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

## Supplemental Risk Management Analysis

United States
Department
of Agriculture

Animal and
Plant Health
Inspection Service

May, 2009

# Executive Summary

The Animal and Plant Health Inspection Agency (APHIS) conducted the risk management analysis (RMA) entitled "Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area: Risk Management Analysis" (USDA, 2007b) in 2007 in response to the decision by APHIS to halt citrus canker disease eradication efforts and declare the entire state of Florida a quarantine area. This document updates the 2007 RMA.

The 2007 RMA and the corresponding 2007 pest risk analysis (PRA) found commercially packed fresh citrus fruit is not an epidemiologically significant pathway for the introduction and spread of the citrus canker bacterium (*Xanthomonas* citri subsp. *citri*, Xcc). The 2007 document prescribed phytosanitary measures (Code of Federal Regulations, 2008b) for shipments of commercially packed fresh citrus fruit from Florida to non-citrus producing States.

Two recent publications provide additional research addressing key uncertainties identified in the 2007 RMA which justifies revisiting our conclusions regarding both fruit as a pathway for introducing and spreading citrus canker disease and subsequent risk management options. In a 2009 PRA, APHIS determined that new research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009) reduces the level of uncertainty that commercially packed and disinfected citrus fruit is not epidemiologically significant in transmitting and establishing citrus canker disease.

Considering the newly published scientific evidence above, this Supplemental RMA updates the sections of the 2007 RMA pertaining to the biology and epidemiology of Xcc and examines the following: 1) the effect of post-harvest treatments on the viability of bacteria on fruit; 2) the mortality of bacteria on fruit after it is harvested; 3) the low potential for spread from fruit to suitable hosts; 4) the likelihood that discarded fruit rinds will spread the disease; and 5) whether fruit parts, even those that are in direct contact with susceptible trees, spread the disease.

APHIS concluded that although citrus fruit may remain a conceptually possible pathway for transmitting and establishing citrus canker disease, research shows that extreme, artificial conditions are required to successfully transmit the pathogen from infected fruit to a susceptible host, and even under these extreme conditions, transmission is unlikely. APHIS concluded that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for transmitting and establishing citrus canker disease in regions currently free of Xcc.

Based on these conclusions, the Supplemental RMA identifies several options for modifying APHIS regulations on the interstate movement of citrus fruit from regions quarantined for citrus canker disease. The risk management options evaluated are:

**Option 1** ***Allow unrestricted distribution of all types and varieties of commercially packed citrus fruit to all U.S. States.*** Option 1 is the least restrictive option and would remove all current restrictions on the movement of citrus fruit from areas quarantined for citrus canker disease. A substantial body of evidence suggests that citrus fruit, even if untreated with disinfectants, does not serve as an epidemiologically significant pathway for the introduction of Xcc. The recent research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009) support this conclusion. Even so, the Gottwald *et al.* (2009) study only goes so far as to conclude "*packinghouse-disinfested*, citrus fruit with canker lesions" are an unlikely pathway.

**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. No packinghouse phytosanitary inspection is required.*** Option 2 echoes the conclusion of the Gottwald *et al.* (2009) study and would require an APHIS-approved packinghouse disinfection treatment while allowing distribution to all U.S. States.

**Option 3** ***Allow distribution of all types and varieties of commercially packed citrus fruit to all U.S. States except commercial citrus-producing States. No packinghouse phytosanitary inspection is required. For all U.S. citrus-producing States, allow distribution of all types and varieties of commercially packed citrus fruit with APHIS approved disinfectant treatment and some additional requirement (e.g., inspection).*** Option 3 would require an APHIS-approved packinghouse disinfection treatment and would take a more stringent approach differentiating between the risk to non-citrus producing States and commercial citrus-producing States by adding the additional requirement of an inspection for fruit shipments to the latter.

**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 subject to packinghouse disinfectant treatment. No packinghouse phytosanitary inspection required.*** Option 4 would be more restrictive still and prohibits altogether fruit shipments to commercial citrus-producing States.

**Option 5** ***Leave the current regulations for the interstate movement of citrus fruit from citrus canker disease quarantined areas in place and unchanged.*** Option 5 completely discounts the additional evidence from epidemiological studies recently conducted in Japan, Florida and Argentina (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009) and would leave the current regulations unchanged.

# Table of Contents

Executive Summary ..... i
1 Introduction ..... 1
2 Purpose and Scope ..... 3
3 The Movement of Commercially Packed and Disinfected, Fresh Citrus Fruit as a Pathway for the Introduction of Xcc ..... 5
3.1 Fresh Citrus Fruit Production and Harvesting Techniques Reduce the Prevalence of Xcc-infected Fruit ..... 6
3.2 Commercial Citrus Fruit Packing Techniques Reduce the Prevalence of Infected or Contaminated Fruit ..... 8
3.3 Mortality of Xcc Associated with Fresh Citrus Fruit and/or Packing Materials Following Harvest and Packing ..... 9
3.4 Environmental and Epidemiological Conditions for Xcc Establishment ..... 12
3.5 Conclusions and Summary of Evidence Regarding Fruit as a Pathway for Xcc Introduction ..... 15
4 Risk Management Options ..... 17
4.1 Option 1 – Unrestricted movement ..... 17
4.2 Option 2 – Unlimited distribution, disinfectant ..... 19
4.3 Option 3 – Two-tier distribution requirements ..... 21
4.4 Option 4 – Limited distribution (all varieties) to non-commercial citrus producing States, disinfectant ..... 22
4.5 Option 5 – No change ..... 23
4.6 Conclusion ..... 24
5 Literature Cited ..... 25

# 1 Introduction

In response to the widespread occurrence of citrus canker disease in the State of Florida, APHIS published an amendment to the citrus canker disease regulations (7 CFR 301.75-1 through 301.75-14, referred to below as the regulations) on August 1, 2006 (FR, 2006) listing the entire State of Florida as a quarantined area for citrus canker disease [caused by *Xanthomonas citri* subsp. *citri* (Xcc)[1]] and amending the requirements for the movement of regulated articles from Florida. Prior to the amendment, the regulations had required every tree in a given orchard in a quarantined area be inspected not more than 30 days before harvest and found free of citrus canker disease, that regulated fruit be accompanied by a limited permit, and that regulated fruit not be distributed to U.S. commercial citrus-producing States and Territories (Arizona, California, Hawaii, Louisiana, Texas, American Samoa, Guam, the Northern Mariana Islands, Puerto Rico, and the U.S. Virgin Islands). By designating the entire State as a quarantined area, the August 2006 interim rule made all fresh Florida citrus fruit produced for interstate movement subject to regulations previously required only in specified quarantine areas.

As a consequence of the 2006 amendment to the regulations, APHIS evaluated the epidemiological significance[2] of commercially packed[3] fresh citrus fruit as a pathway for the introduction of Xcc to new areas. In 2007, APHIS concluded that while commercially packed and disinfected citrus fruit was unlikely to be an epidemiologically significant pathway for introducing Xcc, it was not possible to design an operationally practicable system that ensured only uninfected fruit would be shipped from quarantined areas, and there was insufficient evidence to adequately remove uncertainties surrounding the ability of fresh citrus fruit produced in an Xcc infested grove to serve as a pathway for the introduction of Xcc into new areas (USDA, 2007a; b).

After considering accrued evidence for commercially packed citrus fruit as a pathway for the introduction of Xcc, and the available mitigation measures, APHIS evaluated five risk management options for the interstate movement of fresh citrus fruit from Florida and selected one. Its implementation began with a final rule that amended the regulations

---

[1] The classification of Xanthomonads was revised in 2006. The taxonomic designation for the pathogen that causes citrus canker disease is now *Xanthomonas citri* subsp. *citri* (Xcc); this pathogen was formerly known as *Xanthomonas axonopodis* pv. *citri* (Xac) **Euzeby, J. (2007).** Validation list no. 115. *International Journal of Systematic and Evolutionary Microbiology* **57**, 893-897, **Schaad, N. W., Postnikova, E., Lacy, G., Sechler, A., Agerkova, I., Stromberg, P. E., Stromberg, V. K. & Vidaver, A. K. (2006).** Amended classification of xanthomonad pathogens on citrus. *Systematic and Applied Microbiology* **29**, 690-695.

[2] The term "epidemiologically significant" refers to minimum conditions required for successful Xcc infection. Our judgment of whether fruit is an epidemiologically significant pathway for disease transmission is based on the likelihood that the fruit itself will be infected with the disease, that the infection will occur in a way or at a level sufficient for transmission of the disease, and that such an infected fruit will encounter the biological conditions required for transmission of the disease.

[3] "Commercially packed" is defined as fruit packed in a commercial packinghouse. A commercial packinghouse is defined as an establishment in which space and equipment are maintained for the primary purpose of disinfecting and packing citrus fruit for commercial sale. In addition, a commercial packinghouse must be licensed, registered, or certified with the State in which it operates and meet all the requirements for the license, registration, or certification that it holds.

published on November 19, 2007 and effective from that same date (FR, 2007). The regulations were amended to allow the interstate movement of fresh fruit under certain conditions. Those conditions include the following requirements:

- Packers must enter into a compliance agreement with APHIS;
- Fruit must receive an APHIS approved disinfectant treatment with sodium hypochlorite, peroxyacetic acid and/or sodium orthophenylphenate;
- Fruit must be inspected by an APHIS inspector at a rate such that the inspection has a 95 percent confidence of detecting a 0.38 percent infection rate based on visual inspection;
- Fruit is prohibited from distribution to U.S. commercial citrus producing States and Territories (American Samoa; Arizona; California; Florida; Guam; Hawaii; Louisiana; Northern Mariana Islands; Puerto Rico; Texas; and the Virgin Islands of the United States; referred to from here on as commercial citrus producing States); and
- Fruit containers must be labeled to indicate the distribution restriction.

