangerines, from areas with citrus canker disease to U.S. commercial citrus producing States. Option 4 would amend CFR 301.75 (a)(2), substituting the packing house inspection described in Appendix 1 for the preharvest grove inspections currently in the regulation. Option 4 compensates for uncertainty in the rate of illegal fruit movement by requiring a disinfectant treatment and phytosanitary inspection in addition to the distribution restriction. These measures ensure that even if a given shipment were illegally moved to a prohibited State, it has a low likelihood of containing symptomatic fruit.

A packinghouse-based inspection could ensure the same level of phytosanitary security as the preharvest grove survey required by Option 5, would be easier and potentially less costly to implement and enforce, and would be more reliable and less easily circumvented. In addition, a phytosanitary packinghouse inspection creates a performance standard for packed fruit that allows citrus producers greater flexibility to determine the most efficient and effective means of producing a compliant product.

Table of Contents

1 Purpose and Scope

On January 10, 2006, the U.S. Department of Agriculture (USDA) announced the termination of its Florida eradication program for Asiatic citrus canker disease, caused by *Xanthomonas axonopodis pv. citri* ("Xac"). A letter from U.S. Deputy Secretary of Agriculture Chuck Conner to Florida Agriculture Commissioner Charles Bronson (2006) stated that the decision was made "in light of…expert analysis on the distribution of the disease and the infeasibility of eradication."

As a result, the USDA's, Animal and Plant Health Inspection Service (APHIS) published an interim rule (FR 2006) quarantining the entire State of Florida for citrus canker disease and amending the regulatory requirements for the movement of fresh fruit from Florida. APHIS considered allowing interstate movement of Florida citrus fruit to any domestic location, but did not have sufficient epidemiological information at the time to justify such a decision.

Since then, APHIS has prepared an analysis entitled "*Evaluation of asymptomatic citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease*" (USDA 2006), which has been made available for public comment and peer review (FR 2006). That analysis assumed that commercially produced citrus is cultivated under specific pest management practices including field treatments with copper-based pesticides for controlling the incidence of citrus canker; grove sanitation; fruit culling procedures during harvest and packing; and, a post-harvest surface disinfectant dip treatment. The evaluation concluded "…that asymptomatic, commercially produced citrus fruit that has been treated with disinfectant dips and subjected to other mitigations is not epidemiologically significant[5] as a pathway for the introduction of citrus canker."

Where the previous analysis (USDA 2006) assumed that citrus fruit was commercially produced under a specific set of pest management practices, the present document, while recognizing that effective pest management measures for Xac are available to private and commercial growers (Chamberlain *et al.* 2001; Timmer *et al.* 2006), and are normal production practices for many of these growers, does not assume that measures in the grove are mandatory. Furthermore, the previous analysis (USDA 2006) focused on the role of *asymptomatic* fruit only as a pathway for Xac introduction while the present analysis expands the scope of the aforementioned analysis and evaluates all commercially packed fresh citrus fruit.
The present document summarizes available scientific, technical and historical information relevant to the movement of fresh citrus fruit from citrus canker disease quarantine areas as a potential pathway for the introduction of Xac into areas where citrus canker disease does not occur. Based on that information, the analysis identifies and evaluates operationally feasible options for regulating interstate movement of fresh citrus fruit that reduce the potential for that fruit to serve as a pathway for the introduction of

[5] The term "epidemiologically significant" refers to minimum conditions required for successful Xac infection .

Xac. Together, USDA (2006) and this analysis provide the scientific basis for a proposed change in APHIS regulations for the interstate movement of fresh commercial citrus fruit from regions with citrus canker disease.

2 Definitions

Abaxial: Directed away from the stem of a plant; pertaining to the lower surface of a leaf (see adaxial) (D'Arcy *et al.* 2001).
Adaxial: Directed toward the stem of a plant; pertaining to the upper surface of a leaf (see abaxial) (D'Arcy *et al.* 2001).
cfu (Colony Forming Units): The number of colonies formed per unit of volume or weight of a [bacterial] cell or spore suspension (D'Arcy *et al.* 2001).
Endoparasite: Parasitic organism that lives and feeds from inside its host (D'Arcy *et al.* 2001).
Epidermis: The superficial layer of cells occurring on all plant parts (Agrios 1997).
Epiphytic bacteria: Those bacteria that could be washed from the plant surface (Rybak and Canteros 2001).
Establishment: Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2006a).
Inoculum: The pathogen or its parts that can cause infection. That portion of individual pathogens that are brought into contact with the host (Agrios 1997).
Introduction: The entry of a pest resulting in its establishment (FAO 2006a).
Latent infection: Infection unaccompanied by visible symptoms (D'Arcy *et al.* 2001).
Lesion: Localized diseased area or wound (D'Arcy *et al.* 2001).
Lot: The inspectional unit for fruit; composed of a single variety of fruit that has passed through the entire packing process in a single continuous run not to exceed a single work day (*i.e*., a run started one day and completed the next is considered two lots); the lot size is used to determine the size of the sample for phytosanitary inspection; regulatory actions (*e.g.,* issuance of limited permits, rejection) are taken at the lot level.
Mesophyll: The tissue of a leaf, located between the upper and lower layers of epidermis (Stern 1982).
Pathway: Any means that allows the entry or spread of a pest (FAO 2006a).
Rutaceae: Botanical family comprising about 150 genera and 900 to 1500 species of warm temperate to tropical trees and shrubs. This family includes all of the citrus fruits such as oranges, lemons, limes, grapefruits and tangerines (Anonymous 2003).
Stoma (pl. stomata): A pore in the epidermis of aerial parts of the plant providing a means for gaseous exchange between internal tissues and the atmosphere (Blackmore and Toothill 1984).

3 USDA Regulatory Policy: Xac and Citrus Canker Disease

The USDA regulatory policy on citrus canker disease and its causal agent, Xac, has evolved along two different but related paths. The first path is the regulation of the movement of domestic citrus fruit after detections of Xac in Florida. The second is the regulation of imported citrus fruit to mitigate the likelihood of introducing Xac. This section describes the evolution of USDA's domestic and import regulatory policies for Xac.

3.1 Domestic Citrus Regulations

Xac was probably introduced into the United States around 1910 in nursery stock imported from Japan (Dopson 1964; Stall and Seymour 1983). By 1914, "…the disease had spread so fast and was so virulent in its effects that it was recognized as a threat to the existence of the entire citrus industry of the Gulf States" (Dopson 1964). The following year, Congress appropriated the first Federal funds to eradicate a plant disease and a USDA-Florida cooperative eradication program for canker was initiated. By 1927, at a cost of $6 million and with the destruction of millions of trees, canker was eradicated from Florida (Dopson 1964; Graham and Gottwald 1991b). By 1943, citrus canker disease was eradicated from the rest of the Gulf States (Dopson 1964).

Xac was again detected in Florida in the mid-1980s (Schoulties and Miller 1985) leading the USDA to take emergency action to eradicate the disease and to create a new domestic quarantine for citrus canker disease, 7CFR 301.75 (FR 1984). Focused primarily on eradicating citrus canker disease, the regulation was highly restrictive. The regulation implicitly assumed that all transmission pathways were at least conceptually possible and therefore appropriate to regulate. The regulation defined the limits of the quarantined area, identified regulated articles (which included fruit) and specified requirements for the movement of regulated articles. Under this regulation, fruit could be shipped interstate under limited permit, provided that "the fruit originated in an area found to be free of citrus canker disease based on surveys…the fruit is free of leaves, litter and stems…and…the fruit has been treated by a thorough wetting with a solution containing 200 parts per million active chlorine for at least two minutes" (FR 1985). The fruit was prohibited from moving to American Samoa, Arizona, California, Hawaii, Louisiana, Puerto Rico or Texas. Later, Guam, the Northern Marianas Islands and the U.S. Virgin Islands were added to the prohibited destinations (FR 1985). In 1988, these prohibitions were removed, allowing fruit to ship to all U.S. States and Territories, if it met additional grove survey and inspection requirements (FR 1988). On March 17, 1994, the USDA declared that Xac had been eradicated in Florida and that "Citrus canker is not known to exist in the United States" (FR 1994).

In September 1995, a third outbreak of Xac was detected on a residential citrus planting in Dade County, Florida (Schubert *et al.* 1996), and the citrus canker disease quarantine was reinstated in Florida in January, 1996 (FR 1996), again with the goal of disease eradication. The restrictions on the movement of regulated articles instituted during the previous outbreak and codified at 7CFR 301.75 remained in force. In 1999, provision was made to allow for fruit grown outside the quarantine area to move into the quarantine area for packing and subsequent shipment to all U.S. States and Territories (FR 1999, 2002).

On January 10, 2006, the USDA recognized the infeasibility of eradicating *Xac* in Florida (Conner 2006) and officially terminated the eradication program. On August 1, 2006, APHIS published an interim rule (FR 2006) extending the existing quarantine region within Florida to include the entire State. Being an emergency action taken in response to the recent extensive spread of citrus canker disease and the termination of the eradication program, the interim rule was not based on a formal risk assessment. The rule maintained the existing restrictions on the movement of regulated articles but applied them to articles originating anywhere in Florida. The rule noted that "The exceptionally active hurricane seasons in 2004 and 2005 were devastating to the citrus canker eradication program…surveys show that citrus canker has become so widespread within Florida that approximately 75 percent of commercial groves in the State are now located within 5 miles of a location where the disease has been detected…" The requirements for the movement of fresh fruit from Florida were amended to reflect that the entire State was now designated as a quarantine area.

3.2 Regulations for Imported Citrus

Based on its experience with the 1914 citrus canker disease outbreak, USDA perceived the likelihood and consequences of Xac introduction to be sufficiently high to justify implementing the trade regulations now known as Quarantine 19 (7CFR 319.19) and Quarantine 28 (7CFR 319.28). Quarantine 19, effective January 1, 1915, regulates the importation of citrus plants and plant parts, except fruit and seeds. Quarantine 28, effective August 1, 1917, prohibits the importation of citrus fruit and peel from specified countries and regions where Xac and certain other citrus diseases are known to occur. APHIS, with few exceptions, prohibited the importation of fresh citrus fruit from all regions with Xac. In those few instances where APHIS did allow the entry of fruit from countries or regions with citrus canker disease, the Agency required multiple, independent, and often complex mitigations.

In 1967, Quarantine 28 was amended to permit importation of Unshu oranges from Japan into Alaska, Hawaii, Idaho, Oregon and Washington. In 1987, Quarantine 28 was again amended to expand importation into all areas except citrus producing States, buffer States and U.S. Territories and in 1994, the regulation was amended further to allow importation into buffer States. Upon request from Japan, APHIS conducted an analysis to determine risks associated with Unshu orange importation into citrus producing States (USDA 1995). The analysis identified several quarantine pests of concern including Xac and recommended a variety of risk mitigating measures. These included requirements that, among other things, imported fruit be grown and packed in canker-free export areas, export groves are surrounded by 400m buffer zones, and that APHIS and Japanese inspectors jointly inspect production areas and buffer zones. Based on the analysis, in 2002, Japanese Unshu oranges were permitted entry into citrus producing U.S. States.

The 1995 analysis on which APHIS based its rule allowing the importation of Japanese Unshu oranges into citrus-producing U.S. States did not specifically assess the likelihood that fruit are a pathway for introducing Xac. Rather, the analysis was based on the longstanding position that symptomatic fruit could be an epidemiologically significant pathway for introduction; it then proposed risk mitigation measures to interrupt that pathway based on the Agency's interpretation of the evidence available at the time.

3.3 Policy Shift

The shift in domestic regulatory policy away from citrus canker disease eradication and towards disease management provides incentive to re-evaluate the scientific basis for the Agency's regulations on the movement of fresh citrus fruit. The approach of domestic citrus canker regulations had been to designate as quarantined areas those places where Xac was found and restrict the movement of fruit from these areas while allowing unrestricted movement of fruit from areas not under quarantine. The decision to terminate the citrus canker disease eradication program prompted APHIS to re-evaluate its citrus canker disease regulations for domestic citrus, especially its regulations for the movement of fresh citrus fruit from areas designated as quarantined areas for citrus canker disease. Most importantly, APHIS began to question whether fresh commercially packed citrus fruit is an epidemiologically significant pathway for the long distance introduction and spread of Xac in light of scientific evidence accrued since those regulations were originally promulgated.

4 Citrus Canker Disease

Citrus canker disease is caused by the plant pathogenic bacterium *Xanthomonas axonopodis* pv. *citri* (Xac). The bacterium infects leaves, stems and fruit attached to the tree (Leite and Mohan 1990; Gottwald *et al.* 2002) of species in the plant family Rutaceae, including economically important citrus species (CABI/EPPO 1997). Infections are non-systemic, *i.e*. they do not spread from inside the plant (Silva *et al.* 2002). Xac enters host plant tissues through natural openings, *e.g.*, stomata (Gottwald and Graham 1992) and wounds (Civerolo 1984). Xac enters its hosts naturally by rain splash directly through stomata or by way of wounds. There is no evident epiphytic growth stage (Brunings and Gabriel 2003). Symptom expression of citrus canker disease varies depending on the age of the lesions, the plant part affected

Figure 4-1. Lesions on a citrus leaf and immature fruit caused by Xac (photo by Dan Robl).

and its age, and the species of *Citrus* infected. New leaf lesions develop as pin point spots then expand to lesions 2 to 10 mm in diameter on both surfaces of infected leaves, later become corky and crater-like, and often are surrounded by a yellow halo (Gottwald and Graham 2000; Pruvost *et al.* 2002; Timmer *et al.* 2005) (Figure 1). Xac lesions on fruit and stems generally resemble those on leaves (Gottwald and Graham 2000). Fruit lesions penetrate only the rind (Civerolo 1984) and are variable in size (Gottwald and Graham 2000).

The presence of free moisture triggers release of Xac bacteria as an ooze from lesions (Figure 2) (Pruvost *et al.* 2002). The oozing bacteria are dispersed by rain splashing and wind-driven rain (Pruvost *et al.* 2002) mostly within infected trees or to neighboring trees (Gottwald and Graham 2000). Short distance spread of Xac within trees, and from tree to tree, occurs primarily via wind-driven rain, especially during storms and hurricanes (Civerolo 1984; Goto 1992; Gottwald *et al.* 1997). Longer distance movement of bacteria is attributed to severe weather events or human assisted movement of infected or contaminated plants, plant material, equipment, containers or conveyances (Gottwald and Graham 2000). There is no authenticated record of the movement of fresh fruit infected with Xac being related to the epidemiology of citrus canker disease (CABI/EPPO 1997; Gottwald and Graham 2000). Long distance dispersal of Xac by animals, birds, and insects has not been conclusively demonstrated (Jetter *et al.* 2000).
Strong winds that cause injuries on leaves, twigs, and fruit, and rainstorms (as well as thunderstorms, tornadoes, tropical storms and hurricanes) that disperse the pathogen, facilitate infection. Xac infection can be facilitated by feeding activities of the citrus leaf miner (*Phyllocnistis citrella*) (Sinha *et al.* 1972; Gottwald *et al.* 2002).

Major outbreaks of citrus canker disease occur when abundant bacterial inoculum is present in combination with susceptible plant tissues (Gottwald and Graham 1992), and frequent rainfall with warm weather and high winds (Serizawa and Inoue 1974; Gottwald and Graham 1992). Three such outbreaks have occurred in the United States, one affecting the Gulf Coast States (beginning around 1910), and two others confined to Florida (from the mid 1980s to 1994 and the current outbreak which started in 1995).

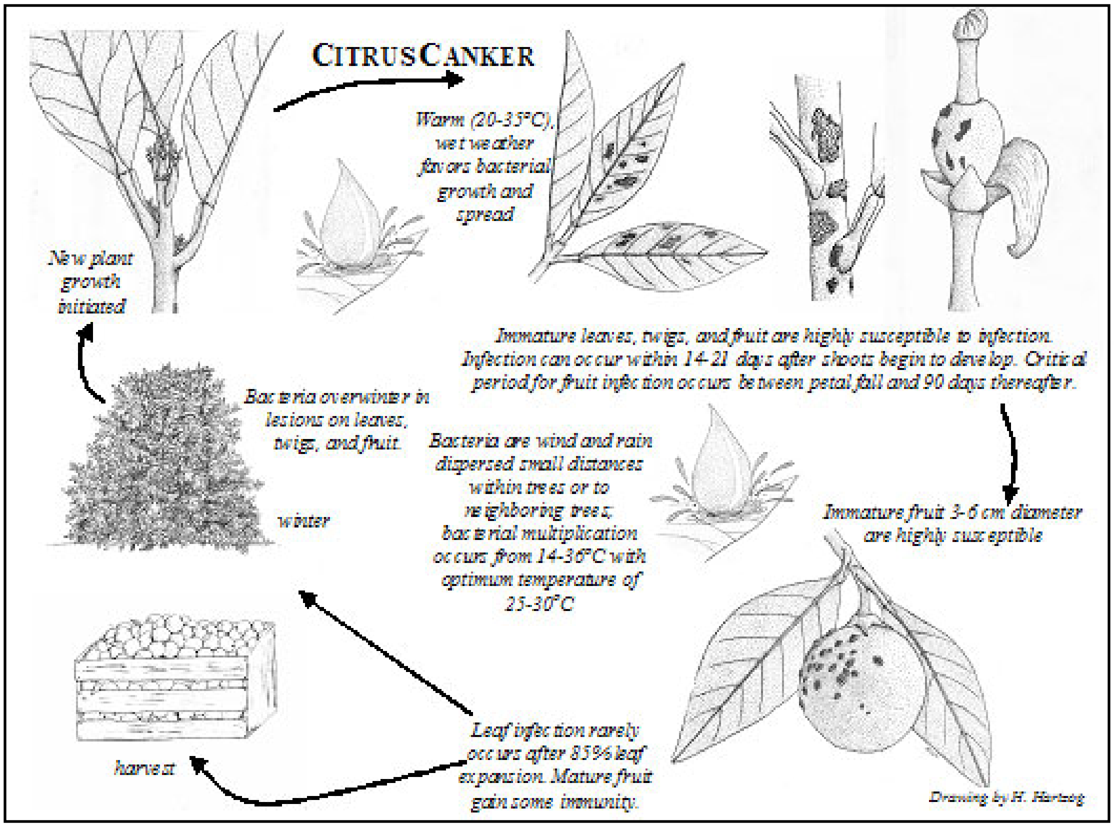

Figure 4-2. Disease cycle of citrus canker disease caused by *Xanthomonas axonopodis* pv. *citri*.

5 The Movement of Fresh, Commercial Citrus Fruit as a Pathway for the Introduction of Xac

This analysis focuses on commercially packed, fresh citrus fruit as a pathway for the introduction of Xac. Previous analyses on the topic (USDA 1995; Schubert *et al.* 1999b; USDA 2006) have concluded that the likelihood of introducing Xac into previously free areas on commercially produced and packed citrus fruit is low for the following reasons: 1) Production practices exist to reduce the prevalence of harvested fruit that are actively infected with Xac; 2) symptomatic fruit are culled and all fruit are treated with disinfectants during commercial packing; 3) epiphytic contamination by Xac is devitalized by a disinfectant treatment of fresh citrus fruit prior to packing; 4) mortality of Xac associated with fresh citrus fruit and/or packing materials occurs during shipping; 5) successful Xac infection that results in disease outbreaks occurs only with a suitable host and in areas with particular environmental and climatic conditions suitable for survival and reproduction; and 6) large quantities of fresh citrus fruit shipped for many years from regions with Xac have not resulted in any known outbreaks of citrus canker disease. The following sections summarize available evidence supporting these conclusions.

This section evaluates evidence summarized from "*Evaluation of asymptomatic citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease*" (USDA 2006) and additional evidence from the scientific literature.

5.1 Production Practices and the Likelihood of Harvesting Xac Infected or Contaminated Fruit

This section evaluates evidence for the likelihood that Xac infected or contaminated citrus fruit will be harvested and delivered to the packinghouse. The magnitude of the hazard at this stage will depend in large part on the proportion of infected fruit, and the nature of the contamination. Groves in infested areas may have citrus canker infected fruit at varying levels, depending on the prevalence of inoculum, the susceptibility of the variety, climatic, environmental, and cultural conditions. Presence of Xac on fruit may be associated with lesions, injuries, or blemishes, or it may be epiphytic (surface contamination). We found no reports of endoparasitic infection inside fruit that does not exhibit symptoms. Infections are non-systemic; i.e., they do not spread from within the plant (Silva *et al.* 2002).
Guidelines. Practices such as pre-harvest grove inspections, designation and exclusion of infected trees, enhanced inspection of fruit in field bins, *etc.*, may reduce the likelihood that symptomatic fruit is harvested and transported to the packinghouse (CHRP 2006; Kinney 2007). The efficacy of these measures and the level to which they are applied is difficult to assess since such practices are not required by any current State or Federal regulations and thus are not monitored.

Chemical and cultural control. Disease management practices in the grove, including the application of prophylactic copper sprays and use of windbreaks, can reduce, but do not eliminate Xac populations in a grove (Stall *et al.* 1980; Stall *et al.* 1981; Gottwald and Timmer 1995; Dixon *et al.* 2000; Canteros 2004; Graham *et al.* 2004). Well-timed field treatments significantly reduce the prevalence of disease, the level of inoculum, and the number of symptomatic fruit in the field. Prophylactic sprays of copper oxychloride (or other copper-containing compounds) provide protection against initial infection in canker-endemic areas during growth flushes and early fruit development (fruit approximately 2-6 cm diameter) (Koizumi 1977b; Kuhara 1978; Stall *et al.* 1980; Medina-Urrutia *et al.* 1985; Leite and Mohan 1990; Das and Shyam 2003; Graham and Leite 2004) and reduce the prevalence of Xac infection in the field (Stall *et al.* 1980; Leite and Mohan 1990; Gottwald *et al.* 2002). Windbreaks "significantly reduced both disease increase and spatial spread of citrus canker [on grapefruit] over time" (Gottwald and Timmer 1995). Grapefruit with no windbreaks peaked at 35 percent disease incidence, while grapefruit with windbreaks never peaked above 5 percent disease incidence (Gottwald and Timmer 1995). Combinations of prophylactic sprays and cultural control practices, such as windbreaks and pruning diseased shoots, further reduce disease incidence (Kuhara 1978; Leite and Mohan 1990; Leite 2000; Das 2003).

Culling and selection in the field. Currently in Florida, harvesting measures to selectively pick fruit free of citrus canker disease lesions are not required by regulation, nor is it assumed by this document that they will be widely practiced. APHIS field personnel indicate that packinghouse buyers scout fields for disease incidence, but it is not known how widespread or effective this practice is. The Florida Citrus Packers (Kinney 2007) indicated the industry proposes to develop best management practices including: collaborative grower and packer grove inspections; diversion of fruit from infected trees to processing; and inspection of fruit in field bins before delivery to packinghouses. These best practices will be voluntary when implemented.

Symptom expression. Culling of Xac infected fruit, whether in the field or subsequently in the packinghouse, is based on the observation of symptoms. Citrus canker is mainly a leaf spotting and fruit blemishing disease (Gottwald and Graham 2000). Conspicuous lesions typically develop on leaves, stems and fruit (Civerolo 1984; Gottwald and Graham 2000). Immature fruit infected early in their development may develop severe symptoms including cracking and malformation (Verniere *et al.* 2003). Koizumi (1972) found that fruit inoculated by pin prick prior to 60 percent expansion developed typical erumpent, corky lesions 2 to 5 mm in diameter while late infections (65 to 85 percent expansion) developed less typical nonerumpent or pinpoint greenish spots 0.1 to 1.5 mm in diameter. Goto (1969) found in a wound inoculation study that "…latent infections were never observed."

Tissue susceptibility. Fruit are susceptible to natural (stomatal) infection from petal fall (Goto 1992) until they are around 6 cm in diameter, and are most susceptible at a fruit diameter of 2 to 6 cm, a period of about 90 to 120 days (Koizumi 1972; Graham *et al.* 1992b; Verniere *et al.* 2003). Mature citrus fruit have natural wax layers on their surface, decreasing susceptibility by reducing access to natural openings, such as stomata (Albrigo 1972; Albrigo 1976; Graham *et al.* 1992b). Mature, aboveground citrus tissues can be infected through wounds (Gottwald *et al.* 2002). Goto (1969) used carborundum rub inoculation to abrade the surface of mature fruit and extend the susceptible period of orange and mandarin fruit on the tree beyond the fruit maturity susceptible to natural (stomatal) infection. The Asian leafminer (*Phyllocnistis citrella* Stainton) interacts with Xac by providing wounds that serve as infection courts in leaves and, to a lesser extent, fruit (Schubert *et al.* 2001; Gottwald *et al.* 2002). Leafminer wounds create suitable microclimates for Xac development (Chagas *et al.* 2001). Leafminer-damaged leaves have more and larger lesions (Sohi and Sandhu 1968; Sinha *et al.* 1972). Verniere *et al.* (2003) states that "the combination of *X. axonopodis* pv. *citri* and the leafminer can lead to significant field infection even on highly resistant cultivars and species of citrus such as, calamondin and kumquat."

Wounds. The term "wound" in this document is meant to describe an injury to any external surface of the plant by its being torn, pierced, cut, or broken. Unlike a lesion, the occurrence of a wound does not imply that disease has developed. In previous decisions (FR 1983), USDA has determined that "It is unlikely that new citrus canker infections would be established in the United States because of the importation of fruit or peel of citrus or citrus relatives carrying bacteria trapped in the pores or wounds. In order for the bacteria to cause an infection an unlikely sequence of events would have to occur. First, bacteria trapped in the pores or wounds of the fruit would have to be released without coming in contact with any of the acid of the fruit since citrus canker bacteria are quickly killed by contact with the acid. Next bacteria would have to come into intimate contact with young live twigs or leaves of host plants and, in addition, such contact would have to occur under optimum temperature and humidity conditions."

Fulton and Bowman (1929) reported that, during inoculation studies, wounding needed to be done with care not to cut oil glands in order for infection to occur. They noted, "The exuding oil had a tendency to injure a portion of the adjacent tissue and to interfere with a normal infection reaction." They also reported that infection only occurred if the wound stayed moist until the time of inoculation. Wounds that were allowed to dry and were inoculated after 26 hours did not result in infection. That is, infections occurred only when oil glands were avoided and inoculum was applied within 26 hours of wounding (Fulton and Bowman 1929). Verniere *et al.* (2003) reported a disease incidence of zero when inoculating mature fruit either by pin prick or spray inoculation.

No published reports were found regarding the prevalence or survival of Xac in naturally occurring wounds without typical lesions of citrus canker disease. Survival of Xac in lesions is discussed below.

Xac survival in lesions. Bacteria survive in lesions formed on above-ground parts of susceptible hosts, including fruit still attached to the tree, leaves, twigs, stems, and the bark of the trunk (Leite and Mohan 1990). Bacteria in leaf and twig lesions are a source of inoculum for secondary infections (Pruvost *et al.* 2002); and stem lesions can act as reservoirs of inoculum for longer periods than fruits and leaves (Leite and Mohan 1990; Verniere *et al.* 2003). Timmer *et al.* (1991) inoculated grapefruit and Swingle citrumelo leaves with Xac in the field, and then collected the leaves at 14, 21 and 49 days after inoculation to assess bacteria concentrations within active lesions. Bacterial populations within the lesions were closely correlated with lesion age. Young lesions (4 to 6 weeks old) exude approximately 10^4 to 10^6 cfu/ml in the first 48 hours of wetting, while older lesions (4 to 6 months old) exude about 10^2 to 10^3 cfu/ml in the same time period (Timmer *et al.* 1991). Bacteria may survive for a few weeks to several months on decomposing plant litter (fallen fruit, leaves, and limbs) on the soil surface (Civerolo 1984; Graham *et al.* 1987; Leite and Mohan 1990; Schubert *et al.* 2001; Gottwald *et al.* 2002), or in plant material buried in the soil (Graham *et al.* 1987). Survival in decomposing leaves, both in and on the soil surface, is dependent on moisture and temperature (Graham *et al.* 1987; Goto 1992). Xac populations within the lesions of infected fruit gradually decline after harvest (Koizumi 1972; Civerolo 1981).

