Bacterial Citrus Canker and the 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*)

A report prepared for the Citrus Canker Risk Assessment Groups for Manatee County, Collier County, and Miami/Dade and Broward Counties Florida Department of Agriculture & Consumer Services


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Introduction

The movement of citrus fruit from citrus production areas afflicted with bacterial citrus canker has historically been prohibited on the assumption that some inoculum on the transported fruit could:

1) occur on the surface of symptomless fruit in the vicinity of canker-infected trees that are overlooked in the orchard inspection process (assuming orchards are inspected for freedom from canker);
2) survive the packing process (which may include disinfection treatments depending on regulations);
3) survive the shipping process;
4) be shipped to a new location with a suitable habitat for disease development;
5) encounter a suitable host in the new habitat;
6) become successfully established and cause disease on that host in the new habitat.

On first consideration, this logic seems relatively sound in the light of clear evidence that the bacterial citrus canker disease has spread from its apparent point of origin in Asia to the majority of citrus production areas in the world that have the hot rainy climate able to support bacterial citrus canker disease development.
In addition to the immediate considerations of possible inoculum dispersal on fresh fruit as mentioned above, several questions of a broader scope need to be addressed in regard to the spread of citrus canker, namely:

1) How does citrus canker inoculum move over long distances?

2) Which of these methods of inoculum dispersal have been proven empirically and which are assumed on the basis of circumstantial evidence?

3) Among those likely methods of dispersal listed above, is it plausible to blame inoculum dispersal on symptomless fresh fruit for disease establishment in a new habitat? Is there any documented evidence that fresh fruit movement from canker-infested areas has ever provided the inoculum to introduce the disease to a new habitat?

The first part of this report will attempt to quantify with approximate values the risks of each of the stages of potential inoculum distribution listed above. The second part will present some answers and additional considerations relative to the questions on how canker inoculum is spread.

Theoretical Steps in the Process of Citrus Canker Inoculum Dispersal on Citrus Fruit

Each of the following entries will elaborate on what must occur for canker inoculum to successfully proceed to the next step in the dispersal process. Any break in the continuity of the process means that the dispersal fails.

Step 1.) Inoculum will occur on the surface of symptomless fruit in the vicinity of canker-infected trees that are overlooked in the orchard inspection process. This presentation assumes orchards are inspected for freedom from canker on a timely basis (such as every 60-90
days) by trained personnel inspecting tree by tree. A ten-person crew can effectively inspect about 50-70 acres per 8-hour day in an average orchard with trees producing fruit suitable for export (average sized trees, mowed rows, and no other access problems). Regulatory experience of almost 15 years with citrus canker in Florida has proven that visual inspection every 60-90 days is a reliable method to detect the disease for eradication purposes, and should suffice for export certification. In the unusual circumstance that infected trees are overlooked in the inspection process (the procedure is not foolproof), infections will become more apparent so that detection occurs with certainty at the next 60-90 day cycle. Inspection cycles could be varied depending on the risk associated with the particular site (closest known infection, citrus variety, history of canker in area, associations with infected orchards and their personnel and equipment, etc.). In considering the biology of new infections, foliar lesions always precede fruit lesions. Thus, it is impossible to imagine how canker-blemished fruit could leave even a poorly inspected grove and enter the fresh fruit product stream destined for export. Therefore, any inoculum on unblemished fruit near an overlooked (i.e., low-level) active foliar infection would be at the low levels commensurate with that unusual situation.

As an aid in estimating superficial inoculum levels that could be expected on fruit from inspected orchards where canker was not detected, consider the following:

1) Inoculum levels associated with heavily infected mature grapefruit trees in Argentina were reported to be about $10^2$ to $10^6$ cfu’s per ml in rainwater under infected trees, and $10^2$ to $10^4$ cfu’s per ml at a distance 1 meter away from infected trees (Stall, et al., 1980).

2) Timmer, et al., (1996) estimated levels of epiphytic canker bacteria to range from $<10^0$ to $10^5$ CFU’s per leaf on symptomless leaves in the immediate vicinity of obviously infected grapefruit leaves in a nursery setting. These authors further concluded that since there is no multiplication in the epiphytic form, canker bacteria residing on asymptomatic leaf surfaces are not an epidemiologically important source of inoculum (i.e., successfully infectious inoculum with few exceptions emerges directly from active lesions and finds its way into the substomatal chamber within a very short time frame).