The preamble to the November 19, 2007 Final Rule (FR, 2007) stated "If, in the future, evidence is developed to support a determination that commercially packed citrus fruit (both symptomatic and asymptomatic) is not an epidemiologically significant pathway for the introduction and spread of citrus canker, we would undertake rulemaking to amend our regulations accordingly."

Two recent publications provide additional research addressing key uncertainties that justifies revisiting our previous findings (USDA, 2007a; b). The first article, by Gottwald *et al.* (2009), documents research on the survival of Xcc on commercially packed citrus fruit and the likelihood that such fruit could serve as a mechanism to spread the disease. The second article, by Shiotani *et al.* (2009), documents research on the survival of Xcc on mandarin fruits and the likelihood of spread of Xcc to trees from harvested mandarins.

APHIS conducted an analysis to determine if the evidence in this new research, along with the existing body of evidence cited in our earlier analyses (USDA, 2007a; b), supports a finding that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for the introduction and spread of citrus canker disease and whether the citrus canker regulations for the movement of fruit should be amended accordingly. That analysis, "An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)" (USDA, 2009), is a supplement to the earlier pest risk analysis (PRA) "Evaluation of asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)" (USDA, 2007a) that, in part, informed previous regulatory decisions regarding the interstate movement of commercially packed and disinfected citrus fruit from citrus canker disease quarantine areas.

The original pest risk analysis (USDA, 2007a) concluded that "asymptomatic fruit is not epidemiologically significant as a pathway for introducing citrus canker when produced under the conditions of a systems approach." The evidence presented in Gottwald *et al.*

(2009) and Shiotani *et al.* (2009), along with the existing body of evidence, inform the risks associated with the movement of asymptomatic as, well as symptomatic, fruit as viable pathways for the introduction and spread of citrus canker disease . The original PRA (USDA, 2007a) did not focus on the risks associated with the interstate movement of symptomatic fruit, but the scientific literature analyzed in it is applicable to characterizing the risks associated with the movement of symptomatic fruit.

The new information of the Gottwald *et al.* (2009) and Shiotani *et al.* (2009) studies in conjunction with the evidence presented in the original PRA (USDA, 2007a), as well as its update (USDA, 2009), consistently argue that infected fruit is not epidemiologically significant as a pathway for introducing citrus canker disease.

The following relevant findings are presented in the PRA update (USDA, 2009): 1) post-harvest treatments reduce the viability of bacteria on fruit; 2) the viability of bacteria on fruit diminishes after it is harvested; 3) the low potential for spread from fruit to suitable hosts has now been reported by several sources (Civerolo, 1997; Gottwald *et al.*, 2009; Shiotani *et al.*, 2009); 4) rinds of infected fruit are unlikely to provide inoculum for disease if they have been discarded in the field at least 8 days; and 5) fruit parts, even those that are in direct contact with susceptible trees, are unlikely to spread the disease.

The updated PRA document concludes that:

- asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker disease, and
- symptomatic fruit subjected to a packinghouse process that includes treatment with disinfectants is also not epidemiologically significant as a pathway for introducing citrus canker disease.

Given those findings, APHIS determined that a review of the risk management measures specified in the current citrus canker disease regulations (Code of Federal Regulations, 2008b) and a supplement to the risk management analysis (RMA) (USDA, 2007b) that informed the regulations were justified.

# 2 Purpose and Scope

*Purpose.* This analysis reflects the adaptive risk management strategy adopted by APHIS in the previous rulemaking (FR, 2007) where the Agency stated "If, in the future, evidence is developed to support a determination that commercially packed citrus fruit (both symptomatic and asymptomatic) is not an epidemiologically significant pathway for the introduction and spread of citrus canker, we would undertake rulemaking to amend our regulations accordingly." This document is not intended to either describe the rulemaking/decision making process or any decision reached but rather to evaluate the scientific and technical conclusions of previous analytical documents (USDA, 2007a; b) in light of new information.

This document is intended to complement previous analytical documents; these other documents are:

- Evaluation of asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (USDA, 2007a);
- An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*) (USDA, 2009); and
- Movement of commercially packed citrus fruit from citrus canker disease quarantine area: Risk management analysis (USDA, 2007b).

The purpose of this document is 1) to provide APHIS decision makers with an evaluation of the impact of new evidence, in addition to the existing body of evidence, on the potential role of commercially packed and disinfected citrus fruit from citrus canker disease quarantine areas in spreading and establishing the citrus canker pathogen to areas previously free of that disease and 2) to develop a range of risk management options to be considered for revisions to APHIS regulations on the movement of fruit from regions quarantined for citrus canker disease based on this analysis as well as separate environmental and economic analyses.

*Uncertainties.* The previous PRA and RMA (USDA, 2007a; b) listed several key uncertainties around the epidemiological significance (or lack thereof) of Xcc associated with symptomatic and asymptomatic commercially packed citrus. Among those uncertainties were:

- Can symptomatic fruit that has been treated (with SOPP, chlorine, or other appropriate disinfectant) transmit the bacteria that cause the disease (*i.e.*, can disease be incited on healthy trees or seedlings from infected fruit that has been treated post-harvest)?
- How effective are different products at reducing the biological activity of bacteria in lesions (*i.e.*, what is the efficacy of various post-harvest treatments (*e.g.*, SOPP, chlorine, etc) at rendering symptomatic fruit not epidemiologically significant)?
- How long after post-harvest treatment can viable, pathogenic Xcc be recovered from infected fruit?
- Can wounds on harvested fruit serve as prolonged sources of inoculum for Xcc infection?

The new research in Gottwald *et al.* (2009) and Shiotani *et al.* (2009) provides additional evidence which addresses these key uncertainties and justifies revisiting conclusions made in the previous RMA (USDA, 2007b). The new evidence relates to several events identified as necessary for the pathogen to be introduced into a new area on disinfected citrus fruit and incite a disease outbreak. Along with the evidence included as part of the PRA update (USDA, 2009), two additional references are cited in this supplement: Golmohammadi *et al.* (2007) and Christiano *et al.* (2007). This supplemental RMA does not recreate or revise the entire body of evidence cited in the previous RMA (USDA,

2007b), but rather it builds on that body of evidence and evaluates those areas of evidence addressed by the new research.

*Assumptions*. APHIS made certain assumptions in conducting this RMA. Those assumptions include:

- The subject studies that prompted this analysis used citrus cultivars that represented the extremes of susceptibility from highly susceptible (grapefruit) to less susceptible varieties (lemon, mandarins). APHIS assumes cultivars not specifically studied would fall within this range of susceptibility and the results are therefore applicable to all citrus cultivars.
- The fruit that will be affected by the rule is intended for consumption. Fruit or fruit parts that are not consumed are discarded by consumers following standard disposal practices. These practices include placing in the trash intended for landfills, placing in compost heaps or flushing through trash disposal units.
- Vectors do not have a role in disease epidemiology and if they do, it is not subject to regulation (e.g., long distance dispersal of viable inoculum by birds).
- Phytosanitary practices are not assumed to be 100% effective but, in addition the measures required by regulation, other practices routinely employed in producing, packing and/or distributing commercially packed citrus, including the time it takes to complete the process from packhouse to consumer, may further reduce the epidemiological significance of infected fruit.
- Risk of introduction of citrus canker into other citrus producing states via the movement of commercially packed citrus from citrus canker quarantine areas is not assumed to be zero.
- We assume that previous Agency experience with successful prevention and safeguarding informs the likelihood of success of future actions.

*Scope.* This current document is limited in scope to a single pathway associated with this particular regulatory action relating to the potential spread of citrus canker disease: the domestic interstate movement of commercially packed and disinfected fresh citrus fruit from areas where the disease occurs to areas where the disease does not occur. Other pathways (e.g., the movement of citrus plants, the intentional movement of infected citrus fruit as an implement of bioterrorism, etc.) are outside the scope of this analysis. The scope of this document is further limited to technical and scientific issues as they are impacted by the new research of Gottwald *et al.*.(2009) and Shiotani *et al.* (2009). Analyses of social, environmental and economic issues, while relevant to the decision making process, are covered in separate analyses and are not treated in this document.

# 3 The Movement of Commercially Packed and Disinfected, Fresh Citrus Fruit as a Pathway for the Introduction of Xcc

This supplemental risk management analysis focuses on commercially packed and disinfected, fresh citrus fruit as a pathway for the introduction of Xcc. Previous analyses

(Schubert *et al.*, 1999; USDA, 1995; 2007a; b) concluded that the likelihood of introducing Xcc into citrus canker disease-free areas on commercially produced and packed citrus fruit is low for the following reasons:

1. Fresh citrus fruit is produced and harvested using techniques that reduce the prevalence of Xcc-infected fruit;
2. Symptomatic fruit are culled and all fruit are treated for epiphytic contamination by Xcc with disinfectants during commercial packing;
3. The mortality of Xcc associated with fresh citrus fruit and/or packing materials that occurs following harvest and packing;
4. For a successful Xcc infection that results in disease outbreaks an unlikely sequence of epidemiological events would have to occur; and
5. Large quantities of fresh citrus fruit have been shipped for many years from regions with Xcc to areas free of the pathogen without any reports of disease outbreaks linked to fresh fruit.

The following sections summarize the new evidence from Gottwald *et al.* (2009) and Shiotani *et al.* (2009) that addresses these conclusions and impacts uncertainties surrounding the ability of fresh citrus fruit produced in an Xcc infested grove to serve as a pathway for the introduction of Xcc into new areas (USDA, 2007a; b).