Epiphytic survival. Epiphytic populations of Xac may aid in pathogen dispersal, but Goto (1962) reported that epiphytic populations of Xac applied to the surface of leaves of outdoor citrus trees lost infectivity after 3 days under spring (May 24) conditions and after only 8 hours under summer conditions (July 15) in Japan. Epiphytic Xac applied to leaves of potted citrus trees declined dramatically within 24 hours but were detectable at low levels for as long as five days (Timmer *et al.* 1996). Timmer *et al.* (1996) states, "we detected epiphytic [Xac] on asymptomatic plants, but the occurrence of epiphytic populations was not related to subsequent appearance of symptoms", and additionally "our evidence indicates that [Xac] is highly unlikely to persist on hosts or non-hosts in the absence of symptoms for long periods." Rybak and Canteros (2001) found in examining field grown fruit "…that populations of Xac are generally low even from highly infected plots in lesionless leaves and fruits and almost always undetectable in low disease intensity groves." Researchers in Brazil sprayed asymptomatic fruit, picked from trees, with a bacterial suspension of 10^6 cfu/ml; no bacteria were recovered after 5 days at room temperature under laboratory conditions (Belasque and Rodriguez Neto 2000). Epiphytic bacteria do not multiply in water on leaf surfaces or on dry leaves (Timmer *et al.* 1996). Graham *et al.* (2000) found that Xac survived for 48 to 72 hours on a variety of inanimate surfaces in sun or shade, respectively.

Equipment decontamination. Current Federal regulations (7CFR 301.75) require decontamination of vehicles and equipment moving between Xac infected groves and packinghouses. The Citrus Health Response Plan (CHRP 2006) recommends similar decontamination procedures. Such decontamination consists of the removal of all leaves, twigs, and other plant parts from the equipment and subsequent treatment with approved disinfectants, such as sodium hypochlorite, quaternary ammonium chloride, hot water and detergent under high pressure, or steam (Schubert *et al.* 1999a; Roberts *et al.* 2004; Code of Federal Regulations 2006b).

SUMMARY

- Disease management practices in the grove, including the application of prophylactic copper sprays and use of windbreaks, *etc.* may reduce, but do not eliminate Xac populations in a grove.
- Commercially produced fruit harvested in areas where Xac exists may be visibly infected or the fruit may carry the pathogen either on its surface or in wounds without showing typical symptoms.
- Commercially harvested fruit from Xac infested areas is likely to be contaminated with epiphytic populations of Xac.
- Infection of citrus fruit by Xac between harvest and packinghouse is not likely.

5.2 Commercial Citrus Fruit Packing

Citrus fruit, once in a commercial packinghouse, are subjected to cleaning and sanitizing processes to minimize surface contaminants and pathogens, and produce clean and attractive fruit for the fresh market (Figure 3). Diseased, damaged, disfigured, and blemished fruits are culled in the packinghouse. These post-harvest measures are largely voluntary, but USDA-APHIS-Plant Protection and Quarantine (PPQ) provides guidance on their use in the Citrus Health Response Plan (CHRP 2006), without mandating their application. Citrus packinghouses in Florida vary in size, scale, and level of mechanization; however, most employ some or all of the following post-harvest measures which can reduce survival of Xac inoculum associated with fruit. This section documents evidence relating to the likelihood that viable Xac will survive packinghouse processes and treatments and will escape detection to be packed in or on commercial citrus fruit.

Fruit handling and packinghouse sanitation. Packinghouses may have one to several steps at which fruit are handled by workers, from initial dump of fruit, to intermediate grading steps for blemishes, size or color, to the final packing stage where finished fruit[6] are placed in boxes or bags for transport. The U.S. Food and Drug Administration's (FDA) has developed Good Agricultural Practices (GAP) (Food and Drug Administration 2003) and the USDA has drafted the Citrus Health Response Plan (CHRP 2006) to provide packinghouse managers with guidelines for the production and packing of citrus. Sanitation measures included in GAP which would likely reduce Xac contamination in packinghouses include: maintenance of sanitizers (*e.g.* chlorine or sodium orthophenylphenate– SOPP) at proper concentrations and pH; use of good hygienic practices by workers; proper use of gloves; cleaning packing areas, storage facilities and bins with approved sanitizers before use; separation of unwashed fruit from clean packed fruit; cleaning, sanitizing, and maintenance of equipment; preparation of packing cartons as needed to prevent contamination; and prevention of fruit injury (Ritenour 2001; Goodrich 2005). To prevent product losses due to decay, producers also seek to reduce inoculum of postharvest decay pathogens on fruit (Narciso 2005).

Debris removal and washing. As fruit is initially emptied onto the packing line, field bins are washed using approved disinfectants (Schubert *et al.* 1999a; Roberts *et al.* 2004; Code of Federal Regulations 2006b). Potential sources of Xac inoculum such as infected stems, leaves, and rotten or split fruit are removed (Miller *et al.* 2001). Washing and sanitizing procedures may be done separately or combined in the packinghouse. If washing is done separately, the citrus fruit is washed with a detergent solution for a minimum of 20 to 30 seconds over rotating brushes (Jarrett and Tugwell 1975; Miller *et al.* 2001). Graham and Gottwald (1991a) reported significant reductions in *X. axonopodis* pv. *citrumelo* survival on citrus fruit by simulating packinghouse processes using brush-aided washing with and without SOPP. Washing removes organic matter and increases the effectiveness of sanitizing treatments, such as chlorine (Brown and Schubert 1987), and reduces surface bacterial populations, including Xac (Canteros *et al.* 2001). In laboratory tests in Argentina, Canteros, *et al.* (2001) noted reductions of one to three orders of magnitude in the number of Xac cells on the surface of artificially inoculated fruit when "fruits were prewashed as in a packinghouse".

[6] Fruit that has completed the packinghouse washing, disinfection, grading and inspection processes.

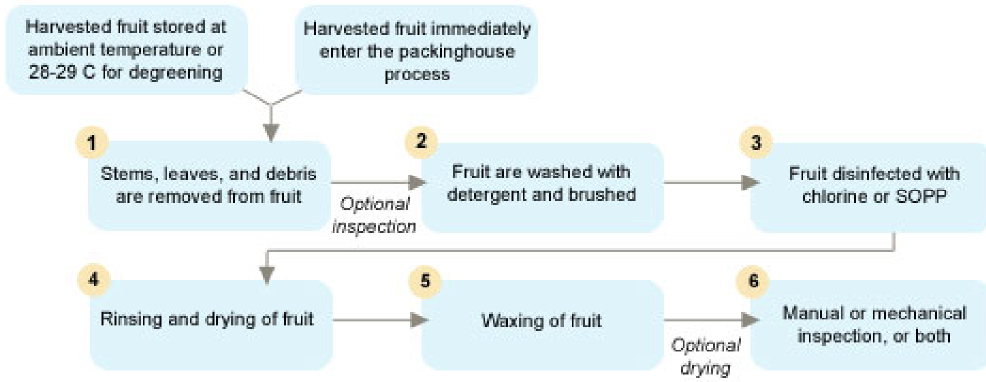

Figure 3. General steps of the standard packinghouse process for fresh citrus fruit. The blue boxes represent the main steps applied to citrus fruit. Italicized text in between the blue boxes represents optional steps that may be taken.

Disinfection of fruit. Two disinfectants are currently approved by USDA for decontamination of fresh citrus fruit: chlorine (treat 2 minutes at 200 ppm sodium hypochlorite, pH 6.0-7.5 where sodium hypochlorite concentrations are verified by monitoring the concentration of available chlorine) and SOPP (45 seconds to 1 minute, depending on detergent concentration, SOPP at 1.86-2.0 %) (Code of Federal Regulations 2006a). Various studies demonstrated the effectiveness of these disinfectants in reducing numbers of Xac cells or similar bacteria to low or undetectable levels (Obata *et al.* 1969; Brown and Schubert 1987; Canteros *et al.* 2001; Rybak and Canteros 2001). Brown and Schubert (1987) studied disinfectants applied alone and during washing, to evaluate impacts on *Xanthomonas campestris* pv. *vesicatoria* (Xcv), as a proxy for Xac. Chlorine dips of artificially inoculated fruit have been shown to eradicate *Xanthomonas campestris* pv *vesicatoria*, a closely related bacteria (Brown and Schubert 1987). “All formulations [of SOPP tested] effectively eradicated cells of *X. c.* pv. *vesicatoria* from the fruit surfaces (Brown and Schubert 1987).”

When the washing process includes a disinfectant, such as 200 ppm chlorine or SOPP, Xac populations are significantly reduced to low or undetectable levels (Obata *et al.* 1969; Graham and Gottwald 1991b).The use of 200 ppm chlorine for 2 minutes reduces natural bacterial populations on citrus fruits by 77 to 99 percent; no Xac was recovered post-treatment, although the authors did not assess the level of Xac (if any) that was present on the fruit prior to treatment (Stapleton 1986). Similar results were obtained using chlorine and/or SOPP treatments; fruit with $<10^7$ cells/ml of Xac dipped in 100 ppm chlorine yielded no detectable levels of bacteria (Obata *et al.* 1969). In vitro tests exposing Xac to as little as 0.1 ppm chlorine eliminated all Xac bacteria (Stapleton 1987).

Successive treatments of sodium hypochlorite and SOPP, common in many packinghouse procedures are highly effective in eliminating epiphytic populations of Xac, although only single sanitizing agents are required in Florida packinghouses,. “Disinfected fruits treated with SH [sodium hypochlorite] followed by SOPP in plates and plants did not yield any living bacteria: 0 (0) cells/fruit (Canteros *et al.* 2001).”

Verdier *et al.*(2006) in an unpublished study submitted with public comments to publication of the 2006 interim rule (FR 2006) reported that when a small number (five replications, total of 72 fruits) of asymptomatic, naturally infested citrus fruits were treated with chlorine and SOPP the number of Xac cfu in the solution used to wash the fruit was dramatically reduced. Only 3 percent (all from a single replication) of the treated fruit were positive for Xac when tested by plating the wash solution on selective media, as compared to 67 percent of the untreated controls and the bacterial population in the wash solution was reduced 99.8 percent from an average of 39.4 cfu/ml on untreated controls to an average of 0.06 cfu/ml on treated fruit.

APHIS proposes to add a third disinfectant treatment option to the currently approved disinfectant treatments. The proposed treatment for use on citrus fruit is a solution containing 85 parts per million peroxyacetic acid (PAA) which is to remain in contact with the fruit surface for at least 1 minute. PAA is effective against a broad range of microorganisms and their spores including the citrus canker bacterium (USDA-APHIS 1999). USDA PPQ Treatment Quality Assurance Unit evaluated data required by the Florida Department of Agriculture and Consumer Services for labeling and provided from third party laboratories on the efficacy of PAA products (Parra 2007).

For those experiments, *X. axonopodis* pv. *citrumelo* cultures were utilized in testing of the PAA products rather than the citrus canker bacteria, *X. axonopodis* pv. *citri*. *X. axonopodis* pv. *citrumelo* is considered to be a suitable surrogate. Experiments were conducted by third party laboratories in conformance with AOAC Method 960.09 for Germicidal and Detergent Sanitizing Action of Disinfectants and ASTM Standard Test Method (E 1153-94) for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces and fulfills EPA requirements. At product rates of 85 ppm and 200 ppm PAA, with exposure times of 30 seconds and 1 minute, the tests met the standard efficacy (99.999% reduction of a known concentration of *X. a.* pv. *citrumelo*) at all tested concentrations (Parra 2007). One test was conducted directly on citrus fruit surfaces for reduction of *Salmonella sp*. on the surface of the fruit. In general, *Salmonella* is more resistant to sanitization by peracetic acid than *Xanthomonas*. The 85 ppm level of PAA provided a 99.999% reduction of Salmonella on the fruit surface (Parra 2007). Based on their evaluation of this data, USDA PPQ Treatment Quality Assurance Unit determined that PAA treatment at 85 ppm for a 1 minute exposure is efficacious against *Xanthomonas axonpodis* pv. *citri* on citrus fruit (Parra 2007).

Bacteria within lesions may be more protected from the detrimental effects of washing, disinfection and drying. Viable Xac has been recovered by APHIS pathologists from citrus canker lesions on fruit culled from packinghouse lines after postharvest treatments. (Riley 2007).

Rinsing and drying. After washing and disinfection, fruit are rinsed and excess water is removed, either mechanically, or with forced air or heat (Miller *et al.* 2001). Hot air drying (58ºC for 2.5 min.) is generally used after wax/fungicide application, but may be used before wax/fungicide application as well (Schubert *et al.* 1999b). Hot air drying, or even air drying, may further reduce viable bacteria on the surface of fruit, since artificially applied Xac inoculum on citrus fruit surfaces dies when exposed to air drying (Stapleton 1986). Survival of naturally occurring inoculum may differ from artificially applied inoculum (Schubert *et al.* 1999b) and inoculum levels present on fruit may also influence survival (Stapleton 1986).

Wax/fungicide application and drying. The fruit typically is coated in wax which may contain fungicide (Miller *et al.* 2001). Waxing itself seems to have limited impact on Xac populations on fruit surfaces. Rybak and Canteros (2001) detected low numbers of Xac cells on lesion-free, non-disinfected fruit of grapefruit, lemon, and orange whether they were waxed or not. Schubert, *et al.* (1999b) noted that an unpublished study by Schubert and Leahy in 1991 found that the combination of wax and hot air drying reduced *X. axonopodis* pv. *citrumelo* inoculum levels on citrus fruit to "very low levels."

Packinghouse inspection. Packinghouse inspections are intended to eliminate fruit that are injured, blemished, misshapen, off-color, non-uniform in size, or otherwise of low quality, including Xac-infected fruit. These inspections occur during various grading and culling steps in the packinghouse by trained personnel and/or electronic optical scanning equipment or combinations of both methods. Under compliance agreements for packinghouses currently issued by the Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), the "packer is responsible for training its graders and field personnel each year in progressive fruit grading techniques for the detection of citrus canker lesions" (Florida Department of Agriculture and Consumer Services 2006; University of Florida - IFAS and FDACS-DPI 2006). This "lesion and symptom detection training is available through UF-IFAS" (Florida Department of Agriculture and Consumer Services 2006; University of Florida - IFAS and FDACS-DPI 2006). The CHRP (USDA-APHIS and FDACS/DPI 2006) also includes provisions for annual training of packers.

Packinghouse processes may be very effective at removing fruit with citrus canker lesions. Studies in Argentina have demonstrated that culling of symptomatic citrus canker fruit is highly effective in packinghouse operations. For example, trays of fruit known to contain either one percent or three percent of symptomatic fruit were visually inspected at three stages throughout the packing process, resulting in extremely low (near 0) numbers of symptomatic, injured or blemished fruit reaching the packing bench, and zero symptomatic fruit packed in boxes (Ploper *et al.* 2004). Other factors may affect the ability to detect blemished fruit including the size and appearance of lesions or blemishes, type of fruit being inspected, quality of lighting at inspection points, number of inspection points, number of personnel inspecting fruit, and the speed of fruit movement through the process (Miller *et al.* 2001).

The size, appearance, and abundance of Xac lesions on fruit entering and exiting the packing line may vary, influencing the ease with which they are detected and the infected fruit is removed. Variations observed in lesion appearance are attributed to many factors, including growth stage at which fruit became infected (Civerolo 1984; Graham *et al.* 1992b; Verniere *et al.* 2003; Graham and Leite 2004), susceptibility of the host (Zubrzycki and Zubrzycki 1986; Graham *et al.* 1992b; Gottwald *et al.* 1993), and association with wounds (Koizumi 1972; Sinha *et al.* 1972; Koizumi 1983; Goto 1992; Graham *et al.* 1992b; Verniere *et al.* 2003). Lesions begin as pin point spots, then depending upon the stage at which fruit are infected, may develop to 2 to 10 mm in diameter, becoming corky and crater-like, uniformly brown, approximately circular, and often are surrounded by a water-soaked margin and yellow halo (Gottwald and Graham 2000; Pruvost *et al.* 2002; Timmer *et al.* 2005; University of Florida - IFAS and FDACS-DPI 2006). Lesions on young grapefruit fruit expanded to 1 to 2 mm diameter after 2 to 3 months, enlarging to 9 mm after 200 days (Stall *et al.* 1980), whereas on fruit infected when more nearly fully expanded, lesions remained as minute (0.1 to 0.15 mm) or small (0.6 to 1.5 mm) greenish spots (Koizumi 1972). It is possible that very small or uncharacteristic lesions may escape detection.

During an evaluation of a diagnostic tool, APHIS plant pathologists collected approximately 75 pieces of fruit eliminated by packinghouse graders for Xac lesions. The average lesion size on these fruit was about 4 mm. APHIS plant pathologists have intercepted fruit in final packed cartons with lesions in the 2-3 mm range and have observed that the majority of the symptomatic fruit that APHIS inspectors intercepted after passing through the packing line undetected by graders have only one lesion (Riley 2007).

Phytosanitary inspection. Under the current regulation (Code of Federal Regulations 2006b), APHIS conducts monitoring phytosanitary inspections for Xac as part of the process for issuing requisite limited permits for interstate movement of citrus fruit. Up to 2 percent of the fruit in each inspected lot is examined by APHIS inspectors. Not every lot is inspected under the current system. This phytosanitary inspection generally takes place on finished fruit after all packinghouse treatments, grading and inspections are completed (Lowe 2007).

Currently, APHIS has approximately 126 inspectors who are trained and rigorously tested in citrus canker disease recognition. Inspectors are trained in citrus canker disease recognition within 3 weeks of hiring. Training sessions are followed by testing. Tests involve a lab practical where the inspector must determine the citrus canker disease status of plant samples. Included in the test are leaf and fruit samples with Xac lesions at various stages of development or samples that contain lesions or blemishes caused by an array of fungal or bacterial diseases that can be easily mistaken for canker (greasy spot, citrus scab, anthracnose, melanose, citrus bacterial spot, *Alternaria*, *etc.*). Inspectors are given approximately 40 seconds to make a determination as to whether the specimen has citrus canker disease. Inspectors must correctly identify at least 80 percent of leaf and fruit samples to receive a passing grade. Inspectors who fail their first test are given the opportunity to re-test after taking additional training. Failure to pass the re-test may be grounds for dismissal from employment. Refresher training and testing is repeated each year for all inspectors. From 2004 through 2006 APHIS inspectors averaged a score of 93.4 percent on these tests.

Summary

- Procedures for cleaning and disinfecting fruit are routinely applied by packinghouses.
- The individual efficacy of each of these procedures for removing or destroying Xac may not be known in detail, but the effect of packinghouse treatments in combination with grading, culling and inspections reduces the prevalence of Xac and the level of inoculum associated with commercially packed fresh citrus fruit.
- Grading and inspection procedures are effective in removing fruit with visible lesions.

5.3 Mortality of Xac during Shipping

The most important factors in shipment and storage which may influence Xac bacterial survival are likely storage duration, temperature, and moisture, as well as safeguarding to prevent contamination of sanitized fruit or containers. This section documents evidence relating to the likelihood that viable Xac will survive shipping processes and conditions in or on commercially packed citrus fruit.

Effect of shipping and storage temperature. Temperatures during shipping and storage influence Xac inoculum survival. In general, to maintain fruit quality, temperatures during storage and shipment of citrus fruit would range from 4 to 10 º C for tangerines and mandarin-type fruits to 10 to 15º C for grapefruit (Sunkist Growers Inc. 1983; Wills et al. 1998). In host plant tissues, Xac infection and subsequent multiplication only occurs at temperatures above 14º C and below 38º C (Koizumi 1976). Dalla Pria *et al.* (2006) reported 20° C temperatures, interfere in the infection process, reducing disease incidence. Bacterial populations in existing lesions decreased from 10^7 to between 10^2 and 10^4 cells when the average maximum temperature was below 20° C and average minimum temperature was below 10°C (Koizumi 1977b). Stall *et al.* (1980) noted “the populations of viable cells in lesions decreased about 100-fold during the winter months.”

Epiphytic survival. Xac has been shown to survive for a short time on the surfaces of contaminated fruit, containers or conveyances as detailed above (Section 5.1). Any Xac remaining on the surface or in lesions are unlikely to infect harvested mature fruit and unlikely to multiply on fruit surfaces (Timmer *et al.* 1996).

Survival in lesions. See the discussion in Section 5.1 Production Practices and the Likelihood of Harvesting Xac Infected or Contaminated Fruit. The multiplication of Xac bacteria associated with lesions is closely related to lesion expansion. In expanding lesions, Xac bacteria multiply abundantly, but as lesion expansion ceases, bacteria multiplication noticeably decreases (Koizumi and Kuhara 1982; Graham *et al.* 1992a). In the late stages of lesion expansion, bacterial multiplication becomes inhibited in the peripheral area of the lesion (Koizumi 1977a). Bacteria survive in the margin of the lesions in citrus leaves and fruit, until they fall or are removed from the tree (Graham *et al.* 2004). Xac bacteria do not increase in number on fruit once the fruit is removed from the tree, but rather populations decline within the lesions of infected fruit following harvest (Koizumi 1972; Civerolo 1981). Fruit-to-fruit post-harvest spread of Xac has not been documented.

SUMMARY

- The cool temperatures at which citrus fruit are stored and shipped will restrict the ability of Xac to reproduce and cause infection.
- Xac bacteria do not increase in number on fruit once the fruit is removed from the tree, but rather populations decline within the lesions of infected fruit following harvest.

5.4 Environmental and Epidemiological Conditions Required for Xac Establishment

This section evaluates evidence relating to the environmental and epidemiological conditions required for Xac establishment. Even if fruit with Xac are shipped to a previously free region, introduction requires proximity of that fruit to a susceptible host, spread of a sufficient amount of inoculum from the fruit to host tissue at a susceptible growth stage and environmental conditions conducive to year-round survival, dispersal, and infection.

Areas at risk. The majority of citrus fruit exported from Florida moves to non-citrus producing States or other countries (Florida Department of Citrus 1997, 1998, 1999, 2000, 2001, 2002, 2003b, 2004a, 2005a, 2006a). For example, in the 2005-2006 shipping season, approximately 96 percent of Florida's domestic and Canadian citrus exports were shipped to non-citrus producing States or Canada. Demographics derived from United States Census data may be useful in predicting the distribution of Florida citrus fruit by indicating population centers where demand is greatest. Two of the four most populous States in the United States, Texas and California (U.S. Census Bureau 2002), are citrus-producing States. If we assume that citrus is proportionally distributed across the United States, in accordance with population, then it is reasonable to assume that some fruit will be shipped to these States; however, only a small portion of each State actually produces citrus (USDA-NASS 2002) (see Appendix 1), and an even smaller portion has a climate suitable for canker disease development (Borchert *et al.* 2007).

Climate. At the present time, Xac is established primarily in tropical and subtropical areas (CABI 2006). In the United States, the pathogen is established in Florida (CABI 2005) and is capable of establishment in the Gulf States (Alabama, Georgia, Mississippi, Louisiana, South Carolina, and Texas) as seen in the initial citrus canker disease outbreak in the early 1900's (Dopson 1964). Using hourly wind speed and precipitation, monthly average temperature, and annual and seasonal precipitation data to determine the expected incidence and severity of citrus canker if introduced into California, Borchert *et al.* (2007) concluded that favorable events in California citrus growing areas occurred "… predominantly during the winter season when precipitation is greatest, but temperatures are less conducive for infection activity and citrus growth. This would likely result in low incidence and severity of citrus canker in California if the disease were introduced…" Peltier and Frederich (1926) suggest that a Mediterranean type climate is unfavorable for the development of citrus canker disease, though they concede that the disease "could develop in all of the citrus regions of the world *sometime* over the growing season". The European and Mediterranean Plant Protection Organization (CABI/EPPO 1997) uses the same rationale in designating Xac a quarantine pest for Europe. The "Mediterranean" climate (dry summers) typical of most of California and the arid climate of Arizona make Xac establishment less likely in those States. However, in microclimates with highly susceptible cultivars such as along the California coast between San Diego and Ventura establishment is still possible, as demonstrated by the occurrence of citrus canker disease in Iran and the Arabian Peninsula on a highly susceptible variety of Mexican lime (Mohammadi *et al.* 2001; Das 2003).

Temperature. Temperature affects both the ability of Xac to cause infection and subsequent disease development (Peltier and Frederich 1926; Koizumi 1976; Koizumi 1977b; Dalla Pria *et al.* 2006). It also affects survival of Xac within lesions (Koizumi 1977b; Stall *et al.* 1980). Temperatures between 15 to 20° C and 35 to 40° C are conducive for infection and development of citrus canker disease (Peltier and Frederich 1926; Dalla Pria *et al.* 2006). Bacteria inoculated on wounded citrus leaves during months with an average maximum temperature below 20° C and an average minimum temperature below 10° C were undetectable soon after inoculation with no reoccurrence the following spring (Koizumi 1977b). At these temperatures, bacterial populations in existing lesions decreased from 10^7 and 10^4 to between 10^4 and 10^2 cells, respectively (Koizumi 1977b); Stall *et al*. (1980) noted "the populations of viable cells in lesions decreased about 100-fold during the winter months..."

Moisture. Wind-driven rain or overhead irrigation facilitate dispersal of Xac within and between citrus trees (Gottwald *et al.* 1988; Pruvost *et al.* 1999; Bock *et al.* 2005); aid the movement of bacteria into stomata (Serizawa and Inoue 1974; Gottwald and Graham 1992); and, enhance the exudation of bacteria from lesions (Timmer *et al.* 1991; Timmer *et al.* 1996). In experiments simulating wind-driven rain, Bock *et al*. (2005) found that the greatest quantity of bacteria was dispersed within the first few minutes of exposure; 70 to 80 percent of the total bacteria collected during the experiments were detected within the first hour. Studies have found that between 10^4 and 10^6 cells are exuded from lesions when exposed to a period (less than 1 hour) of wetting or rainfall (Timmer *et al.* 1991; Bock *et al.* 2005).

Inoculum. Another factor influencing the likelihood of Xac causing infection is the size of the bacterial population in or on the fruit. In experiments simulating wind-driven rain, concentrations less than 10^4 cfu/ml for one bacterial strain were insufficient to cause infection on unwounded grapefruit leaves under an impact pressure of 8.05 kPa, however 10^6 cfu/ml gave consistent and successful infection (Gottwald and Graham 1992). Goto (1962) ascertained that the minimal dose of Xac necessary for stomatal infection was 10^5 cells/ml and that for wound infection, about 10^2 to 10^3 cells /ml were required. Pruvost, *et al.* (2002) reported a threshold of 10^3 cfu/ml inoculum for stomatal infection of Mexican limes. Xac populations within the lesions of infected fruit decline after harvest (Koizumi 1972). After 5 days at room temperature under laboratory conditions, researchers were unable to recover Xac from asymptomatic fruit, removed from trees and sprayed with a bacterial suspension of 10^6 cfu/ml (Belasque and Rodriguez Neto 2000). There was "no evidence that Xcc [Xac] multiplies on the leaf surface…"(Timmer *et al.* 1996). Graham *et al.* (2000) found that Xac survived for 48 to 72 hours on a variety of inanimate surfaces in sun or shade, respectively. Rybak and Canteros (2001) found in examining field grown citrus "…that populations of Xac are generally low even from highly infected plots in lesionless leaves and fruits and almost always undetectable in low disease intensity groves." The rapid decline in Xac populations on surfaces coupled with the Xac population size necessary to cause infection creates a limited window of time when surface populations are high enough to potentially infect susceptible host tissue.