3) Rainwater collected in the canopy of infected Washington navel and Natsudaidai in Japan fell in the range of $10^3$ to $10^6$ cfu’s per ml (Koizumi, 1969).

4) Estimated epiphytic population levels would be very generously estimated at $10^2$ to $10^3$ cfu’s per ml for a short time (8-24 hours in the outdoor environment in summer, 72 hours in late
spring when cooler temperatures prevailed), after which time inoculum viability is lost (Goto, 1969).

Extrapolating from the above studies, one could very generously estimate the epiphytic population on symptomless fruit from inspected groves to be in the range of $10^2$ to $10^3$ CFU’s per ml. This estimated inoculum level of $10^2$ to $10^3$ cfu’s per ml on unblemished fruit would then proceed to the packinghouse for treatment prior to packing. Bacteria infesting the surface of intermediate to fully expanded fruit would not be expected to cause actual canker lesions there because of the age of the fruit tissues (Graham, et al., 1992).

**Step 2.) Inoculum will survive the packing process.**

**Option A. No disinfection.** The normal packinghouse operation would call for immediate dumping of the orchard-run fruit directly into a wash tank. This wash would provide the first step in dislodging any superficial inoculum. Detergent in the wash water plus the mechanical action provided by brushes would greatly assist the inoculum removal process.

Next, in the typical packinghouse line not using a water-based wax, the fruit would enter a drying process of an approximately 2.5 minute exposure to forced heated air at 58°C. No hot air drying is used if the finishing wax is applied to the fruit in a water base. Studies performed in 1991 by Schubert and Leahy (unpublished) using citrus bacterial spot inoculum (*Xanthomonas axonopodis pv. citrulnelo*) as the test subject revealed that the hot air drying process followed by a solvent-based aerosol wax application was extremely effective in reducing bacterial inoculum levels to very low levels. The study was not quantitative, however, and no numerical values could be ascribed to the inoculum reduction efficacy.

Remaining bacteria after the wash and hot air drying would be encased in the finishing wax, providing further measures toward inactivation. However, Stapleton (1986) reported that, unlike the natural fruit cuticle, one commercial water-soluble citrus fruit finishing wax widely used in the Mexican lime industry exhibited microscopic cracks after application, resulting in an incomplete coating seal on the fruit surface.
Option B. With disinfection. The packinghouse could opt to dump fruit received directly from the orchard into a wash tank containing disinfectants. Two disinfectants are suitable for this purpose. The first is sodium hypochlorite at 200 ppm Cl for 2 minutes at a solution pH of 6.0 to 7.5. The second is sodium ortho phenyl phenate (SOPP) at 1.86-2.00% AI for 45 seconds if the solution has sufficient soap/detergent to produce a visible foaming action, or 1 minute if the solution does not contain sufficient soap/detergent to produce a visible foaming action. Both disinfection regimes are capable of reducing the surface *Xanthomonas* population to extremely low levels, especially if coupled with rotating brushes and replenishment of the disinfectant solution by spraying the fruit as it moves down the line.

However, these treatments can not be deemed eradicatory. Stapleton (1986) was able to demonstrate 77 to 99+% reductions of *total* natural populations of fruit surface bacteria (unspecified) with a chlorine dip tank treatment (no brushes). At the recommended 200 ppm Cl level for 2 minutes, 270 - 2,900 bacterial colonies could be recovered per cm² of fruit surface after a dip tank treatment, though no *Xanthomonas* was ever isolated. Using artificially applied *Xanthomonas* inoculum to citrus fruit surfaces posed additional problems: the inoculum was unable to survive the drying process. Obata, *et al.*, 1969 had reported similar findings. Fruits for the *Xanthomonas* disinfection study had to be kept under moist conditions after inoculation in order to insure live bacteria for exposure to the Cl disinfectant. Overnight drying in the open air, or even a short drying period before incubation in a humid atmosphere was lethal to the artificially applied bacterial inoculum on unwounded fruit. Extrapolation of this phenomenon to inoculum derived from natural lesions may be dangerous, because artificially applied inoculum is suspended in sterile water. This process removes much of the polysaccharide slime coating around the bacterial cells, exposing them to the natural elements. Cells exuded from natural lesions might be expected to retain more of their protective slime coating. (A simple scanning electron microscope study of artificial and natural inoculum on symptomless citrus leaves would be useful in this discussion).