## 3.1 Fresh Citrus Fruit Production and Harvesting Techniques Reduce the Prevalence of Xcc-infected Fruit

Disease management practices in infested groves may reduce, but do not eliminate Xcc from the grove or from harvested fruit (USDA, 2007b). Commercially produced fruit harvested in Xcc infested areas may be visibly infected or may carry the pathogen on its surface or in wounds without expressing symptoms (USDA, 2007b).

Current production and harvesting practices seek to minimize citrus canker infection to maintain high quality, marketable fruit, which in turn can minimize potential Xcc inoculum associated with harvested fruit. Harvesting practices such as selectively picking fruit free of citrus canker disease lesions, *etc.*, may reduce the likelihood that symptomatic fruit is transported to the packinghouse (CHRP, 2006; Kinney, 2007). Gottwald *et al.* (2009) reported results from packing line experiments for grapefruit and lemon in which washate from symptomatic fruit produced the highest number of citrus canker disease lesions in bioassays for viable Xcc, compared to asymptomatic fruit and mixed asymptomatic and symptomatic fruit. Harvesting later in the growing season may also reduce Xcc inoculum levels found on harvested infected fruit (Gottwald *et al.*, 2009). Based on packing line results in Gottwald *et al.* (2009), "fewer Xcc bacteria were reisolated from naturally occurring fruit lesions in August compared to April, reflecting the effect of fruit age (and therefore lesion age) on inoculum. These results are supported by observations from Japan (Shiotani *et al.*, 2009) where artificially inoculated, symptomatic and aging Satsuma mandarin fruit developed very low levels of Xcc, with

only a small proportion of lesions producing any inoculum.

Infection of citrus fruit by Xcc, for example via wounding (defined as an injury to any external surface of the plant by its being torn, pierced, cut, or broken), during harvest or between harvest and the packinghouse is unlikely to occur. 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 & Bowman, 1929).

Mature, aboveground citrus tissues still attached to the tree can be infected through wounds (Gottwald *et al.*, 2002); however, susceptibility through wounds may be dependent upon tissue maturity and possibly the source of inoculum. The Asian leafminer (*Phyllocnistis citrella* Stainton) interacts with Xcc by providing wounds that serve as infection courts in leaves and fruit (Chagas *et al.*, 2001; Gottwald *et al.*, 2002; Schubert *et al.*, 2001), and can lead to significant field infection even on normally resistant citrus trees (Cook, 1988; Sinha *et al.*, 1972). Although leafminer damage increases susceptibility of the foliage (Christiano *et al.*, 2007), inoculum from symptomatic Xcc-infected fruit in cull piles did not result in transmission to susceptible 'Duncan' grapefruit trap plants with leafminer injury under natural conditions (Gottwald *et al.*, 2009).

In their studies, Gottwald *et al.* (2009) found that no harvested mature citrus fruit which had been wounded and inoculated with Xcc developed lesions. They further concluded wounds do not appear to provide sites for prolonged Xcc survival. That conclusion was supported by a 2006 study with inoculated grapefruit where recoverable numbers of total bacteria declined substantially within 8 days. A subsequent experiment in 2007 was too variable with time to fit a meaningful model. In that experiment, total bacterial counts recovered after an initial decline.

**SUMMARY**

- Disease management practices in the grove reduce, but do not eliminate, Xcc populations.
- Commercially produced fruit harvested in areas where Xcc exists may be visibly infected or the fruit may carry the pathogen either on its surface or in wounds.
- Citrus canker disease development on citrus fruit between harvest and packinghouse, via wounding, for example, is not likely.

## 3.2 Commercial Citrus Fruit Packing Techniques Reduce the Prevalence of Infected or Contaminated Fruit

The previous RMA (USDA, 2007b) concluded that routine procedures applied in packinghouses for cleaning and disinfecting fruit, along with culling and grading, reduce the prevalence of Xcc and the amount of Xcc inoculum associated with harvested fruit, thereby reducing phytosanitary risk. New studies explore additional packinghouse processes that may further reduce Xcc inoculum levels on fruit.

*Disinfectants.* In the previous RMA (USDA, 2007b), debris removal, washing, and disinfection of fruit were evaluated as measures in the packinghouse process which may further reduce Xcc inoculum on harvested infected or contaminated citrus fruit. Three compounds are currently approved by USDA for disinfection of fresh citrus fruit: chlorine (treat 2 minutes at 200 ppm sodium hypochlorite, pH 6.0-7.5), sodium orthophenylphenate (SOPP) (treat 45 seconds to 1 minute, depending on detergent concentration, SOPP at 1.86-2.0%), and peroxyacetic acid (PAA) (treat for 1 minute at 85 ppm of peroxyacetic acid) (Code of Federal Regulations, 2008a).

Several studies demonstrated the effectiveness of the disinfectants mentioned above in reducing numbers of Xcc cells or similar bacteria to low or undetectable levels (Brown & Schubert, 1987; Canteros *et al.*, 2000; Obata *et al.*, 1969; Verdier *et al.*, 2005). When processing includes a disinfectant, such as 200 ppm chlorine or SOPP, Xcc populations are significantly reduced to low or undetectable levels (Graham & Gottwald, 1991; Obata *et al.*, 1969). Washing, particularly before sanitizing treatments, removes organic matter and increases the effectiveness of sanitizing treatments, such as chlorine (Brown & Schubert, 1987), and reduces surface bacterial populations, including Xcc (Canteros *et al.*, 2000). Brushing during the washing procedure physically dislodges contaminants and pathogens from the surface of fruit (US-FDA, 1998). In laboratory tests in Argentina, Canteros *et al.* (2000) noted reductions of one to three orders of magnitude in the number of Xcc cells on the surface of artificially inoculated fruit when "fruits were prewashed as in a packinghouse".

In recent research, the most effective method for killing bacteria on 'Ruby Red' grapefruit surfaces employed prewashing with water plus detergent for 45 seconds followed by chlorine treatment for 45 seconds, which significantly reduced bacterial

populations (not limited to Xcc), although sample size was small (3 replications of five fruit per treatment) (Gottwald *et al.*, 2009). Treating fruit with chlorine alone did not significantly reduce bacterial populations recovered from symptomless 'Ruby Red' grapefruit. Bacteria (not limited to Xcc) were recovered after all treatments[4]. A similar experiment conducted with 'Lisbon' lemon fruit showed no significant differences in Xcc populations recovered after various packing line treatments[5]. The lemon results may be a consequence of low initial populations on tested fruit. Water prewash only, without subsequent disinfectant treatment or water plus detergent prewash without subsequent chlorine treatment were not studied, so it is difficult to assess whether chlorine impacts the efficacy of the prewash in reducing the number of bacteria recovered. In a study of 'Lisbon' lemon fruit held in cold storage, the number of lesions formed on bioassay plants was reduced by approximately 50 percent over untreated fruit when the lemons were treated with chlorine for 2 minutes followed by a detergent wash for 20 seconds, waxed and dried at 40°C for 100 seconds (Gottwald *et al.*, 2009). A statistically significant effect was not seen in other similar experiments with grapefruits (Gottwald *et al.*, 2009).

***Summary***

- Procedures for cleaning and disinfecting fruit are routinely applied by packinghouses.
- The individual efficacy of each of these procedures for removing or destroying Xcc may not be known in detail, but the effect of packinghouse treatments reduces the prevalence of viable Xcc and therefore the level of inoculum associated with commercially packed and disinfected fresh citrus fruit.
- Packinghouse treatments reduce the prevalence of Xcc and the level of inoculum associated with and disinfected fresh citrus fruit.
- Packinghouse processing that includes prewashing fruit with detergent over brushes followed by a disinfectant treatment further reduces amounts of Xcc inoculum on infected or contaminated fruit.

## 3.3 Mortality of Xcc Associated with Fresh Citrus Fruit and/or Packing Materials Following Harvest and Packing

[4] Grapefruit prewash treatments included: 1. non-treated check, 2. prewash with chlorine (200 µl/l at pH 7.0), 3. prewash with water followed by chlorine (200 µl/l at pH 7.0), and 4. prewash with water plus detergent (Fruit Wash 395, FMC Foodtech, Lakeleand, FL) followed by chlorine. The water and detergent washes were performed for 45 seconds on rotating soft bristled brushes. Fruit were treated with chlorine by immersion in a chlorine solution for 45 seconds (Gottwald *et al.*, 2009).

[5] Lemon prewash treatments included: 1. non-treated check, 2. chlorine (200 µl/l at pH 2.0) for 2 minutes, 3. chlorine for 2 minutes followed by detergent (Neutro Deter N Sinner, 2 %) for 20 seconds, 4. prewash with water followed by chlorine for 2 minutes, and 5. prewash with water followed by chlorine for 2 minutes followed by detergent for 20 seconds (Gottwald *et al.*, 2009).

This section documents evidence relating to the likelihood that Xcc on or in harvested fruit will survive commercial packing processes and shipping conditions and evaluates ways in which new evidence addresses our conclusions. The previous RMA (USDA, 2007b) concluded that: cool storage temperatures will restrict the ability of Xcc to reproduce and infect; Xcc bacteria do not increase in number on harvested fruit, but rather decline; and Xcc populations do not infect mature fruit or survive on mature fruit in appropriate numbers for long enough duration to infect new hosts.