Availability of susceptible host. Most of known hosts of Xac are members of the family Rutaceae (which contains citrus species) (CABI/EPPO 1997; CABI 2005), and many of these are found in the United States (USDA-NRCS 2007). Even if viable Xac cells arrive in an area with suitable environmental conditions, to become successfully established in that area the Xac would still need to come in contact with a susceptible host at the proper growth stage for infection to occur. Species of *Citrus* grow naturally in the United States in Florida, Georgia, Hawaii, New Mexico, Texas, Arizona, and California, among other places (FAO 2006b; USDA-NRCS 2007). *Poncirus trifoliata* has a fairly broad, 16 States, distribution in the United States (USDA-NRCS 2007).

Xac can infect any above-ground parts of citrus; *i.e.*, leaves, stems, and fruit (Goto 1972; Graham *et al.* 1992a; Graham *et al.* 1992b; Pruvost *et al.* 2002). However, susceptibility to infection, at least to natural infection through stomata, decreases with tissue maturity (Gottwald and Graham 1992) (see discussion of tissue susceptibility in section 5.1. Production Practices and the Likelihood of Harvesting Xac Infected or Contaminated Fruit).

Leaves can become infected within 14 to 21 days after shoots begin to develop (Stall 1982) with maximum susceptibility when leaves are between 50 and 75 percent expanded (Gottwald and Graham 1992). Fruit are susceptible to infection from petal fall until they are around 6 cm in diameter, and are most susceptible at a fruit diameter of about 2-4 cm (Goto 1972; Koizumi 1972; Graham *et al.* 1992b; Verniere *et al.* 2003). Koizumi (1972) indicates that mature fruit can be infected via wounding, but form different types of lesions than fruit infected at earlier stages. Verniere, *et al.*(2003) state "The age of tissues at the time of infection was a good predictor for disease resulting from the spray inoculation method on fruits and leaves, which represents natural rain splash deposition of inoculum... The age of tissue was also a significant factor for determining disease on fruits following a wound inoculation." In this study, designed to mimic wounds caused naturally by thorns, "The needle-prick method of inoculation increased the susceptibility of the fruit over a longer period. However, it did not overcome a general resistance of fruit that was nearing maturity."

Several ways by which fruit could be brought into close proximity with potential host trees have been suggested in comments on the March 2006 analysis (USDA 2006) made available in the August 1, 2006 interim rule (FR 2006), including the transport of citrus peel by squirrels, the use of citrus fruit as outdoor tree ornaments, and the use of citrus peel as an outdoor cat deterrent. APHIS notes that even if citrus peel is transported by squirrels, used as an outdoor tree ornament or as cat deterrent, the citrus, for reasons discussed elsewhere, is unlikely to contain viable canker bacteria; further, even if it did contain viable bacteria, those bacteria would still need to be transported to and successfully infect susceptible host tree tissues. APHIS believes these would be extremely rare events. We have no data for the frequency of these events, nor does APHIS have any reports of these events resulting in the successful establishment of Xac.

SUMMARY

- As a condition for successful establishment, Xac in amounts sufficient to cause infection, must encounter not only an environment with a temperature, relative humidity, and rain events conducive to infection, it also must encounter host plant tissue that is either at a susceptible growth stage or is wounded and then must successfully enter this tissue.

5.5 Host Resistance- Tangerines

Xac is virulent on all plants in the genus *Citrus* (Koizumi 1981). According to Graham (2001), "The disease affects all major [citrus] varieties, especially grapefruit and early oranges (Hamlin and Navels) that comprise more than 50 percent of the trees in Florida." In the scientific literature, commercially important *Citrus* species are classified into the susceptibility categories. For example, grapefruit (*C. paradisi*), limes (*C. aurantifolia*, *C. limettoides*), and *P.*[*oncirus*] *trifoliata* are highly susceptible; sweet oranges (*C. sinensis*), sour oranges (*C. aurantium*), lemons (*C. limon*, *C. jambhiri*) are moderately susceptible; and thick-skinned East Indian pummelos (*C. grandis*) and mandarins and tangerines (*C. reticulata*) are less susceptible to moderately resistant (Civerolo 1984; Gottwald *et al.* 2002). The available evidence was evaluated regarding the relative resistance of tangerine varieties and its potential as a mitigation measure.

APHIS has employed the reduced susceptibility of Unshu orange (*C. reticulata*) as part of a systems approach to mitigate the risk of introducing Xac on fruit imported from Japan where Xac is considered endemic (Code of Federal Regulations 2006c). That regulation requires, among other things, that only resistant cultivars are planted in and around groves producing fruit destined for export to the United States. In the State of Parana, in Brazil, the use of susceptible varieties is prohibited by the State government and growers are encouraged to plant resistant varieties (Graham 2001).

The taxonomic classification of the genus *Citrus* is complicated and has been the subject of debate (Moore 2001). Tangerines are generally grouped in the species *Citrus reticulata* but many if not most of the tangerine varieties grown in Florida are hybrids of *C. reticulata* with other *Citrus* species (Morton 1987). Table 1 describes the lineage of some Florida citrus varieties.

Table 1. Lineage of selected Florida citrus varieties (Morton 1987)

Variety	Lineage
Dancy tangerine	*Citrus reticulata*
Clementine	*C. reticulata*
Orlando tangelo	Duncan grapefruit X Dancy
Minneola tangelo	Duncan grapefruit X Dancy
Murcott (Honey tangerine)	*C. reticulata* X *C. sinensis*
Temple orange (Tangor)	*C. reticulata* X *C. sinensis*
Fallglo tangerine	(Clementine X Orlando) X Temple
Robinson tangerine	Clementine X Orlando
Osceola tangerine	Clementine X Orlando
Sunburst tangerine	Robinson X Osceola

Crosses between *C. reticulata* and *C. sinensis* (sweet orange) created hybrids like Temple referred to as tangors, possessing characteristics of both tangerines and oranges (Morton 1987). Likewise crosses between *C. reticulata* varieties and grapefruit or pummelo produced the hybrid tangelos (Morton 1987).

The extensive crossing among citrus varieties may explain why varying degrees of susceptibility to citrus canker disease occur within a single variety. Canteros (2004) reports, "…some tangerines and some oranges can be affected to moderate degree, other oranges and tangerines are very resistant." What has not been reported in the literature is the absolute immunity to citrus canker disease of tangerines or any other citrus variety.

APHIS records indicate that during the 2005-2006 growing season grove surveys, Xac was detected on 274 samples from tangerine, tangor and tangelo groves. APHIS pest interception data indicate that between 1985 and 2006, Xac was intercepted 632 times on *C. reticulata* fruit.

SUMMARY

- Taxonomy in the genus *Citrus* is complex; defining "tangerine" would also be complex.
- Tangerines, *sensu lato*, are susceptible to citrus canker; this susceptibility ranges from highly susceptible to highly resistant, but none are completely immune.

5.6 International and Interstate Movement of Citrus Fruit and Its Relation to the Introduction of Xac

There are no accounts in the published literature indicating that fresh citrus fruit or seeds can serve as pathways for the dissemination of Xac. Long-distance dissemination of the pathogen occurs primarily through the movement of propagative material, such as budwood and rootstock seedlings or budded trees from nurseries (CABI/EPPO 1997).

5.6.1 The Origins of Citrus Canker Disease Outbreaks

Xac is widely accepted as having been introduced into the United States on trifoliate orange and Satsuma orange trees from Japan (Wolf 1916; Dopson 1964; Civerolo 1984). In his 1916 treatise (Wolf 1916), F.A. Wolf writes, "Citrus canker is not of American origin, but beyond doubt was introduced into the Gulf States from Japan…it appeared in the United States several years ago simultaneously with the importation of Satsuma and trifoliate stock into Texas in order to supply the large demand for trees for Citrus plantings…Since its introduction into Texas it has been disseminated by the shipment of diseased trees to other States and has further been introduced by shipments to these States direct from the orient…"

Citrus canker disease was again detected in Florida in the mid-1980s (Schoulties and Miller 1985). The source for this outbreak is not known and although most scientists believe Xac was reintroduced "…a few speculate that this outbreak might have resulted from perennial holdover from 1910…" (Schubert *et al.* 2001). In the mid 1990s, new outbreaks were detected in the same area of the west Florida coast (Manatee County) where the 1980s outbreak occurred and in Dade County (Schubert *et al.* 1996; Gottwald *et al.* 2001). In reporting the Dade County outbreak, Schubert *et al.* (1996) stated, "No information is available about the origin of the inoculum responsible for the current outbreak of citrus canker." In their review of the outbreaks, Gottwald *et al.* (2001) report that, "Genomic analysis of bacterial isolates from both time periods indicates that the latest Manatee County outbreak is a hold over from the 1980s outbreak that escaped the eradication program."

In a literature review of citrus canker outbreaks in Australia, Broadbent *et al.* (1992) speculated that a 1912 outbreak in northern Australia had originated from Japan or China due to the fact that citrus trees and fruit were being imported from these sources. They did not clearly state if they considered trees or fruit the more likely source of the inoculum. With regards to an outbreak in 1991, the same authors stated, "The origin of the outbreak is unknown…Because few pummelo cultivars have been legally imported into Australia, and given that the pummelo is indigenous to the Malayan and East Indian archipelagos, where canker is endemic, it is possible that an illegal introduction may have resulted in the current outbreak." The origins of a 2004 outbreak of citrus canker disease occurred in Queensland, Australia are also unknown; however, Australian authorities investigated reports of illegally imported trees on the property where the outbreak was first detected (DAFF 2004).

In her review of citrus canker disease in Latin America, Rossetti (1977) states, based on information in Bitancourt (1957), "Citrus canker was introduced in the state of São Paulo, probably in 1953 or 1954 on smuggled, infected budwood brought from Japan in violation of Brazilian legislation, either by boat through the port of Santos or by air through the São Paulo airport." Citing Sánchez (1968), she furthermore suggests that the introduction of Xac into Paraguay may have resulted from the introduction of infected trees, either from Japan or from Brazil.

Citrus canker disease was first reported from Yemen in 1984 on trees that had been imported as part of a consignment of trees from India where Xac is endemic (Dimitman and Gassert 1984).

Other citrus canker outbreaks in Argentina (from 1972 to 1975) (Civerolo 1984); Uruguay (1979) (Rossetti 1977); Australia (in 1981 and again in 1984) (Shivas 1987; Catley 1988); United Arab Emirates (1984 to 1985) (El-Goorani 1989); and Bolivia (in 2002) (Braithwaite *et al.* 2002) are of unknown origin.

In summary, there is an unfortunate lack of conclusive information regarding the origins of previous outbreaks. Most published accounts are little more than speculation. However, whatever the lack of certainty may be regarding the theories of Xac introduction pathways, they all agree that trees or propagative tree parts are most likely the original source of Xac introduction. Conclusive evidence that fresh fruit is a pathway for the introduction of Xac has never been presented.

5.6.2 International and Interstate Movement of Citrus Fruit

That there is no authenticated record of fresh fruit as a pathway for Xac is especially significant in light of the fact that citrus fruit ranks very high in international fruit trade, with production and trade having been increasing steadily over the last decades (UNCTAD 2006) and much of the traded fruit originating in countries where citrus canker is present.

For example, substantial amounts of citrus fruit are exported from South American countries with citrus canker, such as Argentina, Uruguay, Bolivia, *etc*. to the European

Union (EU) where Xac is a quarantine pathogen. In 2004 the EU imported 18 percent of its citrus from Argentina (FAS 2006); and between 2003-2005, Spain, Europe's dominant citrus producer, imported 642,769 tons of citrus (an equivalent of approximately 3.8 billion pieces of fruit) from Argentina (GTIS 2005). During that same time, Spain imported 86,124 tons of citrus fruit (548 million pieces of fruit) from Uruguay (GTIS 2005). Despite these large volumes of citrus fruit imported into Spain from citrus canker affected countries, there have been no reported outbreaks of Xac in Spain.

It could be argued the lack of outbreaks is the result of EU regulations, that require imported citrus fruit originate in an area or grove officially recognized as Xac-free (EU 2000). However, it should be noted that Xac-infested fruit have been intercepted by Spain in spite of these regulations. In 2003, "Spain informed the other Member States and the Commission that in plant health checks carried out in 2003, numerous infestations of citrus fruits originating in Argentina or Brazil with …*Xanthomonas campestris* [Xac] …" (EU 2004). And, in 2005, Spain reported 17 interceptions of Xac on commercial shipments of citrus fruit from Uruguay (EPPO 2005).

While Peltier and Frederich (1926) suggest that the Mediterranean climate, such as that of Spain, may simply be unfavorable for the development of citrus canker disease, the same authors also concede that the disease "could develop in all of the citrus regions of the world *sometime* over the growing season". EPPO (CABI/EPPO 1997) uses the same rationale in designating Xac a quarantine pest for the region.

Trade of fresh citrus fruit does occur between countries where Xac is present and countries that do not have Xac but do have climates conducive to its establishment (CABI 2006; FAO 2006b). For example, in 2004, India (where Xac is reported) shipped 8 metric tons of citrus to Ghana and 2 metric tons to South Africa (where Xac is not reported) (FAO 2006b). Similarly, China (where Xac occurs) exported 66 metric tons of citrus to Angola (where Xac does not occur) (FAO 2006b). No outbreaks of Xac have been reported in any of the recipient countries.

In the United States, fresh citrus fruit from Florida was shipped during years of Xac outbreaks (1995, and from 1997 to the present) to other citrus producing States (California, Texas, and Arizona based on USDA-National Agricultural Statistics Service data for 1997 to 2002). An average of just over 1 million 4/5-bushel cartons of citrus (including grapefruit, temple oranges, tangerines, honey tangerines, etc.) were shipped to California and an average of 63,000 4/5-bushel cartons were shipped to Texas (predominantly honey tangerines) each year from 1996 through 2005 (Florida Department of Citrus 1997, 1998, 1999, 2000, 2001, 2002, 2003b, 2004a, 2005a). No outbreaks of citrus canker disease resulted from these shipments. It must be noted, though, that shipments may have originated in areas of low prevalence or free of Xac.

This evidence is not sufficient to prove that fresh fruit cannot possibly serve as a pathway for the introduction of Xac. Nevertheless, no canker outbreaks have ever been associated with the entry of fruit into the United States or anywhere in the world, nor has the ability of fruit to serve as a pathway of Xac dissemination ever been demonstrated in any scientific experiment and it seems very unlikely that fruit would be an epidemiologically significant pathway.

SUMMARY

- There are few instances where the origins of citrus canker disease outbreaks have been conclusively demonstrated and reported.
- Where origins have been reported or suggested, imported or smuggled trees and budwood are reported as the source of infection.
- Despite substantial international trade between Xac infected and noninfected countries, there is no authenticated record of movement of diseased fruit as the origin for a citrus canker disease outbreak.
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6 Conclusions and Summary of Evidence Regarding Fruit as a Pathway for Xac Introduction

APHIS has regulated the importation and interstate movement of citrus fruit for many years to prevent the introduction and/or spread of the bacterial pathogen Xac. APHIS regulations have, with few exceptions, restricted the movement of fruit from production areas within the United States affected by citrus canker disease and the importation of fruit from foreign countries and regions reported or suspected of having citrus canker disease. Implicit in all these regulations has been the assumption that fruit represents a potentially important pathway for the long-distance dissemination of Xac. Multiple lines of evidence now suggest that conclusions about the importance of citrus fruit as a pathway for the introduction of Xac may not be valid.

Commercial citrus fruit production. Citrus fruit is produced and harvested using techniques that reduce the prevalence of Xac-infected fruit. These techniques include the use of prophylactic copper sprays in citrus groves, use of windbreaks to suppress bacterial spread, grove inspections and surveys and decontamination of harvesting equipment. Similar principles and practices apply to noncommercial production as well (Chamberlain *et al.* 2001).

These procedures are not all required by either statute or regulation nor are they are all utilized by every commercial citrus producer. Further, none of these procedures ensure that any individual piece of fruit is not infected or contaminated with canker. However, APHIS concludes that collectively, these procedures reduce the prevalence of Xac in commercially packed fruit, even when that fruit originates from regions with citrus canker disease.

Packing and shipping of commercial citrus fruit. APHIS found that commercial citrus fruit is packed using techniques that reduce the prevalence of infected or contaminated fruit. These packinghouse techniques include the decontamination of packing equipment, washing and disinfection of harvested citrus fruit, and elimination of blemished fruit. These are all normal procedures for most commercial producers and packers.

Under the current regulations (Code of Federal Regulations 2006b), APHIS inspects up to 2 percent of each inspected lot, but not all lots are inspected. APHIS inspectors have averaged above 90 percent in proficiency examinations.

The cool temperatures at which citrus fruit are shipped limits the ability of Xac to reproduce during shipping and any epiphytic Xac populations do not survive long, thus reducing the likelihood that commercially packed fresh citrus fruit is a good pathway to introduce Xac.

These packing and shipping procedures and conditions are not all required by either statute or regulation nor are they are all utilized by every commercial citrus packer or shipper. Further, none of these procedures ensures that any individual piece of fruit is not infected or contaminated with Xac. However, APHIS concludes that collectively, these procedures do reduce the prevalence and inoculum level of Xac in commercially packed fruit, even when that fruit originates from regions with citrus canker disease. APHIS also concludes that a phytosanitary inspection at the packinghouse is an effective measure to detect fruit with Xac symptoms and reduce the likelihood that fruit with symptoms are shipped.

Epidemiological and environmental factors affecting establishment potential of Xac. The environmental and exposure conditions associated with the naturally occurring spread of canker within known infected regions were reviewed. As a condition for successful establishment, Xac, in sufficient amounts to cause infection, must encounter not only an environment with temperatures, relative humidity, and rain events conducive to infection. Xac also must encounter plant tissue of a host that is either at a susceptible growing stage or is wounded and then the bacteria, in sufficient numbers to incite infection must successfully enter this tissue. The review found that that tree-to-tree transmission generally requires wind-driven rain. APHIS concludes that even if Xac infected fruit were shipped out of an area with citrus canker disease and by chance were moved to a location close to susceptible host trees, infection of the host trees is unlikely. APHIS, however, does not have sufficient evidence to conclude that such infection is impossible.

Origins of citrus canker disease outbreaks. While many outbreaks of citrus canker disease have been of unknown or unreported origin, the source of others has been reported with varying degrees of confidence. In every citrus canker disease outbreak in which the source has been determined or suggested, that source has been propagative material such as nursery stock or budwood. There are no authenticated reports in the scientific literature of citrus canker disease outbreaks attributed to commercial fresh fruit movement.

APHIS concludes that the absence of reports of citrus canker disease outbreaks linked to commercial fresh fruit combined with the multiple reports of outbreaks due to propagative material is important evidence. This evidence is not sufficient to prove that fruit cannot possibly serve as a pathway for the introduction of Xac. The evidence is sufficient to conclude that if such a pathway exists at all, it is rarely successful in natural environments compared to other pathways of Xac introduction.

International and interstate movement of citrus fruit. Large quantities of commercial citrus (i.e., billions of pieces of fruit) have moved in trade from countries and regions with citrus canker disease to regions without citrus canker disease. While the precise citrus canker disease status of the exporting region is difficult to determine, the presence of at least some infected fruit in this trade is certain. European phytosanitary inspection of imported citrus fruit has detected symptomatic fruit in commercial shipments from South America multiple times. Nonetheless, APHIS is not aware of any reports of citrus canker disease outbreaks in the importing countries.

APHIS concludes that the absence of citrus canker disease outbreaks in countries importing fruit from countries and regions with citrus canker disease, while not sufficient to prove that fruit cannot possibly transmit canker, is nonetheless important evidence to support the hypothesis that fruit is not an epidemiologically significant pathway for introducing Xac.

Host resistance as a regulatory measure: tangerines. Planting of disease resistant varieties is an accepted production measure to reduce disease incidence (Agrios 1997). Tangerines are widely reported to have some level of resistance to citrus canker disease. APHIS assessed the potential for employing tangerine's putative resistance to citrus canker disease. APHIS evaluated evidence that tangerines are less susceptible to citrus canker. Published literature indicates that "tangerines" are regarded as ranging from moderately resistant to moderately susceptible to citrus canker disease. Clearly, though, tangerines are not immune to citrus canker as APHIS records indicate that during the 2005-2006 growing season grove surveys, Xac was detected on 274 samples from tangerine, tangor and tangelo groves. APHIS pest interception data indicate that between 1985 and 2006, Xac was intercepted 632 times on *C. reticulata* fruit.

The level of resistance was expressed as a continuum across tangerine varieties rather than as a discrete immunity for all varieties. This creates a regulatory problem when an overlap occurs in the level of resistance expressed by, for example, a more susceptible tangerine variety and a more resistant non-tangerine citrus variety.

Based on this evidence, APHIS concludes that tangerines may be less susceptible to canker than other species and varieties of citrus. However, APHIS was not able to conclude that tangerine groves are never infected with canker or that sufficient evidence exists to exclude all tangerines from regulations applicable to other Florida citrus varieties.

In summary, fruit produced and packed utilizing the various measures described in this document to reduce the prevalence of viable Xac is unlikely to serve as a pathway for the introduction and spread of the bacterium. This similar to the conclusion reached in the previous analysis for asymptomatic fruit (USDA 2006) except that the present document acknowledges that it is not possible to design a viable system that ensures only uninfected fruit moves from quarantined areas. Furthermore, resource constraints and other practical considerations make it difficult to implement a grove focused regulatory systems approach in Florida that ensures full compliance with the conclusions of the evaluation described above.

Finally, the evidence is not currently sufficient to conclude that fresh citrus fruit absolutely cannot serve as a pathway for the introduction of Xac into new areas. In a similar situation regarding the importation of Mexican citrus fruit (FR 1983), USDA determined that "It is unlikely that new citrus canker infections would be established in the United States because of the importation of fruit or peel of citrus or citrus relatives carrying bacteria trapped in the pores or wounds. In order for the bacteria to cause an infection an unlikely sequence of events would have to occur." But the rule went on to state, "Even though it was determined that the risk was small, it was determined that action should be taken because of the possibility of live citrus canker bacteria being present in the pores or wounds of restricted articles."

In the present case, even though it was determined that commercially packed citrus fruit is an unlikely pathway for the introduction and spread of Xac, however, the evidence is not currently sufficient to prove that such fruit cannot possibly serve as a pathway for the introduction of Xac. That has led us to develop and evaluate several risk management options for the interstate movement of fresh commercially packed citrus fruit from regions with citrus canker disease to regions without the disease. Because resource constraints and other practical considerations make it difficult to implement a regulatory systems approach in Florida, APHIS shifted its emphasis from the grove to the packinghouse as a critical control point in developing the risk management options for mitigating the risk of Xac introduction and spread via commercially packed citrus fruit. A packinghouse-based inspection could ensure the same level of phytosanitary security as a traditional systems approach, but would be easier to implement and enforce than grove measures, and because it focuses on the end product, would be more reliable and less easily circumvented.

7 Risk Management Options

APHIS published an interim rule on August 1, 2006 (Code of Federal Regulations 2006b) listing the entire State of Florida as a quarantined area for citrus canker and amending the requirements for the movement of regulated articles from Florida. For citrus fruit, that rule requires that every tree in a given orchard is inspected not more than 30 days before harvest and found free of canker, that regulated fruit is accompanied by a limited permit, and that regulated fruit may not be distributed to Arizona, California, Hawaii, Louisiana, Texas, American Samoa, Guam, the Northern Mariana Islands, Puerto Rico, and the U.S. Virgin Islands. Under the current regulation (Code of Federal Regulations 2006b), APHIS conducts monitoring phytosanitary inspections for Xac as part of the process for issuing requisite limited permits for interstate movement of citrus fruit. Up to 2 percent of the fruit in each inspected lot is examined by APHIS inspectors. Not every lot is inspected under the current system. This phytosanitary inspection generally takes place after all packinghouse treatments, grading and inspections are completed (finished fruit).

It is not possible to design a viable system that ensures only uninfected fruit moves from quarantined areas. Furthermore, the evidence is not currently sufficient to conclude that fresh citrus fruit produced in a Xac infested grove absolutely cannot serve as a pathway for the introduction of Xac into new areas.

After considering the evidence for commercially packed citrus fruit as a pathway for the introduction of Xac, and the available mitigation measures, APHIS evaluated five risk management options for the interstate movement of fresh citrus fruit from Florida. Those options and details of the phytosanitary inspection are outlined in this section.

7.1 Option 1– Unrestricted movement

- Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States[7] including commercial citrus-producing States[8].

The evidence discussed in preceding sections of this document suggests that fresh citrus fruit may not be an epidemiologically significant pathway for introducing Xac into previously free areas. If, in fact, fruit is not an epidemiologically significant pathway, the rationale for regulating fruit movement disappears. Accordingly, Option 1 would remove all APHIS restrictions on the movement of commercially packed fruit from regions quarantined for citrus canker disease.

In support of the hypothesis that commercially packed fruit is not an epidemiologically significant pathway for introducing citrus canker disease, evidence was considered regarding fruit production and harvest; commercial citrus fruit packing; epidemiological and environmental factors; the origins of citrus canker disease outbreaks; and international and interstate movement of citrus fruit. This evidence suggests that fruit is unlikely to be an epidemiologically significant pathway; no canker outbreaks have ever been associated with the movement of commercial fresh fruit into the United States or anywhere in the world, nor has the ability of commercial fresh fruit to serve as a pathway of Xac dissemination ever been demonstrated in any scientific experiment.

However, the evidence is not currently sufficient to prove that such fruit cannot possibly serve as a pathway for the introduction of Xac. This uncertainty weighs against an option

[7] For clarity, the term “State” is defined here as any of the 50 U.S. States or U.S. Commonwealths, Trusts and Territories

[8] American Samoa; Arizona; California; Florida; Guam; Hawaii; Louisiana; Northern Mariana Islands; Puerto Rico; Texas; and the Virgin Islands of the United States

that allows unrestricted distribution of fruit from areas with citrus canker disease. Consequently, this option was rejected. It is described here only for the sake of completeness in illustrating the spectrum of regulatory options considered.

7.2 Option 2– Unlimited distribution, disinfectant, phytosanitary inspection

- Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States including commercial citrus-producing States.
- Require packinghouse treatment of citrus fruit with APHIS approved disinfectant treatment and APHIS inspection of finished fruit (all types and varieties) for citrus canker disease symptoms.

Option 2 would amend CFR 301.75 to allow the movement of commercially packed fresh citrus fruit to all U.S. States with APHIS approved disinfectant treatment and a mandatory packinghouse phytosanitary inspection.

Substantial evidence exists that commercially packed citrus fruit is not an epidemiologically significant pathway for introducing Xac to previously free regions. Pathways, by which citrus fruit could introduce Xac, though unlikely, are possible. The probability of such introductions is “unknown,” in the sense that a specific numerical value or even a range of values cannot be calculated. Recognizing these uncertainties, Option 2 proposes to mitigate the risk of Xac introduction with a mandatory packinghouse disinfectant treatment of fruit and a mandatory phytosanitary inspection of finished citrus fruit. The purpose of the inspection will be to ensure, within limits of statistical certainty, that fruit with injuries or lesions indicative of citrus canker disease is not moved out of the quarantine zone (*i.e.,* Florida).