Brown and Schubert (1987) were also able to demonstrate significant reductions of *Xanthomonas* inoculum artificially applied to citrus fruits using semi-quantitative methods for checking bacterial survival after Cl and SOPP treatments. Graham and Gottwald (1991) reported similar inoculum reductions in a study of *Xanthomonas campestris* (axonopodis) pv.
citrumelo survival on fruit surfaces using a simulated brush-aided washing treatment both with and without SOPP. Obata, et al. (1969) had studied the same two surface disinfectant treatments using more quantitative methods on the fruit/citrus canker model in an effort to gain quarantine clearance for Japanese Unshu oranges to US markets. They documented essentially eradicant activity for both Cl and SOPP at the prescribed times and concentrations. Both these treatments have great value for post-harvest fungal disease control anyway, so the imposition of either of these disinfection regimes should have an overall beneficial effect on product shelf life.

Step 3.) Inoculum will survive the shipping process. The extended drying period during shipping could be expected to take a small additional toll on superficial bacterial populations. Though one might expect the bacterial cells caught in the matrix of the finishing wax to be at a dead end epidemiologically, this wax might actually keep the bacteria hydrated and thereby prolong their viability. On the other hand, bacteria that are stuck so firmly to a fruit surface that they are not dislodged in the washing process are not likely to be liberated later to reach suitable host tissue for starting a new infection. All things considered, the time lag after completing the packing process to the time of consumption by the end user would probably do little to significantly reduce the residual superficial bacterial population, but whose epidemiological significance is presumably negligible.

Step 4.) Inoculum-bearing fruit will be shipped to a suitable habitat for disease development. Regulations could be imposed that would forbid the transport of citrus fruit from the quarantine area to any other citrus-producing area of the United States (including FL). Regulations limiting the distribution of a commodity because of quarantine risk work reasonably well for major packer-shipper operations. However, such regulations can be hard to enforce if repackaging occurs at the initial destination and the fruit loses it origin certification. Also problematic is when the product is purchased at Florida roadside stands by auto travelers.

Suitable US habitats for bacterial citrus canker development would be in states along the Gulf Coast (especially LA and TX). Other humid citrus production areas around the world could be considered at risk also. Hot, dry climates such as the arid Southwest US (CA, AZ) are inhospitable to bacterial diseases in general, but these states would undoubtedly insist that they be placed on the prohibited list if such a measure was used as a part of the quarantine protocol.
The added danger of citrus leaf miner wounds encouraging canker development, even in dry climates, should be taken into account here.

**Step 5.) Inoculum will encounter a suitable host in the new habitat.** Climate alone does not set the stage for successful establishment in a new location. Inoculum must reach a suitable citrus host in the right stage of growth for natural infection, or be provided with a wound for establishment on a host in the new area. The most likely scenario for such an event to occur would be inoculum-bearing fresh fruit packed as described above, then consumed by a horticulturally-minded homeowner with dooryard citrus in the new habitat. However, the Tim’s Thai Restaurant incident in Gainesville, FL (J. W. Miller, personal communication; Schoulties, *et al.*, 1987), amply demonstrates that scenarios other than the infested fruit to homeowner to dooryard citrus can successfully establish canker in a new location.

In South and Central Florida, about 1/3 to ½ of all homeowners in neighborhoods with single family detached dwellings have citrus growing on their property. This amounts to a citrus density of considerably less than 10 trees per acre on average. A significant percentage of these citrus trees in the south Florida dooryard setting seldom, if ever, receive any hands-on caretaking. The likelihood that persons growing their own citrus would buy commercially-packed citrus is also difficult to estimate.