*Xcc survival in lesions.* Bacteria survive in lesions formed on above-ground parts of susceptible hosts, including fruit (Leite & Mohan, 1990). Bacteria may survive for a few weeks to several months on decomposing plant litter on the soil surface (Civerolo, 1984; Gottwald *et al.*, 2002; Graham *et al.*, 1987; Leite & Mohan, 1990; Schubert *et al.*, 2001), or in buried plant material (Graham et al., 1987). Research by Belasque Jr. and Rodriguez-Neto (2000); Graham *et a*l. (1992); and Koizumi (1972) found the viability of bacteria on fruit and in lesions drops off once fruit is harvested. The multiplication of Xcc bacteria associated with lesions is closely related to lesion expansion and lesion age. Young lesions exude more Xcc bacteria than older lesions (Timmer *et al.*, 1991), and lesions resulting from late season infections fail to expand, remain small, and lack bacterial proliferation (Graham *et al.*, 1992), and thus are considered to be not an epidemiologically significant source of inoculum. This is corroborated by Gottwald *et al.* (2009) who isolated fewer Xcc bacteria from naturally-occurring fruit lesions on late season harvested fruit (August) compared to early season fruit (April).

Xcc populations do not increase on fruit once harvested, but rather populations decline within the lesions following harvest (Civerolo, 1981; Gottwald *et al.*, 2009; Koizumi, 1972). Fulton and Bowman (1929) found that there is apparently a marked difference between the behavior of Xac when inoculated into mature fruit attached to the tree as opposed to mature fruit removed from the tree. They speculated that physiological changes in the fruit were responsible for the difference and they noted changes to the fruit postharvest favor the growth of strongly saprophytic fungi that cause postharvest rots. They went on to say that it was not "inconsistent to presume that these changes would in equal degree hinder the development of an organism having definitely parasitic habits like *Pseudomonas citri* [Xac]."

In a 2007 study using naturally infected 'Ruby Red' grapefruit, Gottwald *et al*. (2009) concluded that viable Xcc populations generally declined with time when the fruit was stored at 5 to 8°C. However, in those experiments, general bacterial population data were variable and although bioassay plants formed no lesions when inoculated with washates from fruit stored for 21 days, there was no statistical difference among the pretreatment controls and the 21 day results. In a related experiment using the lemon cultivar 'Lisbon', there was a statistically significant reduction in both total bacterial populations and lesion formation in bioassays after infected fruit was stored for 7 days at 5 to 8°C (Gottwald *et al.*, 2009).

In another study, no infections developed in bioassays from inoculations using Xcc-infected 'Ruby Red' grapefruit peels left in the field for more than eight days (Gottwald *et al.*, 2009).

Based on transmission studies of Xcc from infected/contaminated Satsuma mandarin fruit to naval orange trees, Shiotani *et al.* (2009) suggested that "there are low numbers of bacteria within lesions on mature fruit of Satsuma mandarin" and "the bacteria appear to be short-lived after fruits are detached from the tree

*Epiphytic survival*. Epiphytic populations of Xcc may aid in pathogen dispersal, but substantial evidence indicates that bacterial populations do not infect mature fruit or survive on mature fruit long enough to infect other hosts. Shiotani *et al.* (2009) inoculated the surfaces of apparently healthy Satsuma mandarin fruits with a traceable strain of Xcc to monitor survival and dispersal of the pathogen in navel orange trees. Shiotani *et al.* (2009) demonstrated that after three days under natural conditions, no Xcc bacteria were recovered from the surface of the artificially contaminated fruit. No traceable Xcc was detected in rainwater traps beneath inoculated fruits in the grove and no lesions developed in trees. These results corroborate previous research that Xcc only survives for a limited amount of time [8-72 hours in sun and shade] (Goto, 1962; 1969). Bacterial suspensions of $10^6$ cfu/ml sprayed on asymptomatic fruit, disappeared after five days at room temperature under laboratory conditions (Belasque Jr. & Rodrigues-Neto, 2000). The survival of epiphytic Xcc populations on asymptomatic fruit under natural conditions would be expected to be further reduced due to harsher environmental conditions.

*Wounds*. Unlike a lesion, wounding does not imply disease development. Wounds inflicted on fruit during harvest or in the packinghouse are unlikely to become infected by Xcc. Shiotani *et al.* (2009) wound inoculated young fruits of Satsuma mandarin with a traceable strain ($1 \times 10^8$ cfu/ml) of Xcc and harvested them after 100 days. Six fruits were retained for sampling, and the strain was recovered from three of 14 lesions at bacterial populations which ranged from $2 \times 10^2$ to $3 \times 10^3$ cfu/lesion. Attempts to inoculate naval orange seedlings with the wound-inoculated fruit did not result in any infection or recovery of the strain from rain traps positioned under the inoculated fruit, suggesting that little or no exudation occurred and the potential for transmission is low.

In wound-inoculation studies, Gottwald *et al.* (2009) found injured fruit remaining on the tree sustained higher populations of bacteria longer than harvested fruit, although bacteria were not confirmed as Xcc. In their studies, Gottwald *et al.* (2009) found that no harvested mature citrus fruit which had been wounded and inoculated with Xcc developed lesions. They further concluded wounds do not appear to provide sites for prolonged Xcc survival. That conclusion was supported by a 2006 study with inoculated grapefruit where recoverable numbers of total bacteria declined substantially within 8 days. A subsequent experiment in 2007 was too variable with time to fit a meaningful model. In that experiment, total bacterial counts recovered after an initial decline.

*Effect of shipping and storage temperature and duration*. Temperatures during shipping and storage, as well as duration of storage influence Xcc inoculum survival. In a 2007

study using naturally infected 'Ruby Red' grapefruit, Gottwald *et al*. (2009) concluded that viable Xcc populations generally declined with time when the fruit was stored at 5 to 8°C. However, in those experiments, general bacterial population data were variable and although bioassay plants formed no lesions when inoculated with washates from fruit stored for 21 days, there was no statistical difference among the pretreatment controls and the 21 day results. In a related experiment using the lemon cultivar 'Lisbon', there was a statistically significant reduction in both total bacterial populations and lesion formation in bioassays after infected fruit was stored for 7 days at 5 to 8°C (Gottwald *et al.*, 2009).

Golmohammadi *et al.* (2007) were able to detect viable Xcc on symptomatic fresh citrus fruit in 11 of 15 shipments from Argentina and Uruguay to Spain. Shipments from which Xcc-infected citrus fruit were sampled were certified to have been treated with bactericides [chlorine or sodium orthophenylphenate (SOPP)]. These results indicate disinfection protocols are not 100 percent effective. Some samples were only positive by PCR protocols. The authors concluded this was probably due to the disinfection treatments which would reduce bacterial populations, and may induce the noncultivable state in the analyzed lesions. They further suggested that the bacterial cells in the lesions could be stressed after the fruit treatments (washing, disinfection, chemical treatments, transport, and storage at low temperatures for variable periods of time). Pathogenicity tests were successfully conducted by artificial inoculations but the epidemiological significance of these results was not evaluated.

***SUMMARY***

- The viability of bacteria on fruit and in lesions and wounds diminishes after the fruit is harvested.
- Epiphytic populations of Xcc may aid in pathogen dispersal, but substantial evidence indicates that bacterial populations do not infect mature fruit.
- Evidence indicates that wounds on harvested fruit containing Xcc inoculum do not lead to citrus canker lesion development, and Xcc populations generally decline, although wounds might occasionally retain Xcc populations that decline more slowly.
- The cool temperatures at which citrus fruit are stored and shipped, and duration of storage reduce the ability of Xcc to reproduce and cause infection.

## *3.4 Environmental and Epidemiological Conditions for Xcc Establishment*

This section evaluates evidence relating to the environmental and epidemiological conditions required for Xcc establishment and impact of new research related to introduction and establishment. Even if fruit with Xcc are shipped to a previously free region, introduction requires proximity of infected or infested harvested fruit carrying sufficient Xcc inoculum to a susceptible host, at a susceptible growth stage, under environmental conditions conducive to year-round survival, dispersal, and infection. Our

previous conclusion that establishment of Xcc is unlikely based on the specialized conditions necessary, is further strengthened by new evidence.

*Inoculum.* Xcc populations decline in lesions, wounds, and surfaces of harvested fruit see discussion above). The decline in Xcc populations on surfaces (in general) coupled with the Xcc population size necessary to cause infection creates a limited window of time when surface populations are high enough to potentially infect susceptible host tissue. Even if Xcc survives shipment, its ability to cause infection is considered low because of the required combination of parameters (*e.g.*, susceptible host material, proper moisture and temperature, and presence of vectors, such as wind) needed for successful infection. In all of the experimental scenarios presented by Gottwald *et al.* (2009), including packing line treatments; infection from diseased fruit in cull piles, diseased citrus peels, or diseased fruit smashed onto healthy seedlings; and survival of Xcc in wounds; no exposed fruit ever developed new Xcc lesions and only one leaf on a susceptible 'Duncan' grapefruit seedling under extreme conditions developed an Xcc lesion. In studies of inoculum longevity by Shiotani *et al.* (2009), after three days in the field, no lesions developed in bioassays. Contaminated fruit discarded in citrus groves also decay rapidly (greater than 50 percent rotted by 21 days), further limiting their ability to act as sources of Xcc inoculum (Shiotani *et al.*, 2009).