In this approach, the citrus growers, harvesters, and packers will be given the flexibility to implement phytosanitary measures that prevent and control the presence of Xac infection in the fruit they produce. APHIS will then inspect randomly selected finished fruit from every lot. Detection of one or more Xac-infected fruit will result in the rejection of that lot. Statistically, an inspection level will be established by the Deputy Administrator that will ensure, with a high level of confidence, that the proportion of undetected symptomatic fruit in a cleared lot is low (see discussion below).

The objective in designing the proposed risk management options was to ultimately ensure that visibly infected fruit is not shipped and does not reach citrus producing States. To that end we set out to design an inspection protocol that would achieve the maximum level of sensitivity (the protocol that would allow the fewest fruit with visible symptoms to escape detection by the APHIS packinghouse phytosanitary inspection) given the constraints of operational feasibility. To assist in evaluating Option 2 and the subsequent options 3 and 4 that all recommend a mandatory phytosanitary inspection by APHIS, we prepared a quantitative model (Appendix 1) based on Florida production and shipping data to evaluate the efficacy of three levels of phytosanitary inspection in ensuring that symptomatic fruit does not enter U.S. commercial citrus-producing States. The model answers the following questions:

1. If commercially packed and APHIS inspected fresh citrus fruit is shipped interstate from Florida, what proportion of that fruit is Xac-symptomatic?
2. If commercially packed and APHIS inspected fresh citrus fruit is shipped interstate from Florida, how many Xac-symptomatic fruit from Florida reach five citrus-growing U.S. States (AZ, CA, HI, LA, TX) per shipping-season?
3. If commercially packed and APHIS inspected fresh citrus fruit is shipped interstate from Florida, how many Xac-symptomatic fruit from Florida reach citrus-growing areas within those citrus-growing U.S. States per shipping-season?

The model was developed for three inspection levels determined by preliminary estimates of PPQ, Citrus Health Response Program staff of inspection levels that might be operationally feasible. The three inspection levels evaluated were 500 fruit per lot; 1,000 fruit per lot; and 2,000 fruit per lot. Statistically, inspection of 500 fruit per lot will ensure, with 95 percent confidence, that the proportion of undetected symptomatic fruit in a cleared lot is no more than 0.75 percent. Inspection of 1,000 fruit per lot will ensure, with 95 percent confidence, that the proportion of undetected symptomatic fruit in a cleared lot is no more than 0.37 percent. Inspection of 2,000 fruit per lot will ensure, with 95 percent confidence, that the proportion of undetected symptomatic fruit in a cleared lot is no more than 0.19 percent.

The outputs of the quantitative model were probability distributions. The model determined, with 95 percent confidence, that the total number of citrus fruit shipped from Florida to five citrus-producing States (AZ, CA, HI, LA and TX) over a single shipping season would be 181,283,744 or less if unlimited distribution is permitted. The model determined, with 95 percent confidence that the number of Xac-symptomatic fruit reaching those five States in a single shipping season would be: 633,152 or less at the 1,000 fruit inspection levels; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level. The model further determined with 95 percent confidence that the number of symptomatic fruit reaching citrus-producing areas within those States in a single shipping season would be: 2,135 or less at the 1,000 fruit inspectional level; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level. According to the sampling algorithm for the probability distribution used in the model, the sampling rates, 500, 1,000, 2,000 fruit per lot, are sufficient to detect symptomatic fruit at levels of *at least* 0.75, 0.38 and 0.19 percent, respectively, regardless of lot size. Indeed for lot sizes less than about 100,000 fruit, the lots are over sampled to achieve those detection rates or, put differently, the detection rates are actually slightly better than the minimum rates listed above. For additional details of the quantitative model see Appendix 1.

PPQ Staff from the Melbourne, Florida office of the Citrus Health Response program conducted a small test of the 2,000 fruit sampling protocol to evaluate its operational feasibility. The study found that the normal complement of two inspectors at the packinghouse chosen for the evaluation were physically unable to achieve the 2,000 fruit per lot inspection level. It was estimated that the number of inspectors would have to have been doubled to four in order to inspect 2,000 fruit per lot, but the packinghouse physically had room for only two inspectors. Based on this test and additional input from PPQ operational staff, it was determined that the higher inspection level that achieves 95 percent confidence of detecting at least 0.19 percent rate of symptomatic fruit (about 2,000 fruit per lot), is only feasible with increased inspectional resources and/or more substantial modifications to the packing/phytosanitary inspection processes, and could be justifiable only if the risk reduction benefits outweighed the cost. An inspection level of 1000 fruit per lot that achieves a detection rate of 0.38 percent with 95 percent confidence was adopted because it provides the maximum level of detection that is operationally feasible with the phytosanitary inspection resources in Florida. Inspection of 500 fruit per lot was rejected because it did not meet the criteria of achieving the maximum level of detection that was operationally feasible.

It is important to recognize that the quantitative analysis described in Appendix 1 estimates that symptomatic fruit may be shipped to citrus-producing States. These values reflect *only* the likelihood that symptomatic fruit reach citrus-producing States and citrus growing areas within those States. For an outbreak to occur, the fruit must be discarded in such a way that Xac, in sufficient amounts to cause infection, survive, encounter not only an environment with a temperature, relative humidity, and rain events conducive to infection, but also must encounter plant tissue of a host that is either at a susceptible growing stage or is wounded and then viable bacteria, in sufficient numbers to incite infection need to successfully enter this tissue.

Despite the determination that commercially packed fresh citrus fruit is an unlikely pathway for the introduction and spread of Xac, and a phytosanitary inspection that ensures, with high confidence, that a low level of shipped fruit has symptoms of citrus canker disease, the model indicates the potential for symptomatic fruit to be shipped to citrus producing States. That potential for symptomatic fruit to reach citrus producing States coupled with the aforementioned uncertainty regarding fruit as a pathway led to the determination that additional mitigations were required.

7.3 Option 3– Limited distribution (except tangerines) to non-citrus producing States, disinfectant, phytosanitary inspection

- Prohibit distribution of all types and varieties of *commercially packed* citrus fruit (except tangerines) to U.S. commercial citrus-producing States. Allow distribution of *commercially packed* tangerines to all U.S. States including commercial citrus-producing States.
- Allow distribution of all types and varieties of *commercially packed* citrus fruits to all U.S. non-citrus producing States.
- Require packinghouse treatment of citrus fruit with APHIS approved disinfectant and APHIS inspection of finished fruit (all types and varieties) for citrus canker disease symptoms.

Option 3 retains the requirements in Option 2, including disinfection of all fruit prior to packing, and mandatory phytosanitary inspection by APHIS sufficient to ensure, with a high level of confidence, that the proportion of packed fruit with visible lesions in each processed lot is low. The quantitative analysis described in Appendix 1 estimates that even with a mandatory phytosanitary inspection in place, symptomatic fruit may be shipped to citrus-producing States and evidence is not currently sufficient to prove that such fruit cannot possibly serve as a pathway for the introduction of Xac. For these reasons, Option 3 also proposes, with one exception described below, to further mitigate the risk of Xac introduction by prohibiting the distribution of fruit from regions with citrus canker disease to those U.S. citrus-producing States.

Option 3, however, would allow the shipment of tangerines to all U.S. States. This exception was evaluated in response to an industry proposal that tangerines have considerably less susceptibility to Xac and therefore are less likely to introduce Xac to previously free regions.

Tangerines are grouped in the species *Citrus reticulata* which is widely regarded as less susceptible to citrus canker disease than other commercially grown *Citrus* species. But many of the "tangerine" varieties grown in Florida are hybrids of *C. reticulata* with other more susceptible *Citrus* species. Tangerines are not immune to citrus canker as APHIS records indicate that during the 2005-2006 growing season grove surveys Xac was detected on 274 samples from tangerine, tangor and tangelo groves. APHIS pest interception data indicate that between 1985 and 2006, Xac was intercepted 632 times on *C. reticulata* fruit.

The level of susceptibility is expressed as a continuum across "tangerine" varieties rather than as a discrete immunity for all varieties. This creates a regulatory problem when an overlap occurs in the level of susceptibility expressed by, for example, a more susceptible tangerine variety and a more resistant non-tangerine citrus variety. While the relative resistance of certain tangerine varieties has been successfully employed as a component of a multicomponent systems approach to mitigate the risk of citrus canker disease, sufficient evidence does not exist to exclude tangerines from regulations applied to other Florida citrus varieties. Mitigating the risk of Xac introduction and spread via interstate movement of commercially packed Florida fresh citrus fruit based on variety clearly was untenable, so APHIS evaluated limited geographic distribution as a mitigation in Option 4.

7.4 Option 4– Limited distribution (all varieties) to non-citrus producing States, disinfectant, phytosanitary inspection

- Prohibit distribution of all types and varieties of citrus fruit (including tangerines) to U.S. commercial citrus producing States.

- Allow distribution of all types and varieties of *commercially packed* citrus fruits to all U.S. non-citrus producing States.
- Require packinghouse treatment of citrus fruit with APHIS approved disinfectant and APHIS inspection of finished fruit (all types and varieties) for citrus canker disease symptoms.

A packinghouse-based inspection could ensure the same level of phytosanitary security as a traditional systems approach, and would be easier and potentially less costly to implement and enforce, and because it focuses on the end product, would be more reliable and less easily circumvented. In addition, a phytosanitary packinghouse inspection creates a performance standard for packed fruit that allows citrus producers greater flexibility to determine the most efficient and effective means of producing a compliant product. Nonetheless, the quantitative analysis described in Appendix 1 estimates that symptomatic fruit may be shipped to citrus producing States (see also Section 7.3 Option 2).

That potential for symptomatic fruit to reach citrus producing States coupled with the aforementioned uncertainty regarding fruit as a pathway led to the determination that additional mitigations were required. Therefore, Option 4 includes all the requirements of Option 3 and further mitigates the risk of Xac introduction by prohibiting the distribution of all types and varieties of citrus fruit, including tangerines, from areas with citrus canker disease to U.S. commercial citrus producing States. Option 4 would amend CFR 301.75 (a)(2), substituting the packing house inspection described in Appendix 1 for the preharvest grove inspections currently in the regulation.

Option 4 prohibits distribution of all types and varieties of citrus fruit, including tangerines, to citrus-producing States. Fruit can, however, be illegally moved, intentionally or unintentionally, to prohibited States, even though fruit boxes are labeled to prevent such movement. USDA-APHIS-PPQ-Smuggling Interdiction and Trade Compliance staff report six known interceptions of Florida citrus fruit since 2006 in citrus-producing States out of an estimated 12,400 shipments.

APHIS staff cannot estimate the frequency of unreported illegal movement of Florida citrus to citrus-producing States or the proportion of reported illegal movement to total illegal movement. Since Option 4 would maintain the current prohibition on movement of citrus fruit to citrus-producing States, the rate of intentional or unintentional movement of Florida citrus fruit to prohibited States is not expected to change under this option.

Option 4 compensates for uncertainty in the rate of illegal fruit movement by requiring a disinfectant treatment and phytosanitary inspection in addition to the distribution restriction. These measures ensure that even if a shipment moves illegally to a prohibited State, it has a low likelihood of containing symptomatic fruit.

Under § 412(a) of the Plant Protection Act (PPA 2000), the Secretary of Agriculture may prohibit or restrict the movement in interstate commerce of any plant or plant product if the Secretary determines that the prohibition or restriction is necessary to prevent the dissemination of a plant pest within the United States. Based on the best available evidence, we have determined that it is not necessary to prohibit the interstate movement of citrus fruit into non-citrus-producing States under the conditions described in this proposed rule. While APHIS has concluded that commercially packed citrus fruit is an unlikely pathway for the introduction and spread of citrus canker, the remaining uncertainty about the precise level of risk associated with the movement of citrus fruit from a quarantined area has led us to maintain the current prohibition on the movement of that citrus fruit into citrus-producing States.

7.5 Option 5– No change

- Leave August 1, 2006 interim rule in place and unchanged.

Option 5 is the most restrictive option. It leaves the current regulations in place and unchanged including the requirement for preharvest grove surveys. APHIS has concluded that a mandatory packinghouse treatment of citrus fruit with APHIS approved disinfectant and phytosanitary inspection, by APHIS, of finished fruit provides an effective safeguard to prevent the spread of Xac via the movement of commercial citrus fruit especially when combined with a limited distribution requirement that excludes shipment to U.S. citrus-producing States.

A packinghouse-based inspection could ensure the same level of phytosanitary security as the current grove certification approach, and would be easier and potentially less costly to implement and enforce, and because it focuses on the end product, would be more reliable and less easily circumvented. In addition, a phytosanitary packinghouse inspection creates a performance standard for packed fruit that allows citrus producers greater flexibility to determine the most efficient and effective means of producing a compliant product. APHIS has determined that the less restrictive measures of Option 4 satisfy the requirements of the Plant Protection Act as outlined in the preceding section.

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9 Appendix 1. Probabilistic analysis of the efficacy of the proposed phytosanitary inspection

This appendix presents the methodology and results of the quantitative analysis.

9.1 Summary

The risk management options for the interstate movement of fresh commercially packed citrus fruit from regions with citrus canker disease to regions without the disease presented in section 7 are summarized below:

Option 1	Allow unrestricted distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States[9].
Option 2	Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States, subject to packinghouse treatment with APHIS-approved disinfectant and APHIS inspection of finished[10] fruit (all types and varieties).
Option 3	Allow distribution of all types and varieties of *commercially packed* citrus fruit (except tangerines) in U.S. States except U.S. commercial citrus producing States[11]. Allow distribution of *commercially packed* tangerines to all U.S. States including commercial citrus-producing States. Require packinghouse treatment of all such citrus fruit with APHIS-approved disinfectant and APHIS inspection of finished fruit (all types and varieties) for citrus canker disease symptoms.
Option 4	Allow distribution of all types and varieties of *commercially packed* citrus fruit in U.S. States except U.S. commercial citrus-producing States and require packinghouse treatment of citrus fruit with APHIS-approved disinfectant and APHIS inspection of finished fruit (all types and varieties).
Option 5	Leave the current regulations for the interstate movement of citrus fruit from citrus canker quarantined areas in place and unchanged.

To assist in evaluating Option 2, APHIS constructed a probabilistic model to evaluate the movement of commercially packed fresh citrus fruit to all U.S. States with APHIS approved disinfectant treatment and a mandatory packinghouse phytosanitary inspection. The model determines the potential quantity of symptomatic[12] *Xanthomonas axonopodis* pv. *citri* (Xac)-infected fruit shipped from Florida to citrus growing areas in the

[9] For clarity, the term "State" is defined here as any of the 50 U.S. States or U.S. Commonwealths, Trusts and Territories

[10] Fruit that has completed the packinghouse washing, disinfection, grading and inspection processes.

[11] American Samoa; Arizona; California; Florida; Guam; Hawaii; Louisiana; Northern Mariana Islands; Puerto Rico; Texas; and the Virgin Islands of the United States

[12] Symptomatic in the context of this probabilistic assessment means that the fruit have visible Xac-lesions 1 mm in diameter and greater.

commercial citrus-producing states of Arizona, California, Hawaii, Louisiana, and Texas during the course of a shipping season for three different scenarios of lot[13] inspection,

Model input parameters, were: a) the number of 4/5-bushel cartons of commercially packed and APHIS-inspected Florida citrus shipped to citrus-producing states per shipping season; b) the number of fruit per 4/5-bushel container; c) the proportion of symptomatic Xac-infected citrus in the shipments; and d) the proportion of the shipments reaching citrus-growing areas in the citrus producing states.

APHIS estimates the true prevalence of symptomatic fruit based on apparent prevalence, adjusted to account for inspection sensitivity. The beta distribution is used to estimate the apparent prevalence, The quantity of fruit shipped from Florida, and the true prevalence of undetected Xac symptomatic fruit, were used to determine the number of Xac-symptomatic fruit that get to citrus producing states.

The model determined, with 95 percent confidence, that the number of Xac-symptomatic fruit reaching those five States in a single shipping season would be: 633,152 or less at the 1,000 fruit inspection levels; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level. The model further determined with 95 percent confidence that the number of symptomatic fruit reaching citrus-producing areas within those States in a single shipping season would be: 2,135 or less at the 1,000 fruit inspectional level; about half that number at the 2,000 fruit inspectional level; and about double that number at the 500 fruit inspectional level.

Table 1 Number of Xac-Symptomatic fruit reaching citrus producing states, and citrus growing areas in those states (95% confidence level results), for three scenarios under risk management option 2.

	Scenario 1	Scenario 2	Scenario 3
	500 fruit sampled per lot	1000 fruit sampled per lot	2000 fruit sampled per lot
Number of Xac-symptomatic fruit reaching citrus producing states	1271193	633152	316891
Number of Xac-symptomatic fruit reaching citrus growing areas in citrus producing states	4291	2135	1071

[13] A lot is described as the inspectional unit for fruit; composed of a single variety of fruit that has passed through the entire packing process in a single continuous run, during the course of one day; regulatory actions (e.g., issuance of limited permits, rejection) are taken at the lot level.

9.2 Purpose and Scope

This Appendix evaluates the effectiveness of the proposed packinghouse phytosanitary inspection; as part of the proposed options for regulating the interstate movement of commercially packed citrus fruit from Florida.

The phytosanitary hazard[14] is the entry of Xac into citrus-growing U.S. States where it is not known to occur after having been moved there on symptomatic Xac-infected Florida citrus that had been commercially packed and undergone a pre-shipment inspection by APHIS.

A model was developed to determine the number of symptomatic Xac-infected citrus (Xac-symptomatic) fruit arriving in citrus-producing states, and citrus growing areas within these states, per shipping-season[15], for three inspection scenarios: inspection of 500 fruit per lot; inspection of 1000 fruit per lot; and the inspection of 2000 fruit per lot.

The model answers the following questions:

1. What proportion of the commercially packed and APHIS inspected citrus fruit shipped interstate from Florida is Xac-symptomatic?
2. How many Xac-symptomatic fruit from Florida reach citrus-growing U.S. States per shipping-season?
3. How many Xac-symptomatic fruit from Florida reach citrus-growing areas in citrus-growing U.S. States per shipping-season?

This appendix does not quantitatively assess the likelihood of Xac establishment, given the shipment of Xac-symptomatic fruit to citrus growing areas. As a condition for successful establishment, Xac, in sufficient amounts to cause infection, must encounter not only an environment with a temperature, relative humidity, and rain events conducive to infection, it also must encounter plant tissue of a host that is either at a susceptible growing stage or is wounded and then the bacteria, in sufficient numbers to incite infection needs to successfully enter this tissue.

9.3 Methodology

APHIS is proposing to regulate the interstate movement of citrus from Florida (where citrus canker exists), by using a performance standard approach to mitigating the likelihood of Xac infected citrus fruit movement. In this approach, the citrus growers (including backyard citrus growers) and harvesters will voluntarily implement phytosanitary measures that prevent and control the presence of symptomatic citrus canker infection in the fruit they produce. APHIS will inspect a specified number of randomly sampled fruit from each produced lot[16]. A lot will be cleared[17] for interstate shipment, on condition that no symptomatic fruit are detected. If any symptomatic fruit are detected, then no fruit from the lot can move interstate.

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[14] A hazard is: something that has the potential to cause harm, and that we do not want to happen,

[15] The shipping-season in Florida is August 1st till July 31st of the next year.

[16] A lot is described as the inspectional unit for fruit; composed of a single variety of fruit that has passed through the entire packing process in a single continuous run, during the course of one day; regulatory actions (e.g., issuance of limited permits, rejection) are taken at the lot level.

[17] If during a pre shipment inspection, the PPQ-APHIS inspection does not detect that there are symptomatically infected fruit in the lot, it will be released for interstate movement

A probabilistic model was developed to determine the potential quantity of Xac-symptomatic fruit shipped from Florida to citrus growing areas in the commercial citrus-producing states of Arizona, California, Hawaii, Louisiana, and Texas during the course of a shipping season for three scenarios of lot inspection: inspection of 500 fruit per lot; inspection of 1000 fruit per lot; and the inspection of 2000 fruit per lot.

The development of the model involved four steps:

1. Developing a risk pathway tree, labeling it and assigning units;
2. Stating assumptions;
3. Estimating Parameters: Gathering and documenting the evidence, and assigning values to the branches of the risk pathway tree;
4. Performing calculations to summarize the likelihood of the hazards occurring

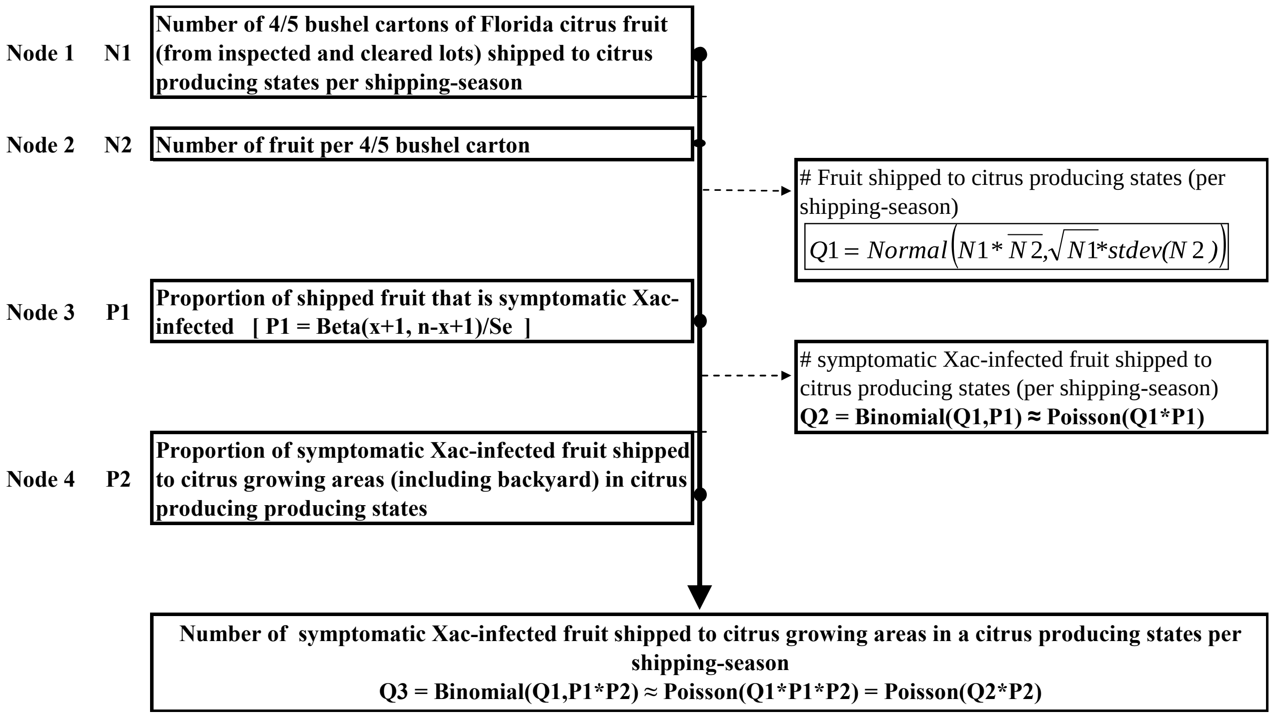
9.3.1 Risk pathway tree

A risk pathway tree (Figure 3.) is a visual representation of the events that could lead to infected citrus fruit from Florida reaching citrus producing areas in other states.

These events were modeled and include:

1. During each shipping-season some quantity of Florida citrus is packed and cleared by APHIS for interstate shipment.
2. Some proportion of the fruit in the cleared shipments is symptomatic Xac-infected. For this to be the case, the following must be true:
    i. Symptomatic Xac-infected fruit were harvested and packed.
    ii. Packed lots containing symptomatic Xac-infected fruit escaped detection during PPQ-APHIS pre-shipment inspection and were released for interstate movement.
3. Some proportion of the shipped symptomatic Xac-infected fruit is shipped to citrus growing areas in citrus-producing States (directly or indirectly)

Figure 9-1 Graphical depiction of the pathway model for interstate shipment of citrus from Florida



9.3.2 Model assumptions

The model assumes the following:

1. If Xac infection exists in a lot, symptomatic Xac-infected citrus fruit are distributed randomly throughout the population of packed fruit, i.e. all fruit have the same likelihood of being Xac-infected[18]
2. Inspection of fruit is a binomial process in which every fruit has an equal chance of being inspected and the size of the sample is small compared to the lot size.
3. The per-capita citrus consumption in the population is assumed to be uniform. No differentiation was made in the interstate and intercounty consumption habits.
4. Fruit consumption is assumed to be directly proportional to the population. The number of citrus fruit reaching citrus growing areas in citrus producing States is directly proportional to the proportion of the population living in citrus producing counties in those States, and the proportion of citrus coverage in the citrus producing counties.

[18] The risk management team considered the impact of clustering on the detection of symptomatic fruit. APHIS field staff report that if clustering exists, it would be at the field bin (equal to about 9 or 10 cartons) level. Processing, which involves sorting by size, mixes fruit from several bins into many cartons, thus dis-aggregating any clustering that may exist in the bins. Field staff observe that processed fruit is well mixed by the time it gets packed in shipping cartons.

5. Xac infected fruit are equally likely to be consumed in citrus growing areas as non Xac-infected fruit. They are no more or less likely to be consumed than non infected fruit.

Under the proposed action, APHIS controls the surface disinfection treatment of the fruit, the phytosanitary inspection and the limited distribution of citrus shipped from Florida. The model presupposes no prior knowledge of the prevalence of Xac-symptomatic fruit in inspected lots.

9.3.3 Estimating parameters

Values for the model input parameters (i.e. of the model nodes) are estimated based on available evidence. Many of these inputs are uncertain[19], and are defined as probability distributions rather than single values. The input parameters and their units are summarized and explained in Table 2.

Table 2 Model parameters and units

NODE	Parameter and description		UNITS
1	N1	Number of 4/5 bushel cartons of Florida citrus fruit shipped to citrus producing states per shipping-season[20]	4/5 bushel cartons shipped to citrus producing states / shipping season
2	N2	Number of fruit shipped per 4/5 bushel carton shipped	fruit shipped to citrus producing states / 4/5 bushel carton shipped to a citrus producing state
3	P1	Proportion of symptomatic Xac-infected fruit shipped to citrus producing states	symptomatic Xac-infected fruit shipped to citrus producing states / fruit shipped to citrus producing states
4	P2	Proportion of symptomatic Xac-infected fruit shipped to citrus growing areas (including backyard) of citrus producing states	symptomatic Xac-infected fruit shipped to citrus growing areas of citrus producing states / symptomatic Xac-infected fruit shipped to citrus producing states

[19] Certain or Uncertain
If the values variables will take in the time frame of your model are known — they are certain, or what statisticians call "deterministic". Conversely, if the values they will take are not known — they are uncertain, or "stochastic". If the variables are uncertain there needs to be a description of the nature of their uncertainty. This is done with probability distributions, which give both the range of values that the variable could take (minimum to maximum), and the likelihood of occurrence of each value within the range.

[20] The shipping-season in Florida is August 1st till July 31st of the next year.

9.3.3.1 Node 1 (N1): The number of 4/5 bushel cartons of Florida citrus shipped interstate per shipping-season

Unit: *4/5-bushel cartons shipped to citrus producing States / shipping season*

The probability distributions for the number of 4/5-bushel containers shipped per growing season for each State destination and variety of fruit, were determined using the minimum, average, and maximum values of the last four years of historical data (Florida Department of Citrus 2003a, 2004b, 2005b, 2006b) on citrus fruit shipping.

The number of 4/5 bushel cartons cleared and shipped interstate is based on the amount of citrus shipped during each of the last four shipping seasons. The analysis assumes that the distribution for the quantity of fruit shipped will not change.