Fruit with inoculum on its surface would have to be handled such that inoculum is transferred to the hands, the hands left unwashed, then manipulative husbandry performed while tending the uninfected plants in the new location for the pathogen to successfully establish. If the contaminated fruit were consumed fresh, the acid juice would likely serve as a bactericide on the hands, thus eliminating any chance of manually transmitting the bacteria successfully to the new host plant. If the contaminated citrus peel was discarded carelessly or even composted in the vicinity of a suitable host, a very slight chance of successful transmission might occur, but rapid decomposition would destroy *Xanthomonas* viability within a matter of 2-3 weeks. Also, it is unlikely that any inoculum would be available for splash dispersal from the discarded peel on the orchard floor to susceptible citrus canopy above unless active lesions were present on the discarded peel. Such a coincidence of events and factors all occurring in proper sequence at just the right place at the right time for new disease initiation is extremely improbable.
Step 6.) Inoculum will become successfully established and cause disease on that host in the new habitat. Any pathologist who has performed pathogenicity tests can recount how difficult it can be to generate new infections on susceptible host plants with a virulent pathogen even when a conducive environment and high inoculum levels are provided. For bacterial citrus canker, the effective inoculum dose is estimated at somewhere between $10^2 – 10^3$ cfu’s per ml for natural infection to succeed under normal circumstances (J.W. Miller, personal communication). Circumstances are not always normal, however, and theoretically, one bacterial cell of the pathogen that somehow finds its way into the substomatal chamber or intercellular spaces of a leaf, stem, or fruit of a susceptible host will cause disease. Assuming inoculum is available, the remaining limiting factors for disease development in approximate increasing order of importance are: 1) susceptible host in proper stage of growth for infection; 2) wet conditions (surface water film); and 3) wind speed of 8 meters / second (approximately 18 mph) or more to drive the inoculum into the stomata (Serizawa et al., 1969; Stall et al., 1980). As an example, in the Miami area, susceptible hosts in the right stage of growth for infection can be found within a one city block area virtually any time of year. Wet weather (0.1" of rain) occurs about 80-90 days per year; 70-80 of those events per year are in the form of thunderstorms in which wind velocities of 18+ mph are likely to occur (Gottwald et al., 1997b). The presence of the citrus leaf miner increases the likelihood of infection even more, but since the leaf miner prefers the same age tissue for infestation as canker bacteria prefer for infection, the increase due to wounding on the highly canker-susceptible hosts is not as significant as the increase in susceptibility on the more canker-resistant citrus that can now be riddled with wounds, lowering its natural resistance. Each potential target habitat must calculate its own climatological risk level for canker development. Miami happens to be the most conducive climate in the continental US for citrus canker establishment. It is doubtful that regulations for shipment of citrus fruit from quarantine zones could be customized to any great degree to accommodate low risk habitats in potential fresh citrus markets.

PROBABILITY CALCULATIONS

Most of the events mentioned above would occur at a frequency estimated at well under one in age 9 of 17
one hundred, except for Step 3 (Inoculum will survive the shipping process). This event could be estimated at one chance in two for our purposes. USDA-APHIS-PPQ-BATS (April, 1997) has performed similar frequency estimates in a Monte Carlo Simulation of the risks of importation of citrus fruit from Argentina into the continental United States. Their calculations are also presented with some slight modifications. In the estimations for the Florida situation, the figures have a considerable safety factor built in, partly because the estimate is not easily quantified, and because a more conservative approach to the risks and probabilities than the Argentine Monte Carlo Simulation (AMCS) is easier to defend, especially considering the lack of empirical data on these subjects. The theoretical calculation of the chances of successfully moving canker step-by-step from inspected, canker-free orchards in Florida to a new habitat by means of superficial inoculum on fresh fruit would be:

Step 1: Inoculum will occur on surface of symptomless fruit in the vicinity of canker-infected trees that are overlooked in the inspection process: 1 in 1,000

Step 2: Inoculum will survive the packing process: 1 in 1,000

Step 3: Inoculum will survive the shipping process: 1 in 2

Step 4: Inoculum-bearing fruit will be shipped to a suitable habitat for disease development: 1 in 50

Step 5: Inoculum will encounter a suitable host in the new habitat: 1 in 100

Step 6: Inoculum will become successfully established on that host in the new habitat: 1 in 10

\[ .001 \times .001 \times .50 \times .02 \times .01 \times .10, \text{ or } 1 \text{ in } 100,000,000,000 \text{ (i.e., } 1 \times 10^{-11}) \]
OR, using APHIS estimates of probability ...

\[ 0.000002 \times 0.01 \times 0.75 \times 0.03 \times 0.002 \times 0.000001, \text{ or } 9 \text{ in } 10,000,000,000,000,000,000 \]
(i.e., \( 9 \times 10^{-20} \))

**Conclusion** - It is difficult to justify further restrictions or mitigating efforts (other than those outlined above) and their accompanying expenditures in the light of such low risks. Resources would be better spent by addressing the regulatory deficiencies that have a greater likelihood of introducing the citrus canker pathogen to a new habitat (especially smuggled propagating material and other plant products).

From this attempt at calculating the risk of establishing bacterial citrus canker to a new habitat via fresh fruit movement, the discussion will now consider the three questions posed earlier.