*Transmission studies.* In conventional Florida commercial packinghouses, citrus fruit is graded at several points primarily after washing (Miller *et al.*, 2001). The culled fruit is removed from the packing line and transferred to bins or trucks for secondary uses such as processing for juice or livestock feed. Citrus fruit disposed of by consumers (including importing agents, retailers, *etc.*), may serve as a source of inoculum for nearby host material. To investigate the likelihood of this scenario, Gottwald *et al.* (2009) studied the transmission of Xcc from unprocessed, infected 'Ruby Red' grapefruit and 'Lisbon' lemon and packinghouse processed 'Ruby Red' grapefruit in cull piles to 'Duncan' grapefruit seedlings during natural weather events. During the course of the experiments, citrus canker lesions did not develop on the grapefruit seedlings (488 seedlings total) surrounding diseased fruit, in spite of extensive leafminer damage present on some of the seedlings. Xcc bacteria were not detected in assays of the foliage. Upon the completion of the experiments, the lesions on the fruit in the cull piles were assayed for viable Xcc with none detected (Gottwald *et al.*, 2009). These results suggest that very specific conditions must be met for an Xcc outbreak to occur. Because survival of Xcc on discarded fruit is also limited, commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for introducing citrus canker disease.

Gottwald *et al.* (2009) repeated the cull pile experiment to see if transmission of Xcc from infected, unprocessed 'Ruby Red' grapefruit fruit is possible under simulated extreme wind and rain conditions. Infected fruit were either placed in a cull pile or suspended by vertical strings. One seedling 0 m downwind from the cull pile became infected when subjected to the highest wind speed (25 m/s) and simulated rain, developing one lesion on a single leaf injured by the action of the high-speed fan. The other 191 plants in the study did not develop Xcc lesions. No Xcc lesions developed on the 192 plants placed at the same distance and subjected to the same wind speed (0, 10,

and 25 m/s with water) from Xcc-infected grapefruit suspended from string. Xcc was recovered from one collection screen set up 2 m from suspended fruit but no Xcc was recovered from the other 144 collection screens set up at various distances (0 to 10 meters) from cull piles or suspended fruit.

In another experiment, Gottwald *et al.* (2009) placed processed diseased grapefruit in piles and monitored bacterial populations on the infected fruit after dew and rain to determine how likely Xcc would spread to nearby hosts. They recovered low numbers of Xcc from three of the approximately 37 assays. In one lesion assayed, they recovered 6.4 x $10^2$ cfu. From the surface of one infected fruit, they recovered 1.33 x $10^1$ and 7.67 x $10^1$ cfu/ml on two separate occasions however no adjacent susceptible trees were infected.

Shiotani *et al.* (2009) were unable to observe Xcc transmission from inoculated (soaked in 1 x $10^6$ cfu/ml or pin-pricked with 1 x $10^8$ cfu/ml) mature Satsuma mandarin fruit suspended in polypropylene net bags in naval orange trees. They were also unable to recover bacteria from rainwater collected in traps placed below the bagged fruit in the trees. From their studies they concluded that "…there are low numbers of bacteria within lesions on mature fruit of Satsuma mandarin. The bacteria appear to be short-lived after fruits are detached from the tree. This is demonstrated by the inability to reculture the bacteria from contaminated surfaces in the field, and the lack of infection among susceptible hosts located close to infected fruit". After a typhoon moved through a heavily infested orchard, asymptomatic fruit did not develop lesions despite the conducive environment, indicating that maturing fruit are not as prone to infection (Shiotani *et al.*, 2009).

Citrus peel does not appear to play an important role in the dispersal of viable Xcc. Gottwald *et al.* (2009) were unable to recover viable Xcc (based on bioassay) from diseased 'Ruby Red' grapefruit peels when placed in the field for 8 days. Since peels were sampled on day 1 and at weekly intervals thereafter, viability was lost after more than 1 day and less than or equal to 8 days. No infection of susceptible 'Duncan' grapefruit seedlings occurred after being splashed by pieces of fruit fragments and juice of infected ‘Ruby Red’ grapefruit (2009). "A total of 300 infected fruit were spattered over 300 plants and no canker lesions developed, and no Xcc bacteria were isolated from the surface of these plants.” The washate assay was done for only one of the two repetitions and involved plating washate on KCB medium, not inoculating susceptible seedlings.

There are no accounts in the published literature indicating that fresh citrus fruit or seeds have resulted in introduction of Xcc to new areas. There are few instances where the origins of citrus canker disease outbreaks have been conclusively demonstrated. Where origins have been reported or suggested, imported or smuggled trees and budwood are reported as the source of infection. 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).

The results of the new research (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009) indicate that both asymptomatic and symptomatic fruit commercially packed and disinfected are not

an epidemiologically significant pathway for the transmission of Xcc in the natural environment. Xcc developed a single lesion on a leaf of a susceptible tree at a wound site, from Xcc bacteria transmitted from the fruit, in only one experiment under extreme conditions. In this case, the fruit were severely infected and subjected to extreme conditions of 25 m/sec wind with water. Even in this scenario, the fruit was only able to infect a tree immediately adjacent to the highly infested source and only when the untreated fruit pile was elevated from the ground.

***SUMMARY***

- As a condition for successful establishment, Xcc in amounts sufficient to cause infection, must encounter not only an environment with a conducive temperature, relative humidity, moisture, and wind for infection, but also must encounter host plant tissue that is either at a susceptible growth stage or is wounded and then must successfully enter this tissue.
- Despite substantial international trade between Xcc-infected and noninfected countries, there is no authenticated record of movement of diseased fruit or seeds resulting in the introduction of Xcc to new areas.

## *3.5 Conclusions and Summary of Evidence Regarding Fruit as a Pathway for Xcc Introduction*

Two recently published scientific articles have added to the existing evidence related to the epidemiological significance of citrus fruit as a pathway for introducing the citrus canker disease bacterium into new areas.

Gottwald *et al*. (2009) considered the epidemiological significance of infected fruit following postharvest treatment. Based on a series of experiments, the authors concluded prewashing the fruit to remove dirt and debris reduced surface bacterial populations; recovery of Xcc from fruit surfaces increased when active citrus canker lesions were present but the quantity of viable Xcc declined as fruit remained in cold storage, or as they aged on the trees; In one experiment, bioassays demonstrated that as fruit senesce and lesions age, the ability of fruit to generate or sustain Xcc bacteria was increasingly compromised; in one experiment, the packing line process reduced canker lesion activity by as much as 50% compared to unprocessed fruit; Discarded canker-infected fruit in cull piles was ineffective as a source of inoculum however, with severely infected piles of culled fruit subject to extreme simulated wind and rain conditions, only a single lesion, associated with leaf injury, developed on a trap plant immediately downwind of the cull pile, suggesting a low risk of spread. In summary, the authors concluded that, taken as a group, this series of experiments demonstrates that "harvested and packinghouse-disinfested citrus fruit are not an epidemiologically significant pathway for Xcc to reach and infect susceptible citrus and become established in canker-free areas."

The authors of the second article (Shiotani *et al.*, 2009) explored the survival of Xcc on and its dispersal from infected Satsuma mandarin fruit. From their studies they concluded bacterial numbers on the contaminated fruits decreased significantly in the first 3 days after fruits were discarded; there was no detectable spread of Xcc from contaminated fruit into rainwater collected beneath the fruit; populations of Xcc present in contaminated fruit were not transferred to susceptible hosts, even when the fruits were placed in close proximity to hosts that were in a receptive stage. They summarized the studies by saying, "There was no spread of X. citri pv. citri in this study, indicating that there is a low risk transmission of this bacterium."

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 Xcc. 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 Xcc.

In conducting this analysis, APHIS considered multiple lines of evidence including, but not limited to, evidence from the two recent studies (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009) and the 2007 RMA (USDA, 2007b) in concluding that commercially packed and disinfected fresh citrus fruit is not an epidemiologically significant pathway for the introduction and spread of Xcc:

- Disease management practices in the grove reduce, but do not eliminate, Xcc populations.
- Commercially produced fruit harvested in areas where Xcc exists may be visibly infected or the fruit may carry the pathogen either on its surface or in wounds.
- Citrus canker disease development between harvest and packinghouse, via wounding for example, is not likely.
- Procedures for cleaning and disinfecting fruit are routinely applied by packinghouses.
- The individual efficacy of these procedures for removing or destroying Xcc may not be known in detail, but the effect of packinghouse treatments reduces the prevalence of viable Xcc and therefore the level of inoculum associated with commercially packed and disinfected fresh citrus fruit.
- Packinghouse processing that includes a disinfectant treatment further reduces amounts of Xcc inoculum on infected or contaminated fruit.
- The viability of bacteria on fruit and in lesions and wounds diminishes after the fruit is harvested.
- The viability of Xcc bacteria which survive the packing process will further diminish during shipping.
- Epiphytic populations of Xcc may aid in pathogen dispersal, but substantial evidence indicates that bacterial populations do not infect intact mature fruit.

- Evidence indicates that wounds on harvested fruit containing Xcc inoculum do not lead to citrus canker lesion development, and Xcc populations generally decline, although wounds might occasionally retain Xcc populations that decline more slowly.
- The cool temperatures at which citrus fruit are stored and shipped, and duration of storage reduce the ability of Xcc to reproduce and cause infection.
- As a condition for successful establishment, Xcc in amounts sufficient to cause infection, must encounter not only an environment with a conducive temperature, relative humidity, moisture, and wind events for infection, but also must encounter host plant tissue that is either at a susceptible growth stage or is wounded and then must successfully enter this tissue.
- Despite substantial international trade between Xcc-infected and noninfected countries, there is no authenticated record of movement of diseased fruit or seeds resulting in the introduction of Xcc to new areas.