Evidence:

Table 3 Thousands of 4/5-Bushel cartons of Florida citrus shipped to citrus growing states during 2002 -03 shipping-season (Florida Department of Citrus 2003a)

	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Arizona	0	0	0	0	0	1
California	192	96	0	0	647	313
Hawaii	0	0	0	0	0	0
Louisiana	19	74	4	3	35	49
Texas	10	20	0	1	67	101
Citrus States	221	190	4	4	749	464
Non Citrus	9,715	11,333	450	822	2,284	3,275
US Market	9,936	11,523	454	826	3,033	3,739

Table 4 Thousands of 4/5-Bushel cartons of Florida citrus shipped to citrus growing states during 2003-04 shipping-season (Florida Department of Citrus 2004b)

	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Arizona	0	0	0	0	0	0
California	146	174	0	0	738	306
Hawaii	0	0	0	0	1	0
Louisiana	24	85	2	1	38	45
Texas	4	38	0	1	82	87
Citrus States	174	297	2	2	859	438
Non Citrus	8,821	10,604	514	631	2,687	3,660
US Market	8,995	10,901	516	633	3,546	4,098

Table 5 Thousands 4/5-Bushel cartons of Florida citrus shipped to citrus growing states during 2004-05 shipping-season (Florida Department of Citrus 2005b)

	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Arizona	1	0	0	0	1	1
California	66	113	0	0	492	199
Hawaii	0	0	0	0	0	0
Louisiana	13	87	2	6	35	38
Texas	1	8	0	0	46	73
Citrus States	81	208	2	6	574	311
Non Citrus	4,807	8,607	302	618	1,930	2,877
US Market	4,888	8,815	304	624	2,504	3,188

Table 6 Thousands 4/5-Bushel cartons of Florida citrus shipped to citrus growing states during 2005-06 shipping-season (Florida Department of Citrus 2006b)

	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Arizona	2	0	0	0	0	0
California	85	76	0	0	469	199
Hawaii	0	0	0	0	0	0
Louisiana	6	37	0	3	22	29
Texas	1	14	0	0	70	55
Citrus States	94	127	0	3	561	283
Non Citrus	4,693	8,253	281	651	2,362	3,010
US Market	4,787	8,380	281	654	2,923	3,293

Evaluation of evidence:

For each citrus variety and U.S. State of destination, we calculated the minimum, mean and maximum number of 4/5 bushel cartons shipped over the shipping seasons 2002/2003 through 2005/2006 and then used these values as parameters in a Pert distribution to generate distributions representative of this data.

For example:

For California grapefruit, the minimum, maximum and mean values for the number of 4/5 bushel cartons of fruit shipped between August 1, 2002 and July 31, 2006 (from Tables 3 to 6) are:

- Minimum (192,000, 146,000, 68,000, 85,000) = 68,000
- Maximum (192,000, 146,000, 68,000, 85,000) = 192,000
- Mean (192,000, 146,000, 68,000, 85,000) = 122,750

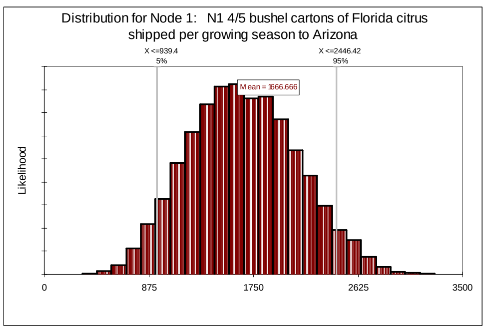
These minimum, mean and maximum values are used in a Pert distribution to describe the number of 4/5-bushel containers of Florida grapefruit that APHIS expects to be shipped to California each shipping season. [$N1_{CA\text{-}grapefruit}$ = RiskPert(68,000, 122,750, 192,000)]

We used these minimum, mean and maximum values in MS- Excel[21], and @RISK[22] to create Pert distributions of the projected number of 4/5-bushel containers that will be shipped to the various destination States (Table 7).

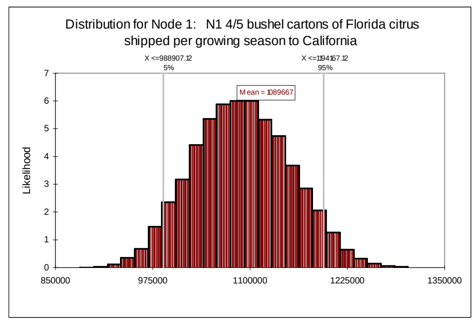
Table 7 Number of 4/5 cartons of each citrus variety shipped domestically from Florida per shipping-season

Number of 4/5 Bushel Cartons of Florida citrus shipped to citrus growing states during 2002-03 to 2005-06 season

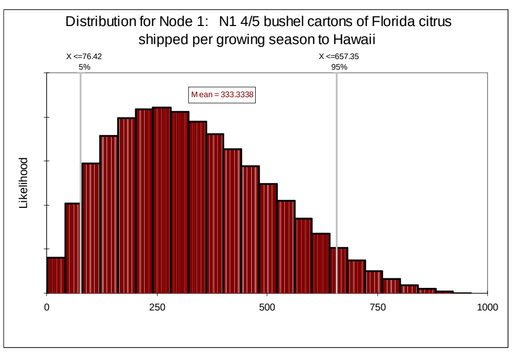
Arizona



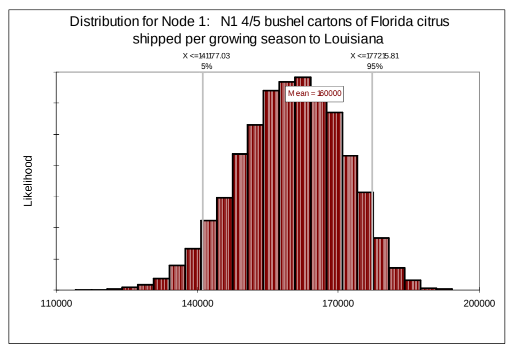
California



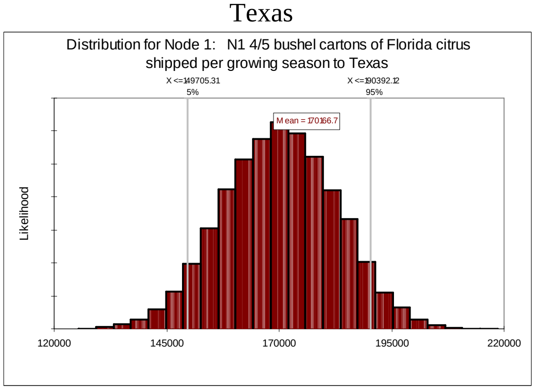
Hawaii



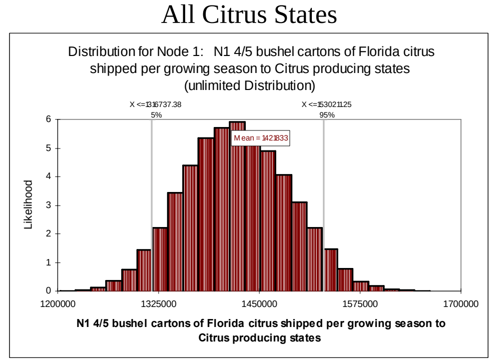
Louisiana



Texas



All Citrus States



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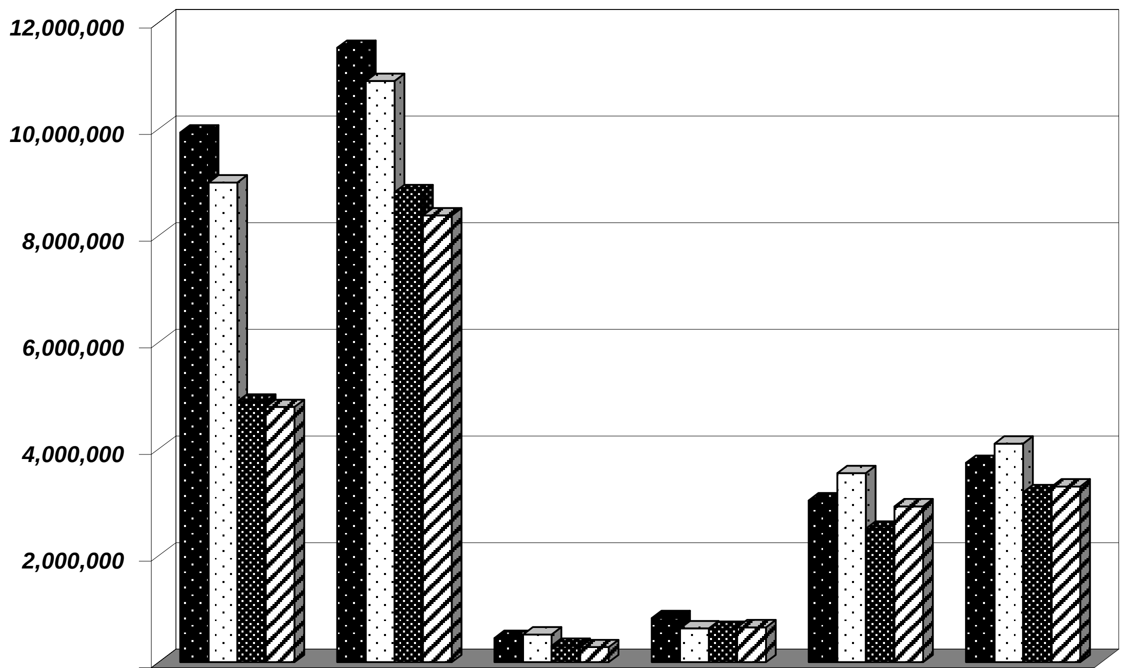
Over the past four shipping seasons, the shipments of fresh citrus from Florida have declined (**Figure** 9-2 9-2), with the exception of tangelos and tangerines, which increased in number over the past season.

Figure 9-2 Florida's shipment of 4/5 bushel cartons of fresh citrus between August 1, 2002 and July 31, 2006



	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Year 02/03	9,936,000	11,523,000	454,000	826,000	3,033,000	3,739,000
Year '03/04	8,995,000	10,901,000	516,000	633,000	3,546,000	4,098,000
Year 04/05	4,888,000	8,815,000	304,000	624,000	2,504,000	3,188,000
Year 05/06	4,787,000	8,380,000	281,000	654,000	2,923,000	3,293,000

9.3.3.2 Node 2 (N2): Number of fruit shipped per 4/5 bushel carton shipped

Unit: *Fruit shipped / 4/5-bushel carton shipped*
The number of fruit per 4/5 bushel carton varies by variety of citrus. Every year in December, USDA does maturity tests to forecast fruit sizes. The Florida field office of the National Agricultural Statistics Service publishes these results **(USDA-NASS 2006)**,

Evidence:

Table 8 Percentage distributions for the number of fruit in 4/5-bushel containers (USDA-NASS 2006) for 2004 through 2006

Number in 4/5-bushel containers	2004	2005	2006	Number in 4/5-bushel containers	2004	2005	2006
Early and mid-shipping-season Oranges (not Navels)**:**				**Navel Oranges:**			
64 and fewer	0.90	0.40	3.90	64 and fewer	51.6	49.9	75.4
80	5.70	3.30	13.90	80	35	33.5	20.4
100	25.00	17.20	32.80	100	10.1	13.1	3.7
125	38.90	37.30	31.30	125	2.7	2.7	0.5
163 and more	29.50	41.80	18.10	163 and more	0.6	0.8	0
White Seedless Grapefruit:				**Colored Seedless Grapefruit:**			
32 and fewer	13.1	16.7	11.1	32 and fewer	6.5	14.1	4.3
36	17.2	19.3	19.7	36	12.2	14.1	12.1
40	26.0	26.3	25.0	40	22.2	23.3	22.5
48	17.9	15.5	17.7	48	22.8	17.4	21.1
56	11.5	9.7	11.0	56	15.1	12.9	16.1
63 and more	14.3	12.5	15.5	63 and more	21.2	18.2	23.9
Sunburst Tangerines:				**Honey Tangerines:**			
80 and fewer	9.2	9.3	10.3	80 and fewer	6.9	2.6	7.5
100	22.8	21.5	27.8	100	21.2	18.5	25.0
120	26.4	25.2	27.8	120	32.3	40.2	34.1
176	15.8	15.3	18.1	176	15.2	18.7	12.5
210 and more	25.8	28.7	16.0	210 and more	24.4	20.0	20.9
Tangelos:							
80 and fewer	18.2	18.9	40.8				
100	28.4	33.3	34.2				
120	33.4	25.6	17.3				
156 and more	20.0	22.2	7.7				

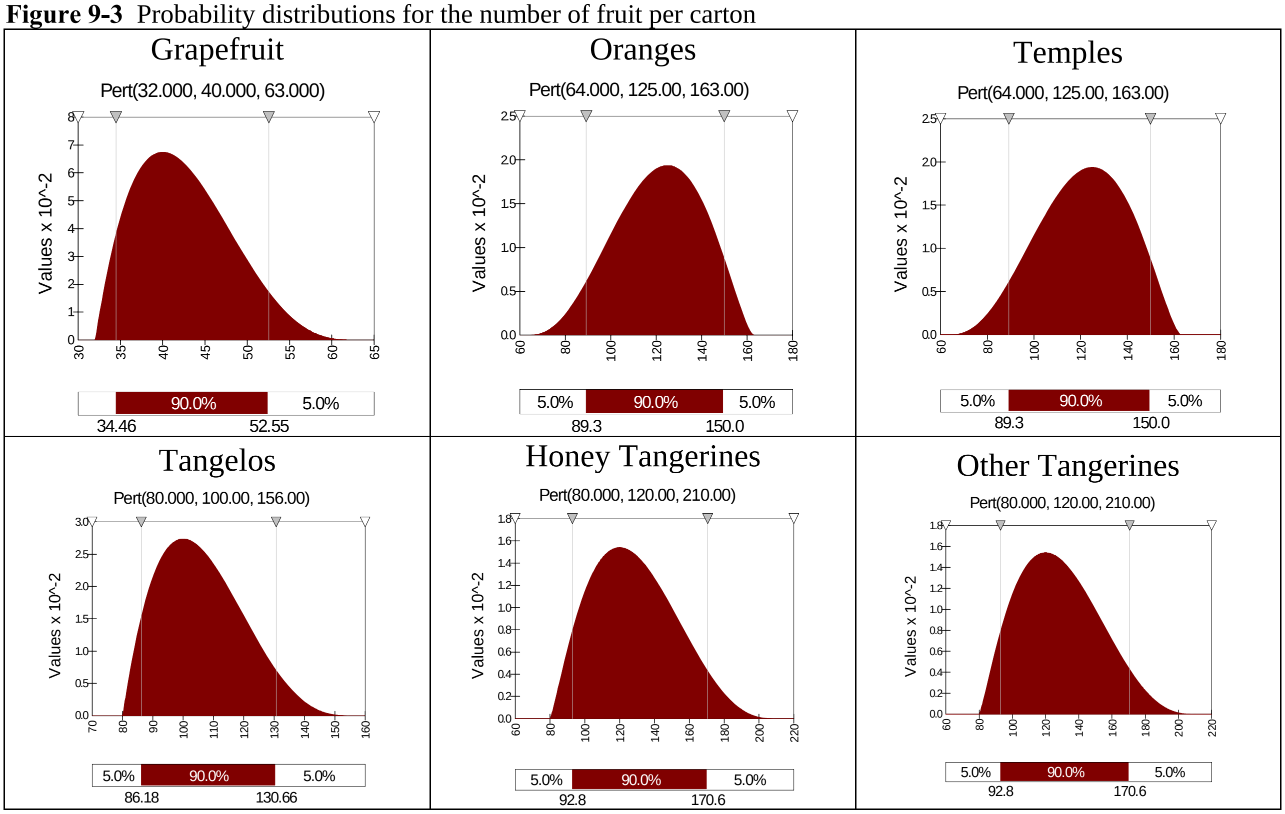
Evaluation of Evidence:

Table 9 summarizes the minimum, mode, and maximum number of fruit per box for each citrus variety. These values were used as parameters in a Pert distribution to define the number of fruit of each variety per 4/5-bushel container (Figure 9-3).

Table 9 Minimum (Min), Mode, and maximum (Max) values for the number of fruit per 4/5-bushel carton

Fruit per 4/5 bushel carton	Grape-fruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Min	32	64	64	80	80	80
Mode	40	125	125	100	120	120
Max	63	163	163	156	210	210
Fruit per Carton (Expected Value)= RiskPert(Min,ML,Max)	42.50	121.17	121.17	106.00	128.33	128.33
The Min, Mode, and Max values were taken from Table 8. In table 8, Oranges were used as a surrogate for “Temples” , and sunburst tangerines were used as a surrogate for “Other Tangerines” References: USDA Citrus December Forecast, Maturity test results and fruit size. Dec 11, 2006. National Agricultural Statistics Service, Florida field office						

Figure 9-3 Probability distributions for the number of fruit per carton



The minimum, most likely and maximum values (Table 9) were used to calculate the mean number of fruit per 4/5 bushel carton ($\overline{N2}$) of each variety of citrus (Table 10), using the following equation (Palisade 2002):

$$\overline{N2} = (Min+4*Mode+Max)/6. \qquad (1)$$

The mean, minimum, and maximum values, were then used to calculate the standard deviation of the Pert distributions for the number of fruit per carton (**S_{N2}**) of each variety of citrus (Table 10), using the equation 2 (Palisade 2002).

$$S_{N2} = ((Mean-Min)*(Max-Mean)/7)^{0.5} \quad (2)$$

The calculated mean and standard deviation for each Pert distribution were used in the mathematical model

Table 10 Mean and standard deviation of the Pert distribution for number of fruit per carton (N2)

Fruit per 4/5 bushel carton	Grape -fruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
Min	32	64	64	80	80	80
Mode	40	125	125	100	120	120
Max	63	163	163	156	210	210
Mean Fruit per Carton, $\overline{N2}$ $\overline{N2}$ = (Min+4*Mode+Max)/6	42.50	121.17	121.17	106.00	128.33	128.33
Standard deviation of N2, S_{N2} S_{N2} = ((Mean-Min)*(Max-Mean)/7)^0.5	5.55	18.48	18.48	13.63	23.75	23.75
The Min, Mode, and Max values were taken from Table 8 References: Palisade 2005.						

9.3.3.3 Node 3 (P1): Proportion of symptomatic Xac-infected fruit in each cleared lot (undetected prevalence of symptomatic fruit)

Unit: *(Xac-infected symptomatic fruit) / Fruit*

APHIS is estimating the true prevalence of symptomatic fruit (p_{true}) based on apparent prevalence ($p_{apparent}$), adjusted to account for inspection sensitivity (Se). The equation is:

$$P1 = p_{true} = \frac{p_{apparent}}{Se} \quad (3)$$

The beta distribution is used to estimate the apparent prevalence, $p_{apparent}$, assuming a sample size of n, and no symptomatic fruit detected in an inspection sample from a lot (x= 0). The equation (Vose 2000) is:

$$p_{apparent} = Beta(x+1, n-x+1) = Beta(1, n+1) \quad (4)$$

Given a minimum, mode and maximum value of sensitivity, the Pert distribution is used to model the probability distribution for the sensitivity of inspection. The equation is:

$$Se = Pert(minimum, mode, maximum) \quad (5)$$

Substituting Equations 4 and 5 into Equation 3 yields:

$$P1 = p_{true} = \frac{p_{apparent}}{Se} = \frac{Beta(x+1, n-x+1)}{Pert(\min imum, \mod e, \max imum)} \quad (6)$$

The model uses equation 6 to evaluate the probability distribution of the true prevalence for three different sampling (i.e., inspection) levels – 500 fruit, 1,000 fruit and 2,000 fruit per lot.

Based on these inspection levels, and the requirement that no symptomatic fruit are found in the inspected fruit (Options 2, 3, and 4), the probability distribution for the true prevalence of Xac-symptomatic fruit in each inspected lot can be calculated by substituting for n and x in equation 6 as follows:

$$P1(n=500, x=0) = \frac{Beta(1{,}501)}{Pert(0.5{,}0.85{,}0.95)}$$

$$P1(n=1000, x=0) = \frac{Beta(1{,}1001)}{Pert(0.5{,}0.85{,}0.95)}$$

$$P1(n=2000, x=0) = \frac{Beta(1{,}2001)}{Pert(0.5{,}0.85{,}0.95)}$$

This determination requires the assumptions, that Xac-symptomatic fruit are randomly distributed within a packed lot, that fruit for inspection are selected randomly, and that the number of inspected fruit is small compared to the size of the entire inspected lot. With these assumptions, the inspection is a binomial process. The determination presupposes no prior knowledge of the prevalence of Xac-symptomatic fruit within an inspected lot.

Figures 9-4, 9-5, and 9-6 represent the cumulative distributions for the true prevalence of Xac-symptomatic fruit leaving Florida under inspection scenarios of 500, 1,000 and 2,000 fruit inspected per lot respectively. The graphs show the probability (i.e., confidence) (vertical axis) that the prevalence of Xac-symptomatic fruit in any (and every) inspected lot is a given proportion *or less* (horizontal axis), given that the lot has passed the inspection.

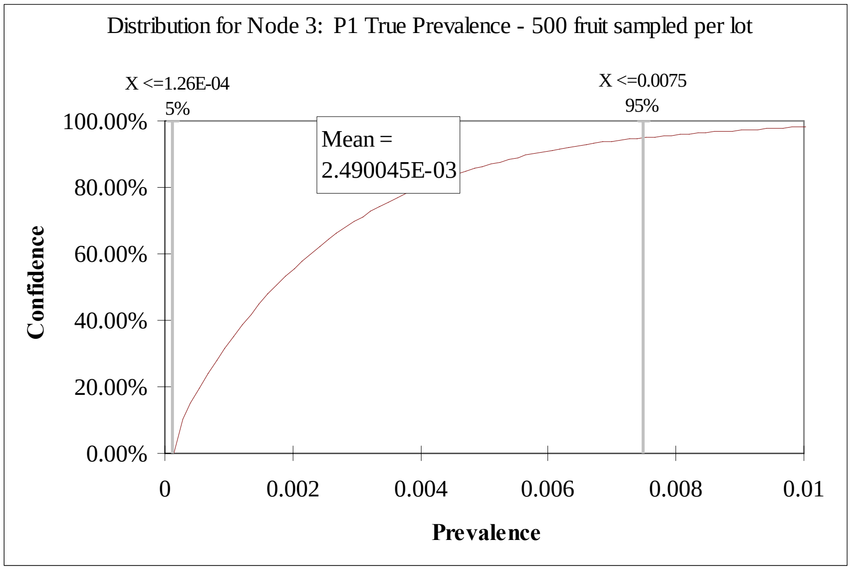


Figure 9-4 Cumulative probability distribution of the true prevalence of Xac-symptomatic fruit in inspected and cleared lots when a sample size of 500 fruit per lot is used.

Figure 9-5 Cumulative probability distribution of the true prevalence of Xac-symptomatic fruit in inspected and cleared lots when a sample size of 1,000 fruit per lot is used.

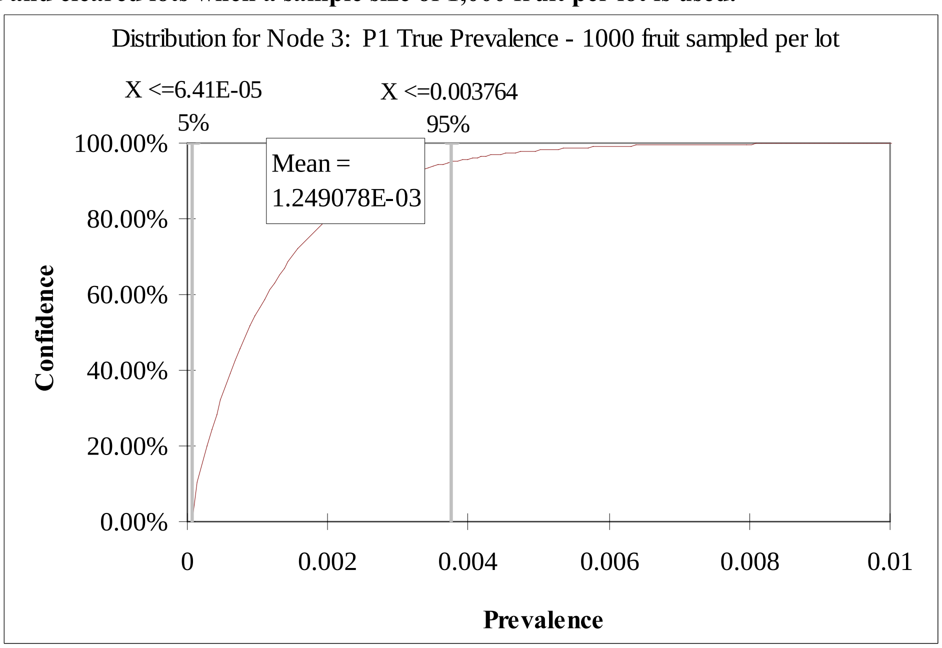
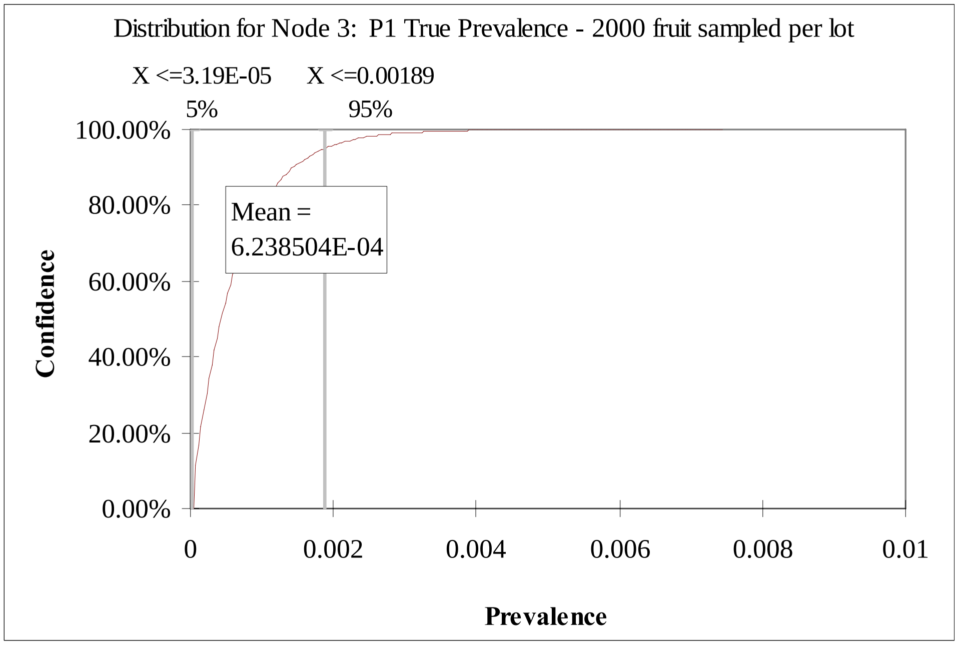


Figure 9-6 Cumulative probability distribution of the true prevalence of Xac-symptomatic fruit in inspected and cleared lots when a sample size of 2,000 fruit per lot is used.



In summary:

a) If in a random sample of 500 fruit no symptomatic fruit are detected, APHIS is 95% confident that the proportion of undetected symptomatic fruit in the cleared lot is no more than 0.748% (748 per 100,000). The mean proportion of Xac-symptomatic fruit is 249 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-4)

b) If in a random sample of 1000 fruit no symptomatic fruit are detected, APHIS is 95% confident that the proportion of undetected symptomatic fruit in the cleared lot is no more than 0.376% (376 per 100,000). The mean proportion of Xac-symptomatic fruit is 125 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-5)

c) If in a random sample of 2000 fruit no symptomatic fruit are detected, APHIS is 95% confident that the proportion of undetected symptomatic fruit in the cleared lot is no more than 0.189% (189 per 100,000). The mean proportion of Xac-symptomatic fruit is 62.4 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-6)

The Beta Distribution:

Beta (alpha1, alpha2) specifies a beta distribution using the shape parameters alpha1 and alpha2. These two arguments generate a beta distribution with a minimum value of 0 and a maximum value of 1.

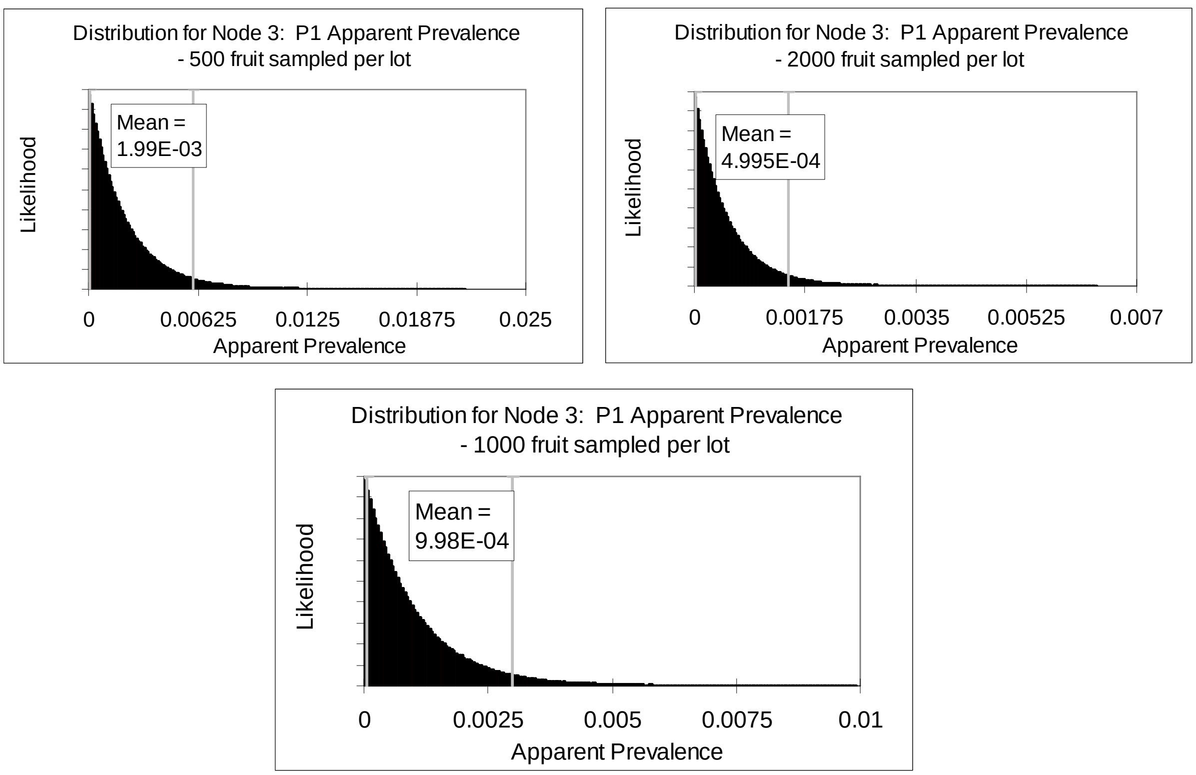
The Beta distribution can be used to define the probability of an event, if we know how many times we have observed the event (x), and we know how many times we have tried to observe the event (n). In this case, alpha1 = x+1, and alpha2 = n-x+1.

Beta(x+1,n-x) specifies a beta distribution using the number of events observed, x and the number of total observation trials, n.

Designed for binomial processes, the beta distribution allows the calculation of the probability of success on a single trial, given a sampling experiment with x successes in n trials.

The Beta distribution is used to determine the apparent prevalence of Xac-symptomatic fruit (Equation 4) in an APHIS inspected and cleared lot of fruit, given the size of the sampled population (the fruit actually inspected) and the number of sampled fruit found to be positive (zero for a cleared lot). Only lots in which no Xac-symptomatic fruit are found are allowed to be shipped under management options 2, 3, and 4. The following diagrams present the apparent prevalence under the fruit inspection scenarios of 500, 1000, and 2000 fruit inspected per lot.

Figure 9-7 Apparent prevalence under the fruit inspection scenarios of 500, 2000, and 1000 fruit inspected per lot.



<u>Why can a constant lot sample size be used for any size of lot?</u>

APHIS is proposing to sample a constant number of fruit from each packed lot in the packing houses, regardless of the size of the lot. This is done for ease of implementation. However, it is important to note the advantages and shortcomings of the approach, and why APHIS believes that a constant sample size can be used for any lot size without compromising the efficacy of the APHIS packinghouse inspection process.

The binomial process of sampling can be modeled in two ways: binomially, involving sampling with replacement; and hypergeometrically, involving sampling without replacement.

The beta distribution, as used in equation 6, estimates the apparent prevalence from the number of detections in a given number of fruit inspected. The underlying assumption is that the probability that a fruit is Xac-symptomatic is the same for every fruit in the lot. This is modeled by sampling with replacement. This means that for a given sample size, the distribution for the prevalence does not change with population size, and therefore the population size does not impact the sample size.

In reality, when the population size is small, the assumption of sampling with replacement does not hold, and the distribution of choice is the hypergeometric distribution, which implements sampling without replacement.

In the case where 1000 fruit are sampled per lot, the use of the Beta distribution in equation 6 yields a 95% confidence that if no Xac-symptomatic fruit are detected in the sample, then the prevalence of Xac-symptomatic fruit in the lot is less than 0.37%.

For illustrative purposes, the binomial and hypergeometric sample sizes are compared using the following equations:

Binomial sample size determination:

$$n = \log(1\text{-conf})/\log(1\text{-prev*sens})$$

Hypergeometric sample size determination:

$$n = (N - (0.5*N * Prev * Sens) + 0.5) * (1 - (1 - Conf) \wedge ^{(1/ (N * Prev * Sens))})$$

Using a confidence (conf) of 95%, a prevalence (prev) of 0.37%, and a sensitivity (sens) of 80%, the population size (N) was varied from 1,000 to 10 million, in increments of 1000, and the binomial and hypergeometric sample sizes were determined.

Figure 9-8 shows that in order to have a 95% confidence of detecting a prevalence of 0.37% or greater in a lot:

- as the number of fruit in the lot increases, the binomially obtained sample size remains constant at 1000
- as the number of fruit in the lot increases, the hypergeometrically obtained sample size approaches the binomially obtained sample size, reaching an asymptote at 1000 when the population is approaching 100,000.

Figure 9-8 Number of fruit per lot that need to be inspected (sampled) in order to provide a 95% confidence that the prevalence is less than 0.37% in the lot

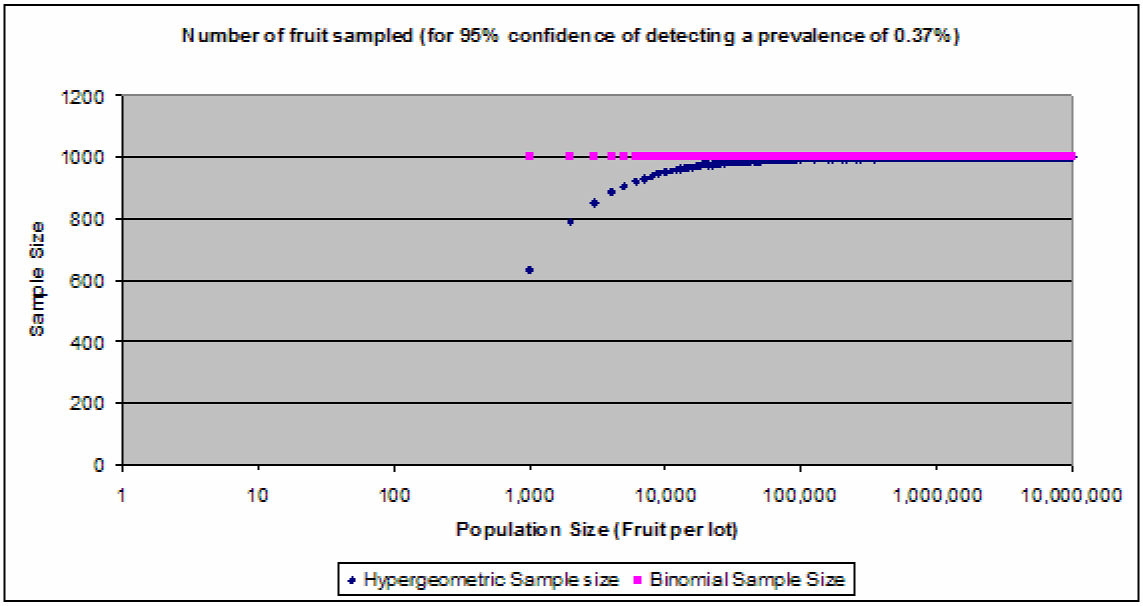


Figure 9-8 also shows that:

- for lot sizes less than about 50,000 fruit, the hypergeometric sampling algorithm can provide sample sizes of less than 1000 fruit, that achieve the same 95% confidence of detecting 0.37% prevalence.
- for small lot sizes (less than 10,000 fruit per lot), the binomial sampling algorithm (on which the beta is based) overestimates the threshold prevalence. The same threshold prevalence can be achieved with fewer fruit inspected per lot, using hypergeometric sampling.

The result of keeping a constant sample size of 1000 (or 500, or 2000), is that at lot sizes less than 20,000 fruit,, there will be greater than 95% confidence of detecting a prevalence of 0.37% in the lot. Another way to look at this is that there would be a 95% confidence of detecting a prevalence lower than 0.37%.

For small lot sizes, the Beta distribution overstates the prevalence of Xac-symptomatic fruit. By keeping the sample size fixed, we are understating the reliability of the efficacy of the APHIS packinghouse inspection process. To the extent that there are many lots less than 50,000,

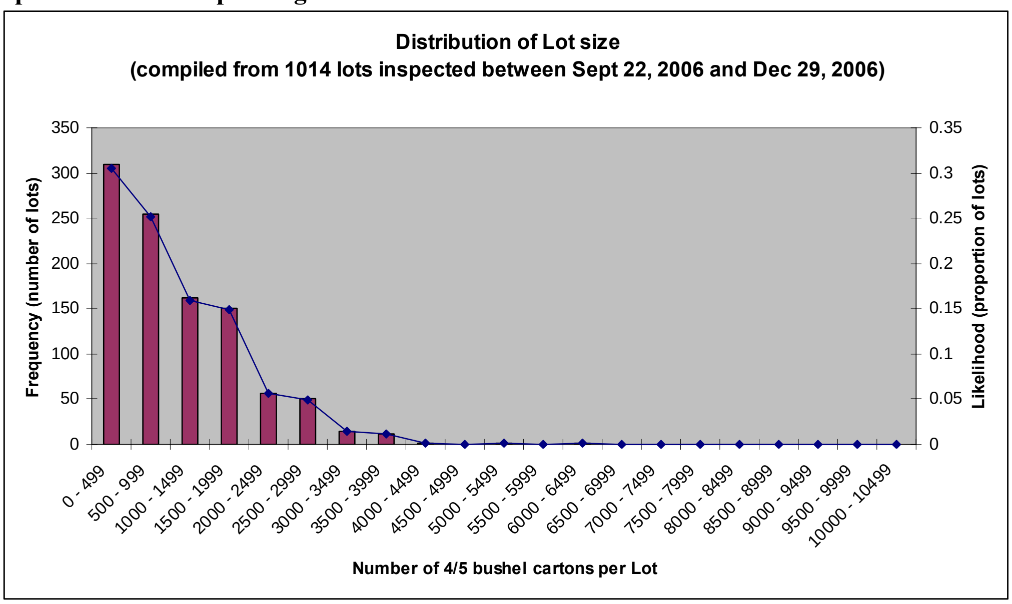
Figure 9-9 presents the distribution of the lot sizes in packing houses in one area of Florida. Out of 1,014 lots inspected by APHIS between September 22, 2006 and December 29, 2006, statistics indicate that the maximum lot size was 11,130 4/5 bushel cartons, and that the average lot size was 1074 cartons (1 truck load is 1,000 cartons). The standard deviation was 923 boxes.

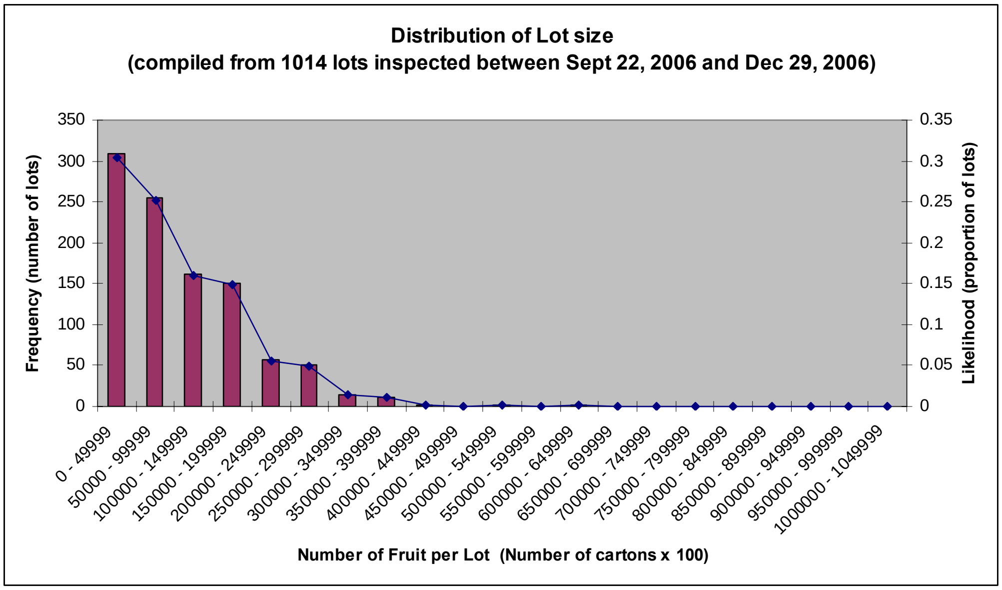
The average lot size of 1,074 boxes (1 truck load is 1000 boxes) per lot, equates to between 50,000 and 200,000 fruit per lot (depending on the variety of fruit). This observation indicates that if the future lot sizes (during implementation of the proposed rule) are similar to those observed in the past, then the actual threshold prevalence will be similar to the true prevalence calculated (based on the assumptions of Beta distribution) using equation 6.

Figure 9-9 indicates that some lots will exceed one truckload. It should be noted that in such cases, no portion of the lot may leave the premises until the entire lot has been inspected. Commingling of inspected and uninspected or preinspected fruit is unlikely for several reasons:

- physical separation of incoming fruit from the field and packed fruit;
- inspected fruit is packed into boxes specifically labeled for interstate movement; preinspection field fruit is in bins while uninspected intrastate fruit may not be packed in boxes for interstate movement;
- inspected fruit is segregated by loading onto trucks, storage in holding or degreening areas or simply by segregation on the packinghouse floor.

Figure 9-9 Probability distributions for the number of 4/5 bushel cartons packed per lot and fruit packed per lot in Florida packing houses.





Inspection sensitivity

APHIS does not assume that inspectors correctly identify every inspected, Xac-symptomatic fruit. The Agency recognizes that even when a Xac-symptomatic fruit is selected for inspection, the symptoms may not be recognized in every case. Inspectors may fail to detect Xac-symptomatic fruit for several reasons. For example, the Xac lesions may be too small to be observed by the naked eye, the lesions may be atypical, the inspectors may fail to observe the entire surface of the fruit, etc. A lesion is visible if it is 1 mm or more in diameter.

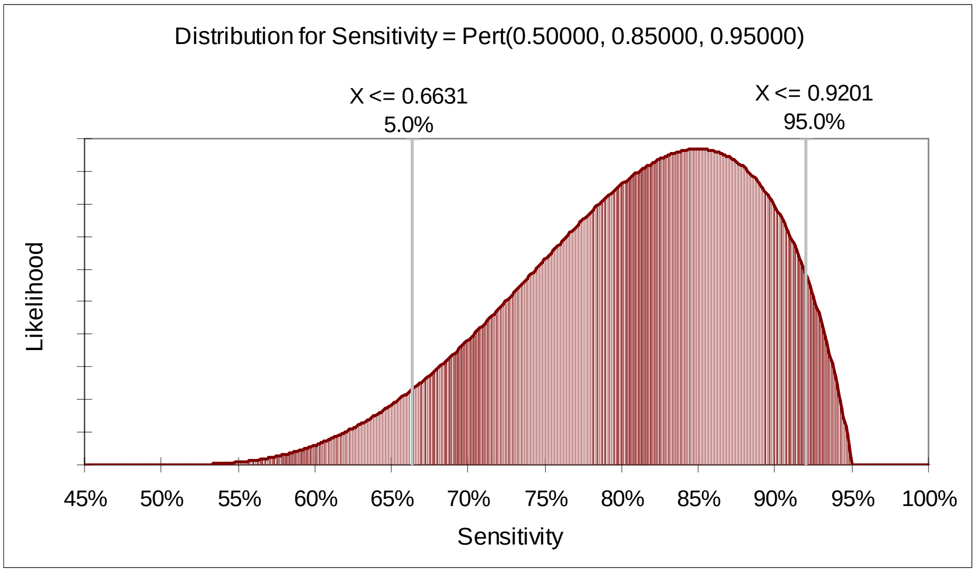
"Sensitivity" is the likelihood that a Xac-symptomatic fruit will actually be detected by inspection. Sensitivity is defined as the proportion of Xac-symptomatic fruit detected by inspection compared to the total number of Xac-infected fruit inspected. Sensitivity equal to 1 means that all inspected Xac-symptomatic fruit is correctly identified as such; sensitivity equal to 0.75 means that ¾ of inspected Xac-symptomatic fruit is correctly identified, etc.

APHIS does not know precisely the sensitivity of the fruit inspection process. However, the sensitivity depends on the training of the inspectors, as well as the visibility and distinctiveness of the Xac lesions on fruit. For this reason a distribution was used to represent the uncertainty in the sensitivity estimate.

PPQ inspectors are trained and tested each season for citrus canker disease symptom recognition. APHIS test records indicate that inspectors on average correctly identify over 90 percent of Xac-symptomatic fruit. APHIS recognizes, however, that test scores may not reflect actual proficiency under packinghouse conditions.

Therefore, agency staff used a pert probability distribution (Figure 9-10) to describe the sensitivity of the inspection process and, based on the evidence presented following, estimated the minimum value of sensitivity equal to 0.50, the most likely value equal to 0.85, and the maximum value equal to 0.95.

Figure 9-10 Probability distribution for the sensitivity of inspection



Inspection sensitivity evidence:

- The size, appearance, and abundance of Xac lesions on fruit entering and exiting the packing line may vary, influencing the ease with which they are detected. Variations observed in lesion appearance are attributed to many factors, including growth stage in which fruit became infected (Civerolo 1984; Graham *et al.* 1992b; Verniere *et al.* 2003; Graham and Leite 2004), susceptibility of the host (Zubrzycki and Zubrzycki 1986; Graham *et al.* 1992b; Gottwald *et al.* 1993), and association with wounds (Koizumi 1972; Sinha *et al.* 1972; Koizumi 1983; Goto 1992; Graham *et al.* 1992b; Verniere *et al.* 2003). Lesions begin as pin point spots, then depending upon the stage at which fruit are infected, may develop to 2 to 10 mm in diameter, becoming corky and crater-like, uniformly brown, approximately circular, and often are surrounded by a water-soaked margin and yellow halo (Gottwald and Graham 2000; Pruvost *et al.* 2002; Timmer *et al.* 2005; University of Florida - IFAS and FDACS-DPI 2006). Lesions on young grapefruit fruit expanded to 1 to 2 mm diameter after 2 to 3 months, enlarging to 9 mm after 200 days (Stall *et al.* 1980), whereas on infected mature fruit, lesions remained as minute (0.1 to 0.15 mm) or small (0.6 to 1.5 mm) greenish spots (Koizumi 1972).
- Training for APHIS phytosanitary inspectors is critical to enable them to detect Xac lesions, and distinguish them from lesions caused by other pathogens (University of Florida - IFAS and FDACS-DPI 2006; USDA-APHIS and FDACS/DPI 2006). Testing of APHIS inspectors occurred two ways.
    - First there is refresher training, followed by testing each year in order to continually improve and measure citrus disease identification skills. A PPQ inspector must score at least 80% on a proficiency test. The average test scores for inspectors is 93% (Lowe 2007).
    - Second, inspectors were tested as part of an evaluation of ELISA Dip Stick tools. When tested to visually diagnose citrus canker symptoms in

culled fruit, PPQ inspectors correctly classified 99% (88 of 89) of the Xac infected fruit as either symptomatic or suspect symptoms. Inspectors also correctly diagnosed 9 out of 10 (90%) of the injured\blemished fruit.

- Training programs for packinghouse and APHIS inspectors focus on distinguishing the overall appearance of typical citrus canker lesions, and it is possible that very small or uncharacteristic lesions may escape detection (University of Florida - IFAS and FDACS-DPI 2006).
- During an evaluation of a diagnostic tool, APHIS plant pathologists collected approximately 75 pieces of fruit eliminated by packinghouse graders for Xac lesions. The average lesion size on these fruit was about 4 mm (Riley 2007).
- APHIS plant pathologists have observed fruit intercepted in final packed cartons with lesions in the 2-3 mm range (Riley 2007).
- APHIS plant pathologists have observed that the majority of the symptomatic fruit that APHIS inspectors intercepted after passing through the packing line undetected by graders have only one lesion (Riley 2007).

9.3.3.4 Node 4 (P2): Proportion of symptomatic Xac-infected citrus shipped to citrus-bearing areas (including backyard) in citrus producing states

Unit: *Xac-symptomatic fruit shipped to citrus growing areas of citrus producing states / Xac-symptomatic fruit shipped to citrus producing states*

The model determines the proportion of symptomatic Xac-infected fruit shipped to citrus-growing areas based on the amount of citrus-bearing acreage (including acreage for backyard trees) in each citrus-producing county, the human population in each citrus-producing county and State, and the area of each citrus-producing county. APHIS considered modeling only the quantity of Xac-symptomatic fruit shipped to citrus-producing counties within citrus-producing States, basing the model on county population. However, because citrus is produced in almost all counties with citrus-producing States, the result would be little different from simply modeling the quantity of symptomatic fruit shipped to citrus-producing States. This approach would greatly overestimate the actual risk and was therefore rejected.

As noted above, the model assumes the proportion of Xac-symptomatic fruit shipped to citrus-growing areas is the same as the proportion of all citrus consumed in citrus-growing areas (i.e., the proportion of fruit that is Xac-symptomatic and the proportion that is shipped to citrus-producing areas are independent variables).

To determine the quantity of symptomatic fruit shipped to citrus-growing areas, the model first determines the quantity of symptomatic fruit shipped to citrus-producing counties. This is calculated by multiplying the quantity shipped to citrus-producing States by the fraction of the State population in each county. Recognizing that this result is still a poor indicator of risk (most consumed citrus, even within citrus-producing counties, will not be consumed in reasonably close proximity to Xac host trees), the model adjusts this result based on citrus-producing acreage within citrus-producing counties and using Schubert, *et al.* (2001) data from Florida. Recognizing that citrus canker disease could be introduced into residential backyard citrus as readily as into commercial citrus, APHIS attempted to model backyard citrus acreage. Tables 11 to 16 present the evidence used for this part of the model, the mathematical approach, and the results. The result for each State is calculated as the sum of the results for all citrus-producing areas in the citrus-producing counties of the State.

Table 11 July, 2006 populations in each citrus producing State, projected from the April 2000 census (US Census Bureau 2006)

State	Projected Population for July 2006
Arizona	6,166,318
California	36,457,549
Hawaii	1,285,498
Louisiana	4,287,768
Texas	23,507,783

Considering that about half the homes in Florida's concentrated citrus producing areas have two to three citrus trees (Schubert *et al.* 2001), the overall (for all citrus producing states) average proportion of homes with backyard citrus (q1) is estimated to be 0.25 (one in four), and the average number of citrus trees per home with citrus (q2) is estimated to be 2.

- A1, the number of owner occupied homes (A1) in each citrus bearing county of each citrus producing state is obtained from 2006 projections of the 2000 United States census statistics (U.S. Census Bureau 2002).
- A2, the number of homes with backyard citrus, is calculated by multiplying the number of owner occupied homes (A1) by the average proportion of homes with backyard citrus (q1). The resultant equation is: A2=A1*q1
- A3, the total number of backyard trees, is calculated by multiplying the number of homes with backyard citrus (A2) by the the average number of citrus trees per home with backyard citrus (q2). The equation is: A3=A2*q2,.
- A4, the acres of backyard trees, is calculated by dividing the total number of backyard trees (A3) by the number of backyard trees per commercial citrus acre, (q3). The equation is: A4=A3/q3
- A5, the commercial citrus bearing acreage is obtained from the US Agricultural Census (USDA-NASS 2000)
- A6, the total citrus bearing acreage in the county, is the sum of the commercial citrus bearing acreage (A5) and the acres of backyard trees (A4). The equation is: A6=A4+A5
- A7, the county area in acres, is obtained from the National Agricultural Statistical Service (USDA-NASS 2000)
- A8, the county population is obtained from the U.S. Census Bureau

- A9, the state population is also obtained from the U.S. Census Bureau

R1, the proportion of the county area under citrus is calculated by dividing the total citrus bearing acreage (A6), by the county area (A7). The equation is: R1=A6/A7.

R2, the proportion of citrus consumed in a county is assumed equivalent to the proportion of state residents living in the county. The proportion of the State population residing in the county is calculated by dividing the county population (A8), by the State population (A9). Thus R2=A8/A9.

P2, the proportion of citrus that goes to a State, and is consumed in a citrus growing area of a county, is calculated by multiplying the proportion of the county area under citrus (R1), and the proportion of citrus consumed in citrus growing areas of the county (R2). The representative equation is: P2=R1*R2.

Summing this proportion over all citrus-bearing counties of the State, yields the proportion of Florida citrus consumed in citrus bearing areas of a State.