# 4 Risk Management Options

As a consequence of the 2006 amendment to the citrus canker disease regulation APHIS evaluated the epidemiological significance of commercially packed fresh citrus fruit as a pathway for the introduction of Xcc to new areas. In 2007, APHIS concluded that while commercially packed and disinfected citrus fruit was not an epidemiologically significant pathway for introducing Xcc, it was not possible to design an operationally practicable system that ensured only uninfected fruit would be shipped from quarantined areas, and there was insufficient evidence to adequately remove uncertainties surrounding the ability of fresh citrus fruit produced in an Xcc infested grove to serve as a pathway for the introduction of Xcc into new areas (USDA, 2007b).

New research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009), summarized above, provides additional evidence that addresses key uncertainties and justifies revisiting our risk management findings. The following five options for revised risk management measures were developed by APHIS considering both the newly published research and the existing body of evidence summarized in the previous analyses conducted by APHIS (USDA, 2007a; b). The efficacy of the various management options is not evaluated from a baseline but rather from the standpoint of the degree to which current restrictions should be adjusted in light of new information.

## *4.1 Option 1 – Unrestricted movement*

- Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States including commercial citrus-producing States.
- No phytosanitary treatment required.
- No phytosanitary inspection required.

If fruit is not an epidemiologically significant pathway for citrus canker disease, the rationale for quarantine regulations is limited to other pathways, such as nursery stock or deliberate movement of the pathogen. 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 (USDA, 2007a; b). This evidence suggests that fresh citrus fruit, when commercially packed and disinfected, is not an epidemiologically significant pathway. 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).

For an outbreak of citrus canker disease to occur, several critical factors must coincide. Infected or contaminated fruit must be discarded in such a way that viable Xcc survives in sufficient amounts to cause infection. The bacteria must encounter an environment with a temperature, relative humidity, and rain events conducive to infection, and also must encounter host plant tissue that is either at a susceptible growth stage or is wounded to allow viable bacteria to successfully enter this tissue in sufficient numbers to incite infection [see discussions in (USDA, 2007a; b)]. New research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009) has added significant evidence that commercial citrus fruit is not a pathway for the introduction of citrus canker disease.

The research published by Gottwald *et al.* (2009) concludes that even under highly conducive conditions with susceptible hosts, successful transmission of Xcc is unlikely. The authors concluded, "…that harvested and packinghouse-disinfested, citrus fruit with canker lesions are an unlikely pathway through which *Xcc* inoculum might lead to infection and *Xcc* establishment in new areas". The experiments addressed the possibility infected fruit escaping the grading process, once discarded might act as a source of inoculum for spread to canker-free locations. The authors found "Culled piles of fruit or discarded fruit do not appear to be significant sources of inocula for infection of susceptible citrus in the field during normal or simulated extreme weather events."

Shiotani *et al.* (2009) also reported that contaminated fruit did not result in infection in the field under natural conditions, nor was viable Xcc detectable on contaminated fruit after brief field exposure to nearby inoculum sources. The authors concluded "…data indicate that mature fruit is not a significant risk as a source of bacteria for infection of citrus, and the epidemiological significance of lesions or surface contamination on mature fruit in the dispersal and spread of bacteria of *X. citri* pv. *citri* is probably minimal."

The evidence presented in the new research is compelling and consistent with a significant body of previous research and observations considered by APHIS. APHIS

concurs with the findings of these studies. APHIS notes, however, that uncertainties remain regarding the epidemiological significance of *untreated* fruit. In the Gottwald *et al.* (2009) study, this uncertainty is linked to the relatively small number of experimental units. This increases the uncertainty and variability of statistical inferences, especially in the single case where transmission occurred from untreated fruit in a simulated wind/rain dispersion experiment. It should be noted that as the authors stated, "The simulated extreme weather cull pile experiment was a highly contrived situation designed to provide every possible opportunity for dispersal of *Xcc* and would be unlikely to occur in most areas, except those locations where hurricanes or tropical storms are common occurrences. The fruit had been individually chosen for severe disease (all fruit had multiple lesions) and it must be stressed they were not processed through the packing line (which appears to reduce lesion activity substantially)."

Gottwald *et al.* (2009) suggests Xcc infected fruit, even if heavily infected and subjected to optimal conditions for infection, is not a likely pathway for the establishment of the disease, and the study concludes "…*packinghouse-disinfested*, citrus fruit with canker lesions are an unlikely pathway through which Xcc inoculum might lead to infection and Xcc establishment in new areas (italics added for emphasis)." The Shiotani *et al.* (2009) study used larger numbers of test plants/fruit than the Gottwald *et al.* (2009) study. But where the Gottwald *et al.* (2009) used grapefruits and lemons representing a range of susceptibilities to Xcc, the Shiotani *et al.* (2009) studies were limited to Satsuma mandarins, a citrus variety highly resistant to citrus canker disease. Thus, the uncertainty regarding the epidemiological significance of untreated Satsuma mandarins is very low.

## 4.2 Option 2 – Unlimited distribution, disinfectant

- 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 with APHIS-approved disinfectant.
- No packinghouse APHIS inspection of every lot of finished fruit required.

Option 2 would amend the citrus canker regulations to allow the movement of commercially packed fresh citrus fruit to all U.S. States when treated with an APHIS approved disinfectant treatment.

Substantial evidence exists that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for introducing Xcc to citrus canker disease-free regions (see discussion in Option 1). The additional evidence provided by the two recent publications (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009) strengthens the determination that commercially packed and disinfected fresh citrus fruit is not an epidemiologically significant pathway for the introduction and spread of Xcc. Gottwald *et al*, (2009) were unable to initiate infection when canker infected grapefruit or lemon fruit was placed in cull piles in close proximity to grapefruit seedlings and exposed to natural rain events.

These results suggest that very specific conditions must be met for an Xcc outbreak to occur, making commercially packed and disinfected citrus fruit not an epidemiologically significant pathway for introducing citrus canker disease.

Shiotani *et al.* (2009) were unable to transmit Xcc from inoculated mature Satsuma mandarin fruit suspended in naval orange trees. They were also unable to recover bacteria from rainwater collected in traps placed below the bagged fruit. From their studies they concluded that "there are low numbers of bacteria within lesions on mature fruit of Satsuma mandarin. The bacteria appear to be short-lived after fruits are detached from the tree." Even after a typhoon moved through a heavily infested orchard, fruit did not develop lesions.

Pathways by which citrus fruit could introduce Xcc, though unlikely, are possible under extreme or contrived scenarios. The probability of such introductions has not been quantified in the sense that a specific numerical value or even a range of values was not calculated. Quantifying all possible, contrived, rare events is not realistic.

In the Gottwald *et al.* (2009) studies, while transmission of Xcc was never achieved under natural conditions, a single grapefruit plant was infected under simulated conditions of high wind and rain using untreated, highly infected fruit as the inoculum source. It should be noted that the "cull pile experiment was a highly contrived situation designed to provide every possible opportunity for dispersal of *Xcc* and would be unlikely to occur in most areas, except those locations where hurricanes or tropical storms are common occurrences" (Gottwald *et al.*, 2009). The fruit used as the inoculum source would be culled during normal grading in the packinghouse, would have received a disinfectant treatment and would not likely be discarded to an elevated position directly adjacent to suitable tissues.

However, this single incident of Xcc transmission could be construed as evidence that such transmission is possible, especially given the low numbers of test plants in the experiment. The low numbers of test plants in the experiment (however contrived) could be used to highlight variability. The Gottwald *et al.* (2009) study suggests Xcc infected fruit, even if heavily infected and subjected to optimal conditions for infection, is not a likely pathway for the establishment of the disease when the fruit is packinghouse disinfected. Recognizing the evidence that fruit is not an epidemiologically significant pathway to introduce Xcc and acknowledging uncertainties regarding untreated fruit, Option 2 proposes a mandatory packinghouse disinfectant treatment of fruit while removing the current prohibition of shipments to commercial citrus producing States and the requirements for phytosanitary packinghouse inspections by APHIS. In this approach, citrus growers, harvesters, and packers would be given the flexibility to implement phytosanitary measures that prevent and control Xcc infection in the fruit they produce.

There is abundant evidence (USDA, 2007a; b) that shows that packinghouse treatments destroy surface bacteria and reduce the viability of all bacteria on fruit. Studies by Gottwald *et al.* (2009) support earlier studies (Brown & Schubert, 1987; Canteros *et al.*,

2000) that found washing fruit prior to disinfectant treatments is more effective at reducing surface bacterial populations, including Xcc, than disinfectant alone or washing after disinfection. Prewashing with water plus detergent for 45 seconds followed by chlorine treatment significantly reduced bacterial populations (not limited to Xcc) by more than half, although bacteria (not limited to Xcc) were still recovered after treatments (Gottwald *et al.*, 2009).

If Option 2 is selected, APHIS would determine whether to continue to require the currently approved disinfectant treatments [2 minutes at 200 ppm sodium hypochlorite, pH 6.0-7.5; 45 seconds to 1 minute sodium orthophenylphenate (SOPP) at 1.86-2.0 percent; and 85 ppm for at least 1 minute peroxyacetic acid (PAA)] (Code of Federal Regulations, 2008a), or apply modifications based on recent research. In Gottwald *et al.* (2009), the authors found that the "…effectiveness of packing line decontamination can be increased by using prewashing treatment that includes detergent (such as SOPP) and brushing to remove dirt and debris that reduce the effectiveness of the disinfestants" and that chlorine treatments alone, had either no or a slight effect. As with the transmission studies, statistical inferences are complicated by the small test populations employed thereby increasing uncertainty and variability regarding the value of pre-wash. Nevertheless, the effectiveness of prewash is supported by observations that plant debris, soil and field trash will bind with any treatment agent, diluting its effectiveness. Washing removes organic matter and increases the effectiveness of disinfection treatments, such as chlorine (Brown & Schubert, 1987). The uncertainty that remains is the determination of whether a prewash has any epidemiological significance. That is, whether it plays any role in reducing the likelihood of disease introduction.

APHIS personnel conducted an informal survey of 134 Florida packinghouses to determine the potential operational impacts of adding a prewash requirement to APHIS approved fruit disinfection treatments. They found 9 of the 134 surveyed packinghouses currently have a prewash with detergent prior to disinfectant treatment and 6 of 134 of the packinghouses surveyed use a detergent prewash with mechanical roller brushes prior to disinfectant treatment.

## *4.3 Option 3 – Two-tier distribution requirements*

- Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. States except commercial citrus-producing States with packinghouse treatment using an APHIS-approved disinfectant treatment.
- Allow distribution of all types and varieties of *commercially packed* citrus fruit to all U.S. citrus-producing States with packinghouse treatment using an APHIS-approved disinfectant treatment and an additional requirement for phytosanitary inspection that ensures that blemished fruit (*or fruit with lesions*) are minimized.

Option 3 would amend the citrus canker disease regulations to allow the movement of commercially packed fresh citrus fruit to all U.S. States under a two-tiered approach. For

distribution to all U.S. States except commercial citrus-producing States, APHIS would require fruit to be commercially packed and disinfected according to an APHIS-approved disinfection treatment. The fruit would then be eligible to move, under limited permit, to any State except the designated commercial citrus-producing States.

Packers wishing to ship to commercial citrus-producing States would be required to satisfy additional conditions. To be considered certified for movement to all U.S. States, fruit would be required to undergo phytosanitary inspection after disinfection using an APHIS approved treatment and commercial packing. The inspection would be to prevent any fruit showing citrus canker lesions from being certified using a protocol similar to if not identical to the inspection required by the current regulation (Code of Federal Regulations, 2008b). APHIS' updated PRA (USDA, 2009) concluded that a requirement for minimal presence of lesions on fruit (*i.e.,* inspection) may be justifiable only when typical packinghouse processes are unavailable.

As already noted, substantial evidence suggests that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway to introduce Xcc to regions free of the pathogen. The evidence and uncertainties for this option regarding the epidemiological significance of commercially packed and disinfected citrus fruit are very similar to those discussed in Option 1 and again in Option 2. Likewise, the efficacy of disinfection methods and the significance of the recent studies by Gottwald *et al.* (2009) have been discussed in Option 2. The efficacy of inspection to detect Xcc infected fruit has been discussed in previous analyses (USDA, 2007a; b). Additional evidence for the efficacy of inspection can be gleaned from the successful performance of the current packinghouse inspection protocols.

Option 3 recognizes that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for the introduction of Xcc by allowing for distribution to all U.S. States. It differs from Options 1 and 2 in taking a more stringent approach to further mitigate uncertainties for fruit shipped to commercial citrus producing States by requiring a packinghouse inspection of finished fruit in addition to an APHIS-approved disinfectant treatment.

## 4.4 Option 4 – Limited distribution (all varieties) to non-commercial citrus producing States, disinfectant

- Allow distribution of all types and varieties of *commercially packed* citrus fruits to all U.S. non-commercial citrus producing States.
- Prohibit distribution of all types and varieties of citrus fruit to U.S. commercial citrus-producing States.
- Require packinghouse treatment of citrus fruit with APHIS-approved disinfectant.
- No phytosanitary inspection required.

Option 4 includes requirements for commercial packing and treatment with an approved disinfectant and further mitigates the risk of Xcc introduction to areas at risk by prohibiting the distribution of all types and varieties of citrus fruit from areas with citrus canker disease to U.S. commercial citrus-producing States. Option 4 would change the current regulations by removing the packinghouse inspection requirements.

At the time of the previous APHIS analyses (USDA, 2007a; b), substantial evidence already existed to suggest that commercial citrus fruit was not an epidemiologically significant pathway to introduce Xcc to new areas. What was lacking were direct experiments to explore the potential for harvested infected or contaminated citrus fruit to act as the inoculum source for transmission of the bacterium to healthy trees under field conditions.

Gottwald *et al.* (2009) and Shiotani *et al.* (2009) provided those types of experiments and the evidence from them has already been discussed in the preceding options as have the uncertainties around the results of these studies. The Gottwald *et al.* (2009) study concluded "…*packinghouse-disinfested*, citrus fruit with canker lesions" (emphasis added) is an unlikely pathway. Shiotani *et al.* (2009) were unable to transmit Xcc from inoculated mature Satsuma mandarin fruit suspended in naval orange trees. They were also unable to recover bacteria from rainwater collected in traps placed below the bagged fruit. From their studies they concluded that "there are low numbers of bacteria within lesions on mature fruit of Satsuma mandarin. The bacteria appear to be short-lived after fruits are detached from the tree." Even after a typhoon moved through a heavily infested orchard, fruit did not develop lesions.

In the Gottwald *et al.* (2009) study, test plant/fruit populations were small, increasing the uncertainty and variability of statistical inferences, especially in the single case where transmission did occur in a simulated wind/rain dispersion experiment using elevated, untreated, highly infected fruit placed directly adjacent to susceptible tissues. The Shiotani *et al.* (2009) study used larger numbers of test plants/fruit than the Gottwald *et al.* (2009) study, however the studies were limited to Satsuma mandarins, a citrus variety highly resistant to citrus canker disease.

Recognizing the potential for fruit with visible symptoms of Xcc to reach commercial citrus-producing States and any uncertainties regarding fruit as a pathway, as discussed in the previous paragraph, Option 4 takes the most restrictive measure- prohibiting distribution- for shipments to commercial citrus-producing States and requires an APHIS-approved disinfectant treatment for shipments to all non-commercial citrus producing States.

## 4.5 Option 5 – No change

- Leave the regulations unchanged.

Option 5 is the most restrictive option. It leaves the current regulations in place and unchanged including the requirements for mandatory packinghouse treatment of citrus fruit with APHIS approved disinfectant and phytosanitary inspection, by APHIS, of finished fruit combined with a limited distribution requirement that excludes shipment to U.S. citrus-producing States. Option 5 completely discounts the additional evidence from epidemiological studies recently conducted in Japan, Florida and Argentina (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009).

## 4.6 Conclusion

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. New evidence considered indicates that prohibition of the interstate movement of disinfected, commercially packed citrus fruit with or without symptoms of citrus canker disease to non commercial citrus-producing States and, perhaps, commercial citrus-producing States may not be scientifically justified.

In the previous RMA (USDA, 2007b), APHIS determined that although commercially packed and disinfected citrus fruit was an unlikely pathway for transmitting and establishing citrus canker disease, there was insufficient evidence to adequately remove uncertainties surrounding the ability of fresh commercially packed and disinfected citrus fruit produced in an Xcc infested grove to serve as a pathway for the introduction of Xcc into new areas.

In this supplemental RMA, based on the new research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009), APHIS determined that the level of uncertainty about the role of citrus fruit in transmitting and establishing citrus canker disease is reduced. APHIS concluded that although citrus fruit may remain a conceptually possible pathway for transmitting and establishing citrus canker disease, research shows that extreme, artificial conditions are required to successfully transmit the pathogen from infected fruit to a susceptible host, and even under these extreme conditions, transmission is rare. APHIS concluded that commercially packed and disinfected citrus fruit is not an epidemiologically significant pathway for transmitting and establishing citrus canker disease in regions currently free of Xcc.

Based on these conclusions, the supplemental RMA identifies several options for revising APHIS' regulations on the interstate movement of citrus fruit from regions quarantined for citrus canker disease. Option 1 is the least restrictive option and would remove all current restrictions on the movement of citrus fruit from areas quarantined for citrus canker disease. A substantial body of evidence suggests that citrus fruit, even if untreated with disinfectants, does not serve as an epidemiologically significant pathway for the introduction of Xcc. The recent research by Gottwald *et al.* (2009) and Shiotani *et al.* (2009) support this conclusion. Even so, the Gottwald *et al.* (2009) study only goes so

far as to conclude "*packinghouse-disinfested*, citrus fruit with canker lesions" are an unlikely pathway. Option 2 echoes the conclusion of the Gottwald *et al.* (2009) study and would require an APHIS-approved packinghouse disinfection treatment while allowing distribution to all U.S. States. Option 3 would require an APHIS-approved packinghouse disinfection treatment and would take a more stringent approach differentiating between the risk to non-citrus producing States and commercial citrus-producing States by adding the additional requirement of an inspection for fruit shipments to the latter. Option 4 would be more restrictive still and prohibits altogether fruit shipments to commercial citrus-producing States. Option 5 completely discounts the additional evidence from epidemiological studies recently conducted in Japan, Florida and Argentina (Gottwald *et al.*, 2009; Shiotani *et al.*, 2009) and would leave the current regulations unchanged.

## Prepared by:

R. Fite, Chief, USDA-APHIS-Policy & Program Development (PPD)-Risk Analysis Systems (RAS)
L. M. Ferguson, Plant Pathologist, USDA-APHIS-Plant Protection & Quarantine (PPQ)-Center for Plant Health Science & Technology (CPHST)-Plant Epidemiology & Risk Analysis Laboratory (PERAL)
A. Lemay, Senior Analyst, USDA-APHIS-PPD-RAS
E. Podleckis, Senior Analyst, USDA-APHIS-PPD-RAS

Technical contact: E. Podleckis

## Contributors:

P. Gomes, National Coordinator, USDA-APHIS-PPQ, Citrus Health Response Program (CHRP)
R. Griffin, Director, USDA-APHIS- PPQ, CPHST, PERAL
W. Gutierrez, Plant Pathologist, USDA-APHIS- PPQ, CPHST, PERAL
T. Holtz, Risk Analyst, USDA-APHIS- PPQ, CPHST, PERAL
M. Hornyak, Federal Director, USDA-APHIS-PPQ, CHRP
D. Munyan, Deputy Director, USDA-APHIS-PPQ, CHRP
S. Poe, Senior Plant Pathologist,USDA-APHIS-PPQ, Emergency and Domestic Programs
R. Sequeira, National Program Science Leader, Risk Analysis, USDA-APHIS-PPQ, CPHST

# 5 Literature Cited

**Belasque, J. & Rodriguez Neto, J. (2000).** Survival of the citrus canker bacterium in non-infected orange fruits. *Summa Phytopathologica* **26**, 128 (Resumo 153).