Tables 12,13,14, 15 and 16, present the method used to determine (P2) the proportion of Xac- symptomatic fruit shipped to citrus-growing areas in each citrus-producing county of Arizona, California, Hawaii, Louisiana, and Texas, respectively

Table 12 Proportion of Florida citrus to Arizona consumed in citrus growing areas of Arizona

ARIZONA	A1	A2	A3	A4	A5	A6	A7	R1	A8	A9	R2	P2
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	**Proportion of County Area under citrus**	County POP2003	State Population	**Prop of state population in county**	**Prop of citrus consumed in citrus growing area of county**
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[23]	A3= A2*q2[24]	A4 = A3/q3[25]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	**R1 = A6/A7**	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	**R2 = A8/A9**	**(P2 = R1*R2)**
Graham	7406	1852	3703	37	3	40	2970311	**1.35E-05**	34628	6166318	**0.005616**	**8E-08**
Maricopa	764547	191137	382274	3823	7883	11706	5903714	**0.001983**	3402358	6166318	**0.551765**	**0.001094**
Pima	213603	53401	106802	1068	12	1080	5875396	**0.000184**	897611	6166318	**0.145567**	**2.68E-05**
Yavapai	51519	12880	25760	258	1	259	5201319	**4.97E-05**	185773	6166318	**0.030127**	**1.5E-06**
Proportion of Florida citrus to Arizona shipped to citrus growing areas of Arizona (P2)												**0.001122**

[23] q1 = proportion of homes with citrus = 0.25
[24] q2 = Average number of citrus trees per home with citrus = 2
[25] q3 = number of citrus trees per commercial acreage of citrus = 100

Table 13 Proportion of Florida citrus to California consumed in citrus growing areas of California

California	A1	A2	A3	A4	A5	A6	A7	R1	A8	A9	R2	P2
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	**Proportion of County Area under citrus**	County POP2003	State Population	**Prop of state population in county**	**Prop of citrus consumed in citrus growing area of county**
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[26]	A3= A2*q2[27]	A4 = A3/q3[28]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	**R1 = A6/A7**	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	**R2 = A8/A9**	**(P2 = R1*R2)**
Butte	48,336	12,084	24,168	242	311	553	1,073,723	0.05%	210072	36,457,549	0.58%	0.00%
El Dorado	44,019	11,005	22,010	220	22	242	1,145,576	0.02%	167761	36,457,549	0.46%	0.00%
Fresno	142,795	35,699	71,398	714	39,202	39,916	3,851,006	1.04%	840410	36,457,549	2.31%	0.02%
Glenn	5,855	1,464	2,928	29	881	910	849,298	0.11%	26852	36,457,549	0.07%	0.00%
Imperial	22,975	5,744	11,488	115	5,249	5,364	2,868,324	0.19%	150907	36,457,549	0.41%	0.00%
Kern	129,609	32,402	64,805	648	60,079	60,727	5,223,062	1.16%	698050	36,457,549	1.91%	0.02%
Los Angeles	1,499,744	374,936	749,872	7,499	247	7,746	2,615,582	0.30%	9873548	36,457,549	27.08%	0.08%
Madera	23,934	5,984	11,967	120	5,096	5,216	1,378,379	0.38%	132570	36,457,549	0.36%	0.00%
Marin	64,024	16,006	32,012	320	4	324	335,977	0.10%	250384	36,457,549	0.69%	0.00%
Merced	37,483	9,371	18,742	187	394	581	1,256,757	0.05%	226611	36,457,549	0.62%	0.00%
Monterey	66,213	16,553	33,107	331	1,526	1,857	2,121,341	0.09%	419850	36,457,549	1.15%	0.00%
Napa	29,554	7,389	14,777	148	6	154	504,400	0.03%	130384	36,457,549	0.36%	0.00%
Orange	574,456	143,614	287,228	2,872	829	3,701	511,465	0.72%	2986914	36,457,549	8.19%	0.06%
Placer	68,372	17,093	34,186	342	279	621	960,289	0.06%	285895	36,457,549	0.78%	0.00%
Riverside	348,532	87,133	174,266	1,743	33,787	35,530	4,673,862	0.76%	1717828	36,457,549	4.71%	0.04%
Sacramento	263,819	65,955	131,910	1,319	89	1,408	636,979	0.22%	1311915	36,457,549	3.60%	0.01%
San Bernardino	340,933	85,233	170,467	1,705	5,067	6,772	12,867,857	0.05%	1825575	36,457,549	5.01%	0.00%

[26] q1 = proportion of homes with citrus = 0.25
[27] q2 = Average number of citrus trees per home with citrus = 2
[28] q3 = number of citrus trees per commercial acreage of citrus = 100

Table 13 Continued Proportion of Florida citrus to California consumed in citrus growing areas of California

California continued	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**R1**	**A8**	**A9**	**R2**	**P2**
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	**Proportion of County Area under citrus**	County POP2003	State Population	**Prop of state population in county**	**Prop of citrus consumed in citrus growing area of county**
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[29]	A3= A2*q2[30]	A4 = A3/q3[31]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	**R1 = A6/A7**	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	**R2 = A8/A9**	**(P2 = R1*R2)**
San Diego	551,461	137,865	275,731	2,757	16,784	19,541	2,712,680	0.72%	2956812	36,457,549	8.11%	0.06%
San Joaquin	109,667	27,417	54,834	548	219	767	916,135	0.08%	615261	36,457,549	1.69%	0.00%
San LuisObispo	57,001	14,250	28,501	285	1,987	2,272	2,124,460	0.11%	258203	36,457,549	0.71%	0.00%
Santa Barbara	76,611	19,153	38,306	383	3,274	3,657	1,759,259	0.21%	408558	36,457,549	1.12%	0.00%
Santa Cruz	54,681	13,670	27,341	273	20	293	286,322	0.10%	261552	36,457,549	0.72%	0.00%
Shasta	41,910	10,478	20,955	210	25	235	2,462,340	0.01%	171170	36,457,549	0.47%	0.00%
Solano	84,994	21,249	42,497	425	71	496	543,280	0.09%	416892	36,457,549	1.14%	0.00%
Sonoma	110,475	27,619	55,238	552	16	568	1,017,948	0.06%	479807	36,457,549	1.32%	0.00%
Stanislaus	89,886	22,472	44,943	449	2,042	2,491	968,809	0.26%	483719	36,457,549	1.33%	0.00%
Sutter	16,632	4,158	8,316	83	43	126	389,358	0.03%	83047	36,457,549	0.23%	0.00%
Tehama	14,214	3,554	7,107	71	42	113	1,895,768	0.01%	57825	36,457,549	0.16%	0.00%
Tulare	67,913	16,978	33,957	340	120,592	120,932	3,096,821	3.91%	386179	36,457,549	1.06%	0.04%
Ventura	164,380	41,095	82,190	822	43,178	44,000	1,188,686	3.70%	794662	36,457,549	2.18%	0.08%
Yolo	31,506	7,877	15,753	158	293	451	654,887	0.07%	182025	36,457,549	0.50%	0.00%
Yuba	11,105	2,776	5,553	56	52	108	411,624	0.03%	61455	36,457,549	0.17%	0.00%
			Proportion of Florida citrus shipped to California and consumed in citrus bearing areas of California									**0.43%**

[29] q1 = proportion of homes with citrus = 0.25
[30] q2 = Average number of citrus trees per home with citrus = 2
[31] q3 = number of citrus trees per commercial acreage of citrus = 100

Table 14 Proportion of Florida citrus to Hawaii consumed in citrus growing areas of Hawaii counties

Hawaii	A1	A2	A3	A4	A5	A6	A7	**R1**	A8	A9	**R2**	**P2**
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	**Proportion of County Area under citrus**	County POP2003	State Population	**Prop of state population in county**	**Prop of citrus consumed in citrus growing area of county**
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[32]	A3= A2*q2[33]	A4 = A3/q3[34]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	**R1 = A6/A7**	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	**R2 = A8/A9**	**(P2 = R1*R2)**
Hawaii	34175	8544	17088	171	313	484	2,578,228	0.02%	156736	1,285,498	12.19%	0.00%
Honolulu	156290	39073	78145	781	142	923	383,572	0.24%	886540	1,285,498	68.96%	0.17%
Kauai	12384	3096	6192	62	73	135	401,475	0.03%	60895	1,285,498	4.74%	0.00%
Maui	25039	6260	12520	125	112	237	742,498	0.03%	137926	1,285,498	10.73%	0.00%
			Proportion of Florida citrus shipped to Hawaii and consumed in citrus bearing areas of Hawaii									**0.17%**

[32] q1 = proportion of homes with citrus = 0.25
[33] q2 = Average number of citrus trees per home with citrus = 2
[34] q3 = number of citrus trees per commercial acreage of citrus = 100

Table 15 Proportion of Florida citrus to Louisiana consumed in citrus growing areas of Louisiana

Louisiana	A1	A2	A3	A4	A5	A6	A7	R1	A8	A9	R2	P2
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	Proportion of County Area under citrus	County POP2003	State Population	Prop of state population in county	Prop of citrus consumed in citrus growing area of county
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[35]	A3= A2*q2[36]	A4 = A3/q3[37]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	R1 = A6/A7	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	R2 = A8/A9	(P2 = R1*R2)
Beauregard	9661	2415	4831	48	5	53	746,226	0.01%	33517	4,287,768	0.78%	0.00%
Lafayette	47798	11950	23899	239	6	245	172,985	0.14%	194752	4,287,768	4.54%	0.01%
Lafourche	24998	6250	12499	125	61	186	762,150	0.02%	91468	4,287,768	2.13%	0.00%
Plaquemines	7117	1779	3559	36	1123	1159	665,489	0.17%	27564	4,287,768	0.64%	0.00%
St. John the Baptist	11573	2893	5787	58	1	59	222,688	0.03%	44571	4,287,768	1.04%	0.00%
St. Martin	14024	3506	7012	70	78	148	522,524	0.03%	50150	4,287,768	1.17%	0.00%
Tangipahoa	26800	6700	13400	134	3	137	526,761	0.03%	104935	4,287,768	2.45%	0.00%
			Proportion of Florida citrus shipped to Louisiana and consumed in citrus bearing areas									**0.01%**

[35] q1 = proportion of homes with citrus = 0.25
[36] q2 = Average number of citrus trees per home with citrus = 2
[37] q3 = number of citrus trees per commercial acreage of citrus = 100

Table 16 Proportion of Florida citrus to Texas consumed in citrus growing areas of Texas.

Texas	A1	A2	A3	A4	A5	A6	A7	R1	A8	A9	R2	P2
Citrus bearing counties	# owner occupied Homes	# homes with back-yard trees	Total back-yard trees	Acres of back-yard trees	Commercial Citrus bearing acreage	Total citrus Bearing Acreage	County Area in Acres	**Proportion of County Area under citrus**	County POP2003	State Population	**Prop of state population in county**	**Prop of citrus consumed in citrus growing area of county**
Reference or Equation	(U.S. Census.Bureau 2002)	A2= A1*q1[38]	A3= A2*q2[39]	A4 = A3/q3[40]	(USDA-NASS 2000)	A6 = A4+A5	(USDA-NASS 2000)	**R1 = A6/A7**	(U.S. Census Bureau 2006)	(U.S. Census Bureau 2006)	**R2 = A8/A9**	**(P2 = R1*R2)**
Brazoria	60674	15169	30337	303	5	308	923,170	0.03%	259937	23,507,783	1.11%	0.00%
Cameron	65875	16469	32938	329	3333	3662	603,872	0.61%	360340	23,507,783	1.53%	0.01%
Hidalgo	114580	28645	57290	573	26631	27204	1,012,851	2.69%	628623	23,507,783	2.67%	0.07%
Liberty	18356	4589	9178	92	5	97	752,928	0.01%	75160	23,507,783	0.32%	0.00%
Starr	11450	2863	5725	57	54	111	786,720	0.01%	56135	23,507,783	0.24%	0.00%
Willacy	4316	1079	2158	22	203	225	384,124	0.06%	20252	23,507,783	0.09%	0.00%
			Proportion of Florida citrus shipped to Texas and consumed in citrus bearing areas									**0.08%**

Table 17 Proportion of Florida citrus consumed in growing areas of Arizona, California, Hawaii, Louisiana and Texas.

	State	Proportion of Florida citrus shipped and consumed in growing counties of a state	Proportion of Florida citrus shipped to a state and consumed in growing areas of a state = P2[41]
Proportion of Florida citrus shipped to state and consumed	**Arizona**	0.7330744	0.1122%
	California	0.7919538	0.4287%
	Hawaii	0.9662380	0.1733%
	Louisiana	0.1275622	0.0094%
	Texas	0.0595738	0.0816%

[38] q1 = proportion of homes with citrus = 0.25
[39] q2 = Average number of citrus trees per home with citrus = 2
[40] q3 = number of citrus trees per commercial acreage of citrus = 100
[41] P2, the proportion of Xac-symptomatic fruit shipped to citrus growing areas of a citrus producing states, is equal to the proportion of Florida citrus shipped to the state and consumed in growing areas of a state

9.3.4 Performing Calculations

Using the input parameters (nodes) described in the previous section, the quantitative model computes a number of output values. These are now described, and an equation relating the output variable to the input parameters is also presented:

a) **Q1,** is the amount of fruit (grapefruit, oranges, temples, tangelos, honey tangerines, and other tangerines) from cleared lots that will move interstate from Florida. This is a function of the number of 4/5-bushel containers (cartons) shipped per growing season (N1), and the number of fruit per carton (N2). The quantity Q1 represents the total number of fruit shipped to citrus producing states summed over all shipped cartons. In a given year, the number of cartons exported (N1) would be fixed, however, the number of fruit per carton (N2) varies among cartons—it is not fixed. Therefore the number of fruit shipped to citrus producing states is not simply the product of N1 and N2. From the central limit theorem[42], the sum of *N1* independent cartons that are identically distributed with respect to the number of fruit per carton (*N2*), is approximately normally distributed with mean = N1* $\overline{N2}$ and standard error = $\sqrt{N1} * S_{N2}$, where N1 = number of cartons, $\overline{N2}$ = the mean number of fruit per carton, and S_{N2} = the standard deviation of fruit per carton. Therefore,

$$\text{Q1}\sim\text{Normal}(\text{N1}*\overline{N2},\ \sqrt{N1}*S_{N2}) \qquad (7)$$

b) **Q2**, is the number of undetected Xac-symptomatic fruit shipped interstate. This is determined for each variety of citrus and for each citrus-producing state, and is a function of Q1, the amount of Florida fruit cleared for interstate movement to citrus producing states, and P1, the undetected proportion of Xac symptomatic fruit in cleared interstate shipments. Assuming Q2 is a binomially distributed random variable, Q2 can be represented as: Q2=Binomial(Q1, P1). However, because Q1 is too large to be used in an @Risk binomial distribution the binomial distribution cannot be used, and an alternative approximation is sought. Because Q1 is very large and P1 is very small, the Poisson distribution is used to approximate the binomial (Vose 2000). This can be simulated using @Risk. Therefore

$$\text{Q2}\sim\text{Poisson}(\text{Q1}*\text{P1}). \qquad (8)$$

c) **Q3**, is the number of undetected Xac-symptomatic fruit consumed in citrus growing areas of citrus producing states. Q3 is determined for each citrus producing state, and is a function of the number of Xac-symptomatic fruit shipped to each state (Q2), and the proportion of symptomatic fruit consumed in citrus growing areas in that state (P2). Like Q2, Q3 is assumed to be a binomially distributed random variable., and can be represented as: Q3 = Binomial(Q2, P2). Because Q2 is too

[42] Central limit theorem: The sum of *n* independent and identically distributed random variables (*x*) is approximately normally distributed with mean = n $\overline{x}$ and standard error = $\sqrt{n}s_x$. Therefore the resulting Value = normal (n $\overline{x}$, $\sqrt{n}s_x$)

large, the binomial distribution cannot be used, and the Poisson distribution is used to approximate the binomial (Vose 2000). Therefore

$$Q3 \sim Poisson(Q2*P2). \quad \textbf{(9)}$$

Table 18 Calculated values, and the equations used in their calculation.

OUTPUT PARAMETER & DESCRIPTION		UNITS	EQUATION
Q1	# Fruit shipped to citrus producing states (per shipping-season)	fruit shipped to citrus producing states -- shipping-season	Q1~Normal(N1* $\overline{N2}$, $\sqrt{N1} * S_{N2}$)
Q2	# symptomatic Xac-infected fruit shipped to citrus producing states (per shipping-season)	symptomatic Xac-infected fruit shipped to citrus producing states ------------------------------------ shipping-season	**Q2~Poisson(Q1*P1)**
Q3	# symptomatic Xac-infected fruit shipped to citrus growing areas in citrus producing cointies of citrus producing states (per shipping-season)	symptomatic Xac-infected fruit shipped to citrus growing areas in citrus producing counties of citrus producing states --------------------------------------- shipping-season	**Q3~Poisson(Q2*P2)**

A stochastic model (based on the aforementioned parameters and calculations) was constructed and 20,000 Monte Carlo iterations (with a fixed seed value of 100) were carried out using MS- Excel[43], and @RISK[44].

The model evaluates three inspection options: inspection of 500 fruit per lot, inspection,of 1000 fruit per lot, and inspection of 2000 fruit per lot

These output parameter results are now presented.

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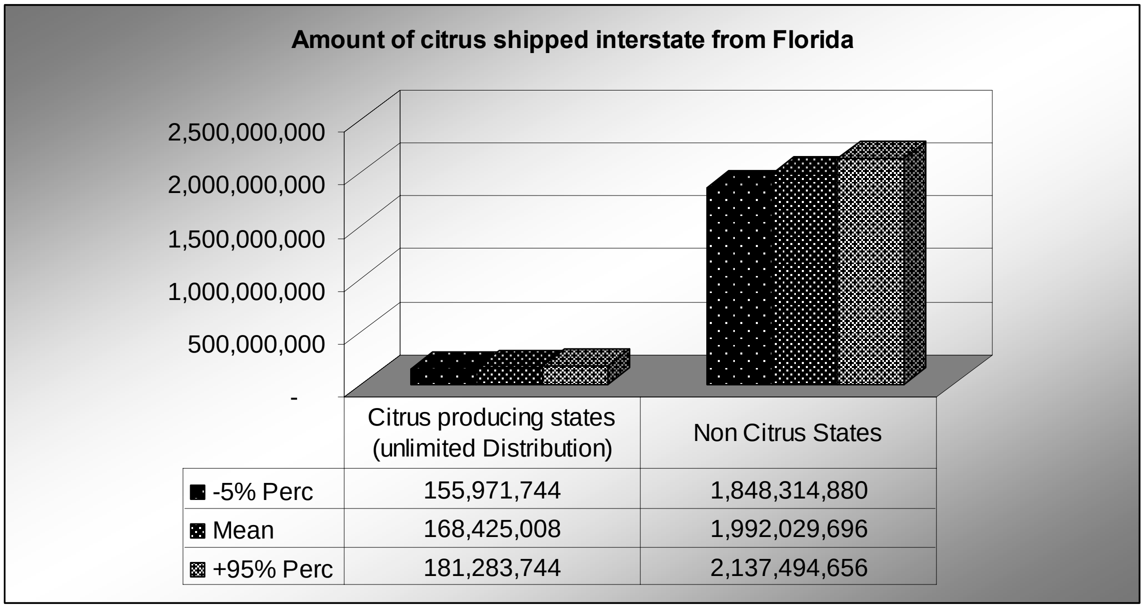
[44] Version 4.5.2 Professional Edition, Copyright © 2002 Palisade Corporation

9.4 Results

Q1 Results: The model first calculates Q1, the number of citrus fruit shipped interstate from Florida each shipping season. Under unlimited distribution (option 2) the simulation results indicate:

- The mean and 95%tile quantities of citrus shipped from Florida to citrus producing states are 168,425,008 and 181,283,744 respectively (Figure 9-11).
- On average, less than 8 percent of the fruit shipped interstate from Florida is shipped to citrus producing states (Figure 9-12).

Figure 9-11 Amount of citrus shipped interstate from Florida



	Citrus producing states (unlimited Distribution)	Non Citrus States
-5% Perc	155,971,744	1,848,314,880
Mean	168,425,008	1,992,029,696
+95% Perc	181,283,744	2,137,494,656

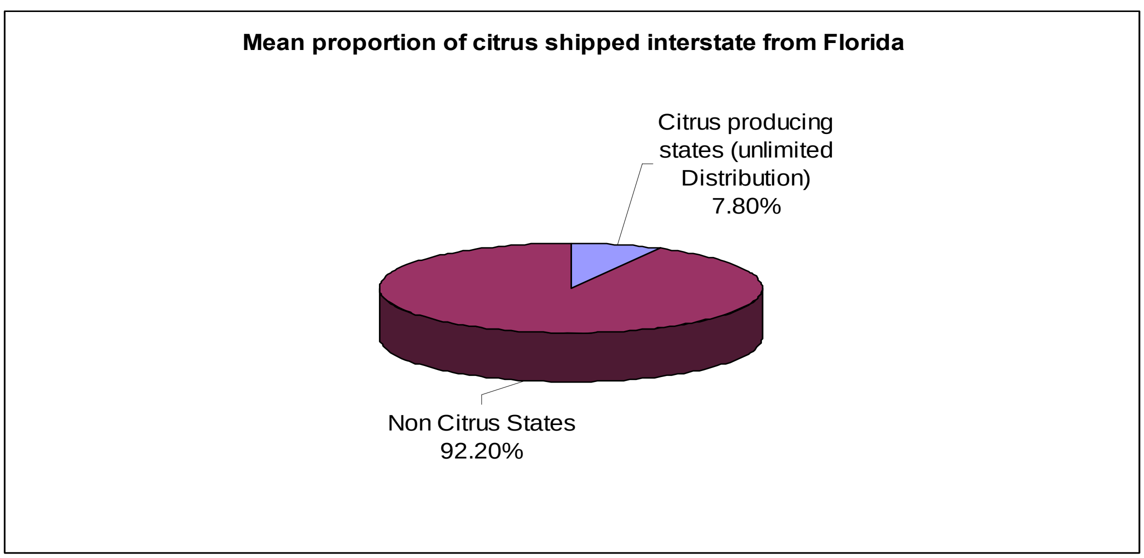
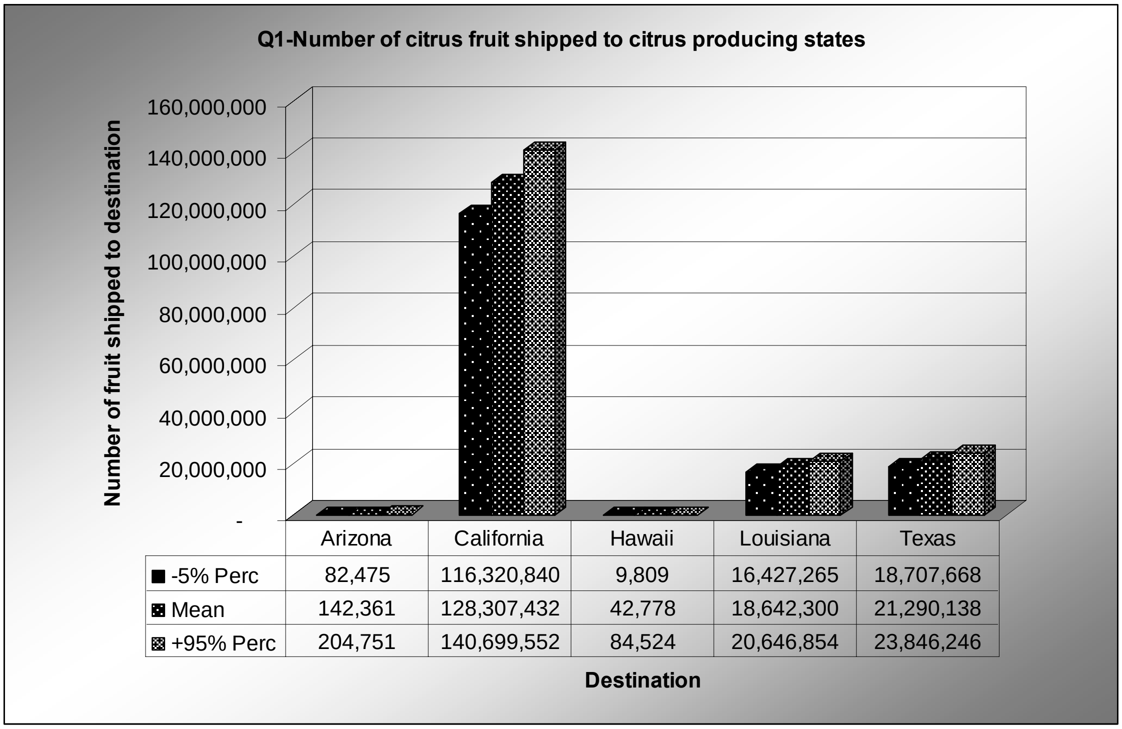


Figure 9-12 Mean proportion of Florida citrus shipped interstate.

- On average, 76.18% of the fruit shipped to citrus producing States, is shipped to California, 12.64% to Texas, 11.07% to Louisiana, and only 0.08% to Arizona and 0.03% to Hawaii (Figures 9-13 and 9-14).



	Arizona	California	Hawaii	Louisiana	Texas
-5% Perc	82,475	116,320,840	9,809	16,427,265	18,707,668
Mean	142,361	128,307,432	42,778	18,642,300	21,290,138
+95% Perc	204,751	140,699,552	84,524	20,646,854	23,846,246

Figure 9-13 Number of citrus fruit shipped to citrus producing States.

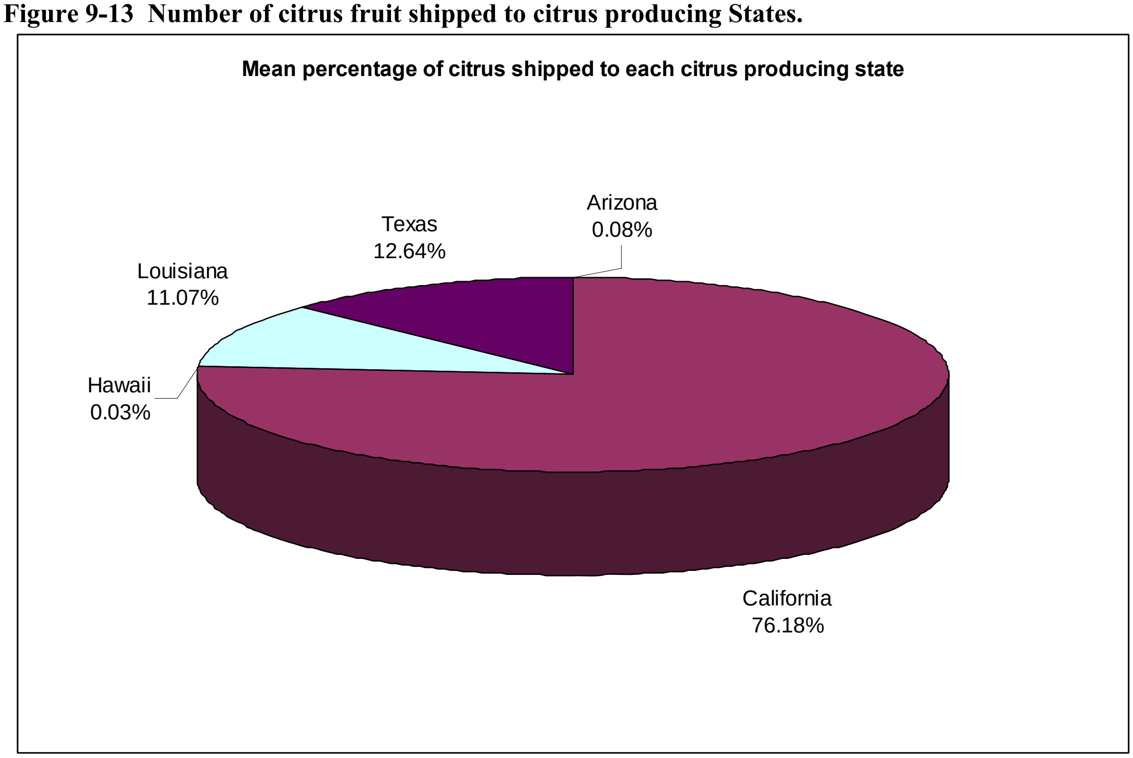
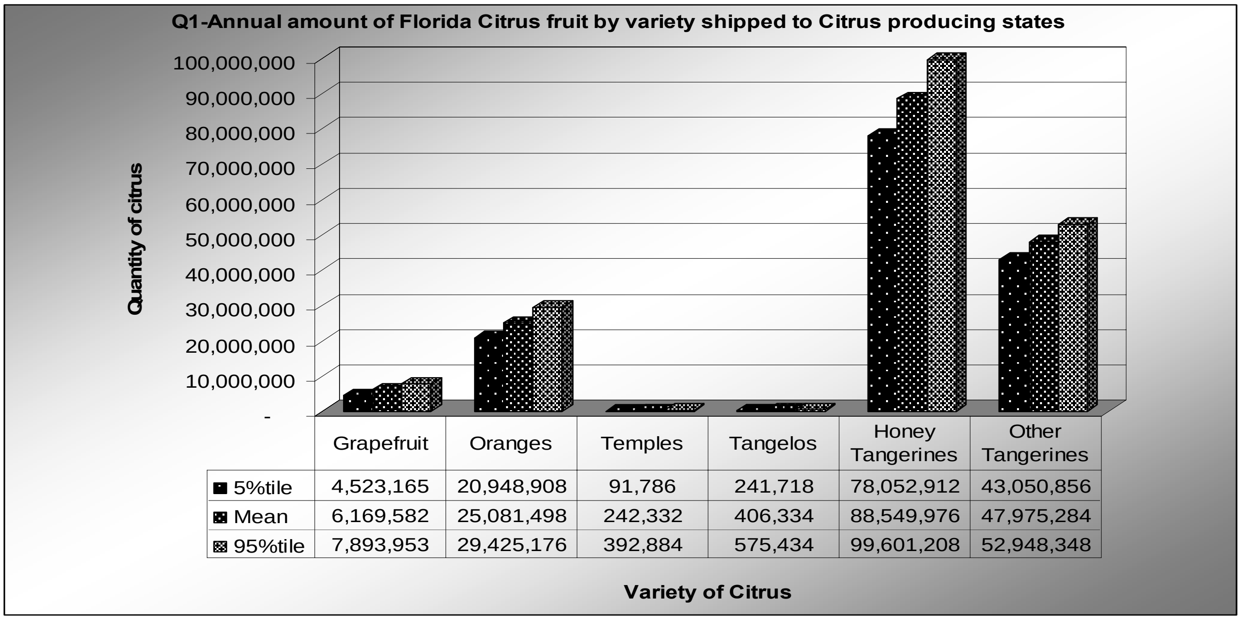


Figure 9-14 Mean percentage of citrus fruit shipped from Florida to selected Citrus producing States.