**Belasque Jr., J. & Rodrigues-Neto, J. (2000).** Survival of the citrus canker bacterium in non-infected orange fruits. *Summa Phytopathological* **26**, 128.

**Brown, G. E. & Schubert, T. S. (1987).** Use of *Xanthomonas campestris* pv. *vesicatoria* to evaluate surface disinfectants for canker quarantine treatment of citrus fruit. *Plant Disease* **71**, 319-323.

**CABI/EPPO (1997).** *Xanthomonas axonopodis* pv. *citri*. In *Quarantine Pests for Europe (second edition): Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization*. Wallingford, UK: CAB International.

**Canteros, B. I., Naranjo, M. & Rybak, M. (2000).** Production of fruits free of *Xanthomonas axonopodis* pv. *citri* in selected plots in areas of endemic canker in Argentina. *Proc Int Soc Citriculture* **IX Congreso**, 1136-1137.

**Chagas, M. C. M., Parra, J. R. P., Namekata, T., Hartung, J. S. & Yamamoto, P. T. (2001).** *Phyllocnistis citrella* Stainto (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* in Brazil. *Neotropical Entomology* **30**, 55-59.

**Christiano, R. S. C., Dalla Pria, M., Jesus Junior, W. C., Parra, J. R. P., Amorim, L. & Bergamin Filho, A. (2007).** Effect of citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. *Crop Protection* **26**, 59-65.

**CHRP (2006).** Citrus Health Response Plan (CHRP): State of Florida 2006-2007: USDA/APHIS and FDACS/DPI.

**Civerolo, E. L. (1981).** Citrus bacterial canker disease: An overview. *Proceedings of the International Society of Citriculture* **1**, 390-394.

**Civerolo, E. L. (1984).** Bacterial canker disease of citrus. *J Rio Grande Valley Hort Assoc* **37**, 127-146.

**Civerolo, E. L. (1997).** Risk of transmission of *Xanthomonas campestris* (=*axonopodis*) pv. *citri* on commercial citrus fruit. In *21st NAPPO Annual Meeting: Colloquium on Quarantine Security*.

**Code of Federal Regulations (2008a).** *7CFR301.75-11d Treatments*.

**Code of Federal Regulations (2008b).** 7CFR301.75- Citrus Canker. In *CFR Title 7 - Agriculture, Subtitle B--Regulations of the Department of Agriculture, Chapter III - Animal and Plant Health Inspection Service, Department of Agriculture*.

**Cook, A. A. (1988).** Association of citrus canker pustules with leaf miner tunnels in North Yemen. *Plant Disease* **72**, 546.

**Euzeby, J. (2007).** Validation list no. 115. *International Journal of Systematic and Evolutionary Microbiology* **57**, 893-897.

**FR (1983).** Citrus Canker-Mexico. *Federal Register* **48**, 53672-53676.

**FR (2006).** Citrus Canker; Quarantine of the State of Florida. In *Federal Register*, pp. 43345-43352.

**FR (2007).** Citrus Canker; Movement of Fruit From Quarantined Areas. In *Federal Register*, pp. 65172-65204.

**Fulton, H. R. & Bowman, J. J. (1929).** Infection of fruits by Pseudomonas citri. *J Agric Res* **39**, 403-426.

**Golmohammadi, M., Cubero, J., Peñalver, J., Quesada, J. M., López, M. M. & Llop, P. (2007).** Diagnosis of *Xanthomonas axonopodis* pv. *citri*, causal agent of citrus

canker, in commercial fruits by isolation and PCR-based methods. *Journal of Applied Microbiology* **103**, 2309-2315.

**Goto, M. (1962).** Studies on citrus canker. *Bull Fac Agric Shizuoka Univ Japan* **12**, 2-72.

**Goto, M. (1969).** Studies on citrus canker in Japan. *Proceedings First International Citrus Symposium* **3**, 1251-1252.

**Gottwald, T., Graham, J., Bock, C., Bonn, G., Civerolo, E., Irey, M., Leite, R., López, M. M., McCollum, G., Parker, P., Ramallo, J., Riley, T., Schubert, T., Stein, B. & Taylor, E. (2009).** The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit. *Crop Protection* **28**, 508-524.

**Gottwald, T. R., Graham, J. H. & Schubert, T. S. (2002).** Citrus canker: the pathogen and its impact. *Plant Health Progress*, 1-32.

**Graham, J. H. & Gottwald, T. R. (1991).** Research perspectives on eradication of citrus bacterial diseases in Florida. *Plant Disease* **75**, 1193-1200.

**Graham, J. H., Gottwald, T. R., Riley, T. D. & Bruce, M. A. (1992).** Susceptibility of citrus fruit to bacterial spot and citrus canker. *Phytopathology* **82**, 452-457.

**Graham, J. H., McGuire, R. G. & Miller, J. W. (1987).** Survival of *Xanthomonas campestris* pv. *citri* in citrus plant debris and soil in Florida and Argentina. *Plant Disease* **71**, 1094-1098.

**Kinney, R. (2007).** Memo: PRA Implementation Procedures, p. 2 pp. Edited by E. To: Podleckis. Lakeland, FL: Florida Citrus Packers.

**Koizumi, M. (1972).** Studies on the symptoms of citrus canker formed on Satsuma mandarin fruit and existence of casual bacteria in the affected tissues. *Bull Hort Res Sta, Japan, Ser B* **12**, 229-244.

**Leite, R. P., Jr. & Mohan, S. K. (1990).** Integrated management of the citrus bacterial canker disease caused by *Xanthomonas campestris* pv. *citri* in the State of Parana, Brazil. *Crop Protection* **9**, 3-7.

**Miller, W. M., Wardowski, W. F. & Grierson, W. (2001).** Packingline Machinery for Florida Citrus Packinghouses, Bulletin 239. edn: University of Florida Extension, Institute of Food and Agricultural Sciences.

**Obata, T., Tsuboi, F. & Wakimoto, S. (1969).** Studies on the detection of *Xanthomonas citri* by phage technique and the surface sterilization of Unshu orange for export to the United States. *Res Bull Plant Prot Japan* **7**, 26-37.

**PPA (2000).** TITLE IV—PLANT PROTECTION ACT. *7 USC 7701*.

**Schaad, N. W., Postnikova, E., Lacy, G., Sechler, A., Agerkova, I., Stromberg, P. E., Stromberg, V. K. & Vidaver, A. K. (2006).** Amended classification of xanthomonad pathogens on citrus. *Systematic and Applied Microbiology* **29**, 690-695.

**Schubert, T. S., Miller, J. W., Dixon, W. N., Gottwald, T. R., Graham, J. H., Hebb, L. H. & Poe, S. R. (1999).** Bacterial Citrus Canker and Commercial Movement of Fresh Citrus Fruit. An assessment of the risks of fresh citrus fruit movement relative to the spread of bacterial citrus canker (*Xanthomonas axonopodis* pv *citri*), p. 13: Florida Department of Agricultural and Consumer Services.

**Schubert, T. S., Rizvi, S., Sun, X., Gottwald, T. R., Graham, J. & Dixon, W. (2001).** Meeting the challenge of eradicating citrus canker in Florida again. *Plant Disease* **85**, 340-345.

**Shiotani, H., Uematsu, H., Tsukamoto, T., Shimizu, Y., Ueda, K., Mizuno, A. & Sato, S. (2009).** Survival and dispersal of Xanthomonas citri pv. citri from infected Satsuma mandarin fruit. *Crop Protection* **28**, 19-23.

**Sinha, M. K., Batra, R. C. & Uppal, D. K. (1972).** Role of citrus leaf-miner (*Phyllocnistis citrella* Staintan), on the prevalence and severity of citrus canker (*Xanthomonas citri* (Hasse) Dowson). *The Madras Agricultural Journal* **59**, 240-245.

**Timmer, L. W., Gottwald, T. R. & Zitko, S. E. (1991).** Bacterial exudation from lesions of Asiatic citrus canker and citrus bacterial spot. *Plant Disease* **75**, 192-195.

**US-FDA (1998).** FDA Technical Scientific Workshop on How Citrus Juice Firms can Achieve 5-Log Pathogen Reduction. Edited by C. U.S. Food and Drug Administration & f. F. S. a. A. Nutrition.

**USDA (1995).** Importation of Japanese Unshu orange fruits (*Citrus reticulata* Blanco var. *unshu* Swingle) into citrus producing states: pest risk assessment. Riverdale, MD: USDA, APHIS, PPQ.

**USDA (2007a).** Evaluation of asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*): United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, Raleigh, NC.

**USDA (2007b).** 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.

**USDA (2009).** An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*): United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, Raleigh, NC.

**Verdier, E., Zefferino, E. & Mendez, S. (2005).** Survival of *Xanthomonas axonopodis* pv. *citri* on the surface of citrus fruit treated with sodium hypochlorite and sodium ortho-phenylphenate *Unpublished*.