- On average, 81% of the fruit shipped to citrus producing States are tangerines (tangerines and other tangerines), 14.89% are oranges, 3.66% are grapefruit, and only 0.24% are tangelos, and 0.14% are temples (Figures 9-15 and 9-16)).



	Grapefruit	Oranges	Temples	Tangelos	Honey Tangerines	Other Tangerines
5%tile	4,523,165	20,948,908	91,786	241,718	78,052,912	43,050,856
Mean	6,169,582	25,081,498	242,332	406,334	88,549,976	47,975,284
95%tile	7,893,953	29,425,176	392,884	575,434	99,601,208	52,948,348

Figure 9-15 Annual amount of Florida citrus fruit, by variety, shipped to selected citrus producing States.

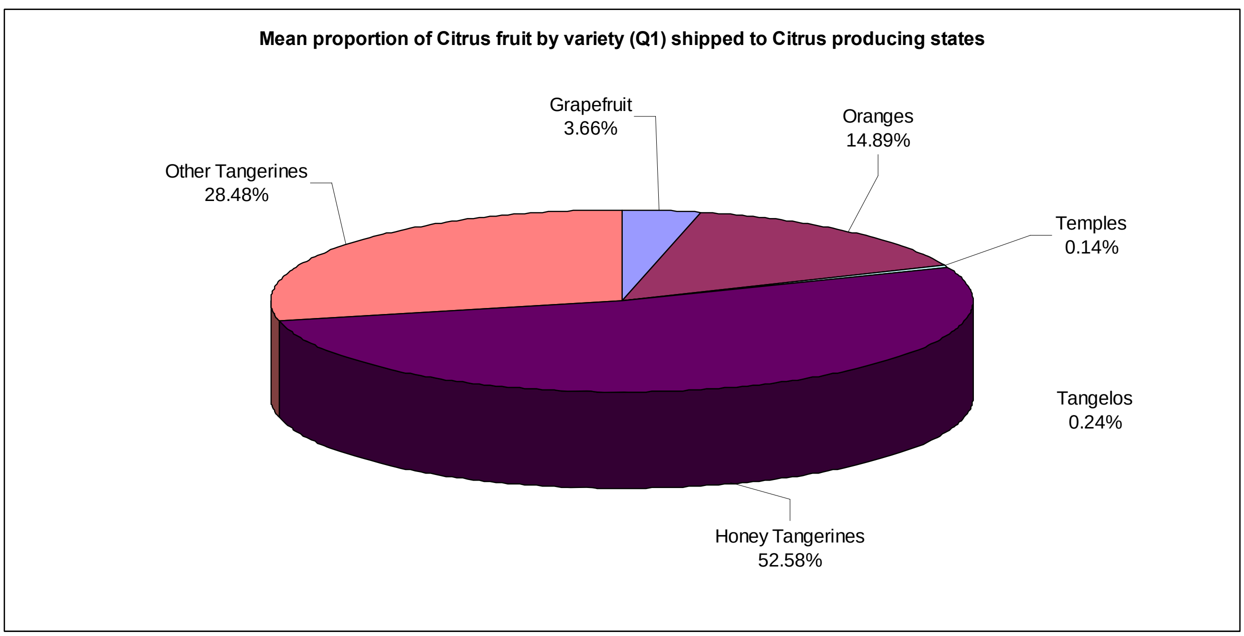
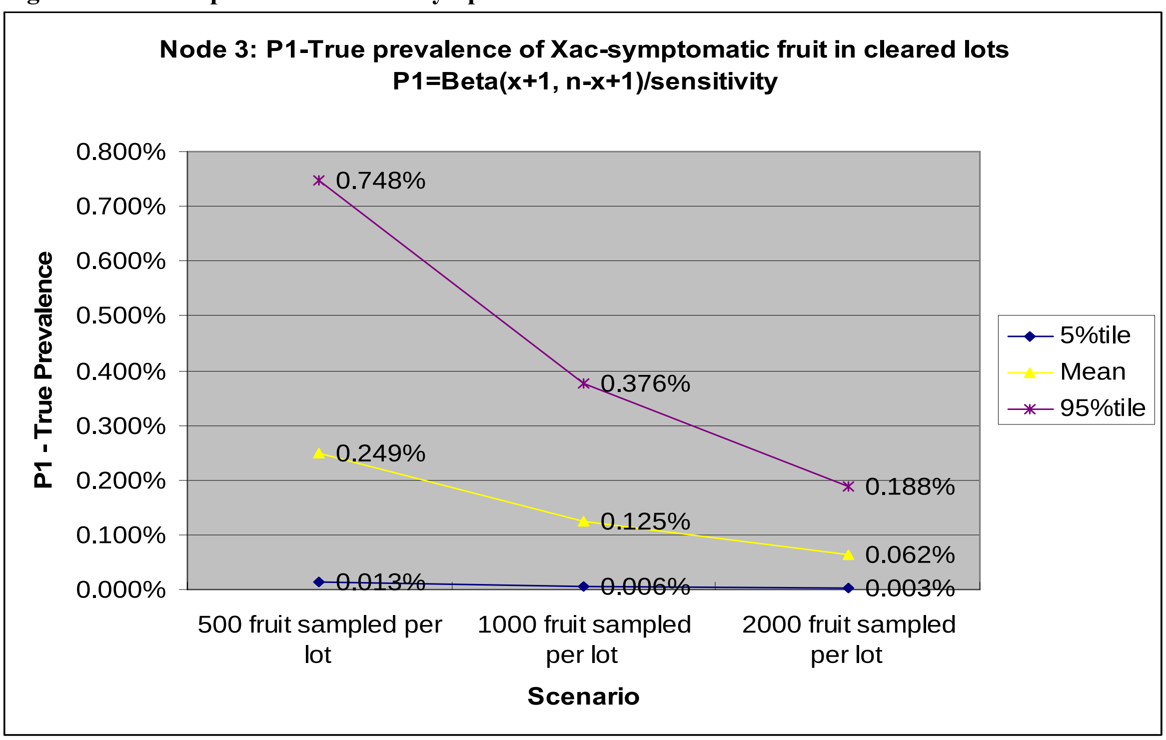


Figure 9-16 Mean proportion of Florida citrus fruit, by variety shipped to seleted citrus producing States.

P1 Results: The model then calculates P1, the true prevalence of Xac-symptomatic fruit in APHIS cleared lots. This is the proportion of symptomatic Xac-infected citrus in interstate shipments of Florida citrus. This is calculated for lot sample sizes of 500, 1000 and 2000, and the results are as follows:

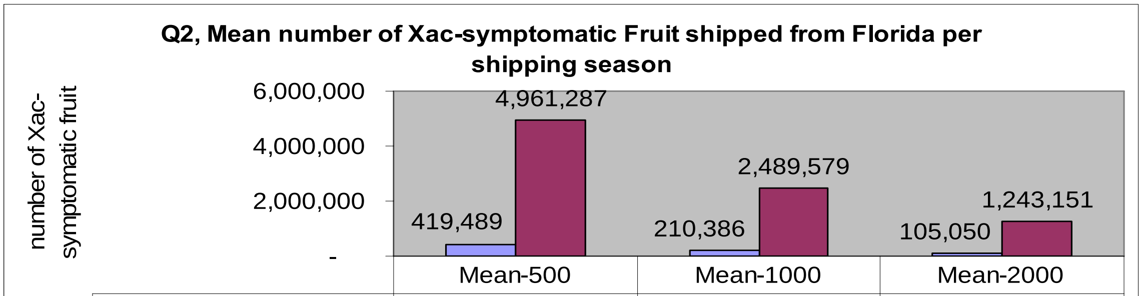
a) When no symptomatic fruit are detected in a random sample of 500 inspected fruit per lot, APHIS is 95% confident that the proportion of undetected Xac-symptomatic fruit in the lot is no more than 0.75% (748 per 100,000). The mean value is 125 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-17)
b) When no symptomatic fruit are detected in a random sample of 1000 inspected fruit per lot, APHIS is 95% confident that the proportion of undetected Xac-symptomatic fruit in the lot is no more than 0.38% (376 per 100,000). The mean value is 125 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-17)
c) When no symptomatic fruit are detected in a random sample of 2000 inspected fruit per lot, APHIS is 95% confident that the proportion of undetected Xac-symptomatic fruit in the lot is no more than 0.19% (188 per 100,000). The mean value is 63 per hundred thousand fruit, and the most likely value is 0 per million fruit. (Figure 9-17)

Figure 9-17 True prevalence of Xac-symptomatic fruit in cleared lots.



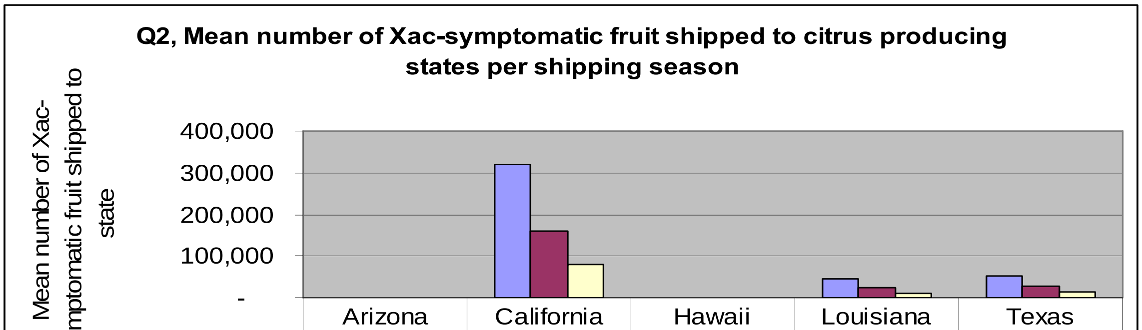
Q2 Results: The model then calculates Q2, the number of Xac-symptomatic fruit that reach citrus producing states.

Figure 9-18 Mean number of Xac-symptomatic fruit shipped from Florida per shipping season.



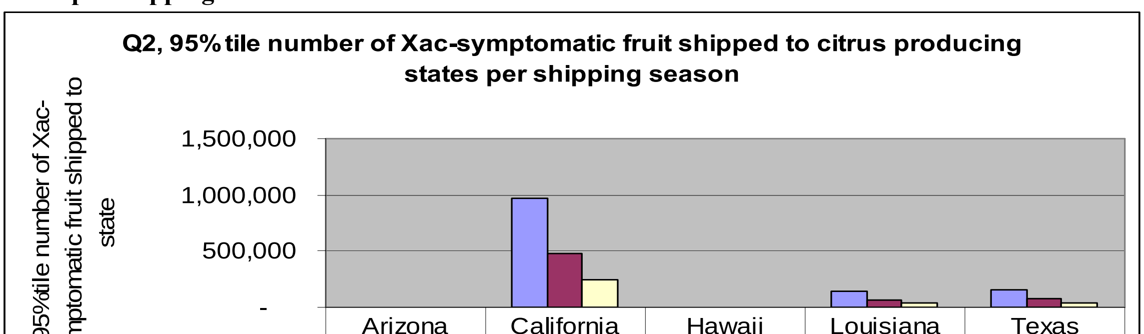
	Mean-500	Mean-1000	Mean-2000
Citrus producing states	419,489	210,386	105,050
Non Citrus States	4,961,287	2,489,579	1,243,151

Figure 9-19 Mean number of Xac-symptomatic fruit shipped to selected citrus producing States per shipping season.



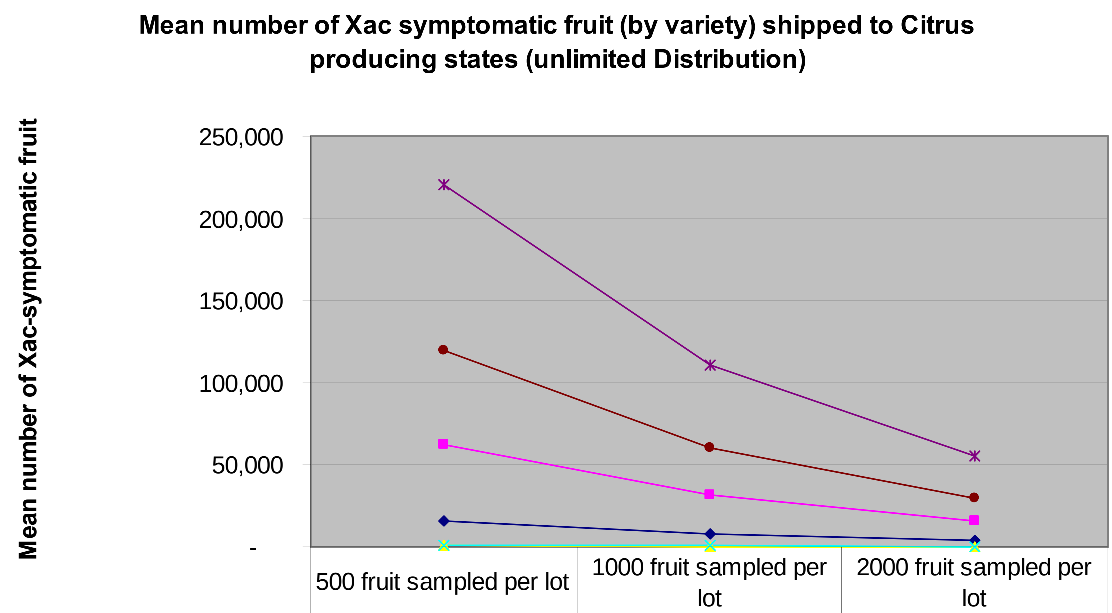
	Arizona	California	Hawaii	Louisiana	Texas
Mean-500	354	319,619	107	46,426	52,984
Mean-1000	178	160,294	53	23,285	26,576
Mean-2000	89	80,030	27	11,629	13,276

Figure 9-20 95th percentile number of Xac-symptomatic fruit shipped to selected citrus producing States per shipping season.



	Arizona	California	Hawaii	Louisiana	Texas
95%tile-500	1,108	967,299	368	140,975	159,329
95%tile-1000	555	483,921	184	70,037	80,374
95%tile-2000	272	242,264	91	35,216	40,134

Figure 9-21 Mean number of Xac-symptomatic fruit (by variety) shipped to seleted citrus producing States per shipping season if distribution to those States is not restricted.

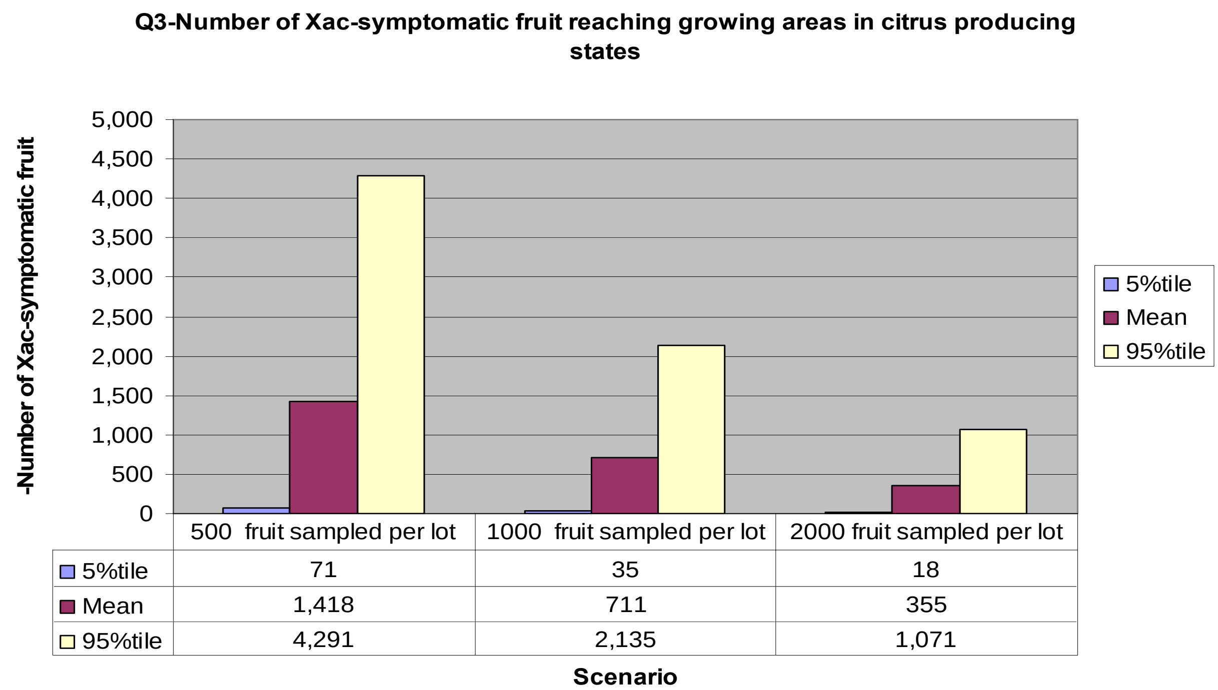


	500 fruit sampled per lot	1000 fruit sampled per lot	2000 fruit sampled per lot
Grapefruit	15,375	7,691	3,850
Oranges	62,437	31,304	15,637
Temples	604	302	152
Tangelos	1,011	507	253
Honey Tangerines	220,611	110,631	55,234
Other Tangerines	119,451	59,949	29,924

scenario

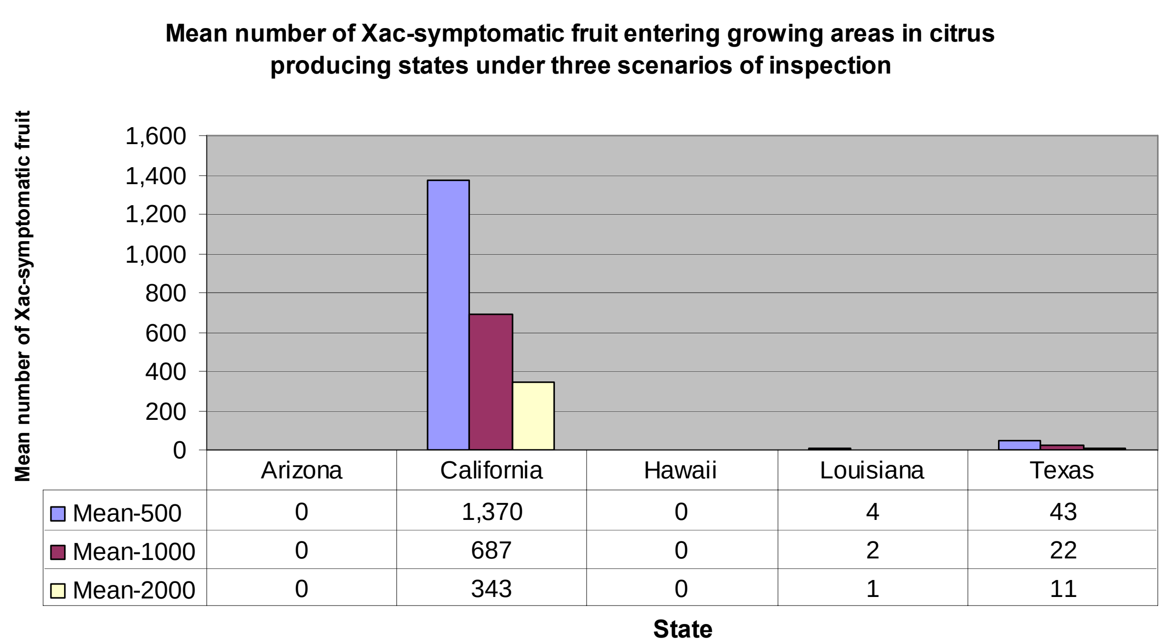
Q3 Results: The model then calculates Q3, the number of Xac-symptomatic fruit that reach citrus growing areas within citrus producing states.

Figure 9-22 Number of Xac-symptomatic fruit reaching citrus growing areas within citrus producing States per shipping season.



	500 fruit sampled per lot	1000 fruit sampled per lot	2000 fruit sampled per lot
5%tile	71	35	18
Mean	1,418	711	355
95%tile	4,291	2,135	1,071

Figure 9-23 Mean number of Xac-symptomatic fruit reaching citrus growing areas within selected citrus producing States in a shipping season.



	Arizona	California	Hawaii	Louisiana	Texas
Mean-500	0	1,370	0	4	43
Mean-1000	0	687	0	2	22
Mean-2000	0	343	0	1	11

Result Summary:

For the three scenarios of inspection, the results were as follows:

Scenario 1: **500 fruit** sampled per lot

The distribution outputs for the model (based on sampling 5,000 fruit) predict that the mean (average) and 95th percentile ("worst case") values for the total number of symptomatic fruit shipped to citrus producing States is 419,489 and 1,271,193 respectively. The predicted number of those fruit to reach citrus growing areas in citrus producing States is 1,418 (mean) and, 4,291 (95th percentile)

Scenario 2: **1000 fruit** sampled per lot

The distribution outputs for the model (based on sampling 1,000 fruit) predict that the mean (average) and 95th percentile ("worst case") values for the total number of symptomatic fruit shipped to citrus producing States is 210,386, and 633152, respectively. The predicted number of those fruit to reach citrus growing areas in citrus producing States is 711 (mean) and, 2,135 (95th percentile).

Scenario 3: **2000 fruit** sampled per lot

The distribution outputs for the model (based on sampling 2,000 fruit) predict a mean (average) of , mode (most likely) and values for the total number of symptomatic fruit shipped to citrus producing States is 105,050 and a 95th percentile ("worst case") of 316,891, respectively. The predicted number of those fruit to reach citrus growing areas in citrus producing States is 355 (mean), and 1,071 (95th percentile).

These values reflect the likelihood that, under management Options 2, 3 and 4, Xac-symptomatic fruit reach citrus-producing States, and citrus growing areas within those States, and **not** the likelihood of Xac establishment in these states.

Table 19. Summary of results.

	Scenario 1		**Scenario 2**		**Scenario 3**	
	500 fruit sampled per lot		1000 fruit sampled per lot		2000 fruit sampled per lot	
	mean	95%tile	mean	95%tile	mean	95%tile
Q1 -Number of fruit shipped to citrus producing states per shipping season	168,425,008	181,283,744	168,425,008	181,283,744	168,425,008	181,283,744
Q2-Number of Xac-symptomatic fruit reaching citrus producing states per shipping season	419,489	1,271,193	210,386	633,152	105,050	316,891
Q3-Number of Xac-symptomatic fruit reaching citrus growing areas in citrus producing states per shipping season	1,418	4,291	711	2,135	355	1,071

If symptomatic fruit reach citrus-producing States, and citrus growing areas within those States, under any management option, in order for an outbreak to occur:

a. the fruit must be discarded in such a way that Xac, in sufficient amounts to cause infection exists, and
b. the Xac must encounter an environment with a temperature, relative humidity, and rain events conducive to infection, and
c. the Xac must encounter plant tissue of a host that is either at a susceptible growing stage or is wounded, and
d. viable Xac, in sufficient numbers to incite infection, need to successfully enter this susceptible/wounded tissue.

These series of events is not likely, and has been discussed in the PRA (USDA 2006).

Management option 4 prohibits distribution of all types and varieties of citrus fruit, including tangerines, to citrus-producing States. Fruit can, however, be illegally moved, intentionally or unintentionally, to prohibited States, even though fruit boxes are labeled to prevent such movement. USDA-APHIS-PPQ-Smuggling Interdiction and Trade Compliance staff report six known interceptions of Florida citrus fruit since 2006 in citrus-producing States out of an estimated 12,400 shipments.

APHIS staff have no information with which to estimate the frequency of unreported illegal movement of Florida citrus to citrus-producing States or the proportion of reported illegal movement to total illegal movement. Since Option 4 would maintain the current prohibition on movement of citrus fruit to citrus-producing States, APHIS expects that the rate of intentional or unintentional movement of Florida citrus fruit to prohibited States will not change under this option. Therefore, the number of Xac-symptomatic fruit reaching citrus growing areas in citrus producing states per shipping season would be expected to be close to zero.

Option 4 compensates for uncertainty in the rate of illegal fruit movement by requiring a disinfectant treatment and phytosanitary inspection in addition to the distribution restriction. These measures ensure that even if a given shipment were illegally moved to a prohibited State, it has a low likelihood of containing symptomatic fruit.

Uncertainty

What APHIS can and cannot estimate reasonably accurately (based on the proposed measures):

- **APHIS cannot** estimate the prevalence of Xac infected groves. The proportion of groves infested, and the levels of fruit infestation within groves, will depend entirely on the grove management practices, and will vary tremendously between groves. The proximity of the groves to Xac sources, and the incidence of hurricanes and conducive climate will also add to the variability and uncertainty in the Xac infestation levels in the groves, trees, and fruit. As a result, APHIS cannot estimate the prevalence of Xac infection in the fruit in groves, or entering the packing houses. This uncertainty will be reduced somewhat over the next few years, as the packinghouse fruit inspection program gathers data.
- **APHIS cannot** estimate (with any degree of certainty) the efficacy of the packinghouse culling process in removing Xac infected citrus. The efficacy of the packinghouse culling could be estimated by measuring the difference between the prevalence in Xac-symptomatic fruit entering the packinghouse (from the groves), and that leaving the packinghouse (in boxes), This requires sampling and inspection of fruit pre culling, and post culling.
- **APHIS can** estimate the proportion of Xac infected symptomatic[45] fruit in each inspected lot, based on the results of a required pre-shipment APHIS inspection of each lot. Even though this is probably an overestimate[46] at present, it is a reliable way to determine the potential proportion of Xac-symptomatic fruit that survive the commercial culling, treatment, and inspection process, that is intended to remove them, and get shipped out of Florida.

[45] Lesion size 1mm and greater

[46] This estimate assumes that nothing is known about the prevalence of Xac-symptomatic citrus in packinghouse finished fruit that is ready for inspection.