1) **How does citrus canker inoculum move over long distances?** Circumstantial evidence for the means of long distance movement of canker inoculum has been published (Civerolo, 1984; Gottwald, *et al.*, 1997a, 1997b). The main culprits are: 1) the illegal movement of infected or infested plant material by people; 2) movement of inoculum on infested personnel, clothing, pruning equipment, pesticide application and mowing equipment, harvesting equipment, packing boxes, and other items associated with harvest and post-harvest handling of the fruit prior to packing house treatments; and 3) weather events. Movement by animals, particularly birds, has not been investigated, but it is conceivable that such movement could occur in a localized area.

Weather events are implicated in many instances of medium distance dispersal, or secondary movement. In thunderstorm events in Argentina, Stall, *et al.* (1980) detected inoculum movement of about 32 meters from the source. Longer distances reported for inoculum spread by weather events range from a few hundred meters (Gottwald *et al.*, 1992) to several miles (Gottwald, *et al.*, 1997b).
However, weather events are inadequate explanations for the initial transcontinental appearances of canker in Monticello, Florida in 1910-11 (Loucks, 1934); Sao Paulo, Brazil in 1953-54 (Rosetti, 1977); Fugi, Itapua, Argentina in 1967 (Rosetti, 1977); Manatee County, Florida in 1986 (Schoulties, et al., 1987), Dade County, Florida in 1995 (Schubert et al., 1996; or perhaps even the 132 km distance bridged from Dade County to Collier County, FL in 1998 (see below). All these events are all best explained via illegal movement of infected or infested plant material. The 1997 Manatee County outbreak is most likely carryover of undetected infections from the former eradication program that ended officially in 1994 (Schubert et al., 1996). Human activity rather than weather events also seem to better explain the appearance of the Miami isolate of citrus canker in Collier County, Florida in 1998.

2) Which of these methods of inoculum dispersal have been proven empirically and which are assumed on the basis of circumstantial evidence? It is very difficult to provide incontrovertible evidence of the source and the ultimate destination of citrus bacterial canker inoculum using present day investigative methods. Only recently have efforts been made to genetically fingerprint canker isolates to permit some empirical tracking capabilities.

One hundred seventy one interceptions of canker infected fruit were made at Florida’s international ports of entry from January, 1971 to June, 1983 (Anonymous, 1983). The authors are aware of only one USDA investigation that successfully identified the source of a citrus canker introduction into the United States. That was the evidently inconsequential Tim’s Thai Restaurant incident, Gainesville, FL, 1985 (DPI Specimen Report X85-7270; J.W. Miller, personal communication; Schoulties et al., 1987), which was apparently due to illegal plant movement and/or handling of illegally imported citrus products bearing canker inoculum, then handling live C. hystrix plants kept on the premises for culinary purposes. The only other successful USDA investigation tracking long distance movement of citrus canker within the United States traced inoculum movement by illegal transport of infected plant material from an area of known canker activity in Manatee County to a residential area in rural Highlands County Florida in 1990 (Selected DPI Specimen Reports X90-7218 through 7408; Gottwald, et al., 1992). A third unproven case with strong circumstantial evidence of illegal plant movement having introduced citrus canker inoculum into the United States was discovered in 1987 in Sarasota County. The Thai owner of a Thai restaurant maintained an isolated residence with...
canker-infected *Citrus aurantiifolia* on the premises. This site represented the only canker site in Sarasota County. The owner also maintained a private residence with infected citrus in the heart of the canker infestation on Anna Maria Island in Manatee County. That introduction, though discovered one year into the eradication program, presents a logical sequence of events that probably sired the 1986-94 eradication program. This program primarily occupied the four county area around Tampa Bay, save for the human transmission to Highlands Co. noted above.

Conclusion: based on the best evidence available today, canker inoculum is carried to a new area by way of illegal movement of infected or infested plant material. Further dispersal is then primarily by way of weather events.

3) Among those likely methods of dispersal listed above, is it plausible to blame inoculum on symptomless fresh fruit for disease establishment in a new habitat? Is there any documented evidence that fresh fruit movement from canker-infested areas has ever provided the inoculum to introduce the disease to a new habitat? The question of legal versus illegal movement of citrus fruit must enter into this discussion. Most illegal movement of plant material by common travelers would be in the form of fruit. Most interceptions at ports of entry are fruit that was intended to be eaten, not plant material intended for propagation.

Legal movement of citrus fruit from an area with canker has been successfully and safely accomplished in the Unshu Orange import program from Japan to the United States. This program utilizes orchard inspections and post-harvest fruit treatment as part of the regulatory protocol. There have been no accidental introductions in that program. Whiteside *et al.* (1988), Anonymous (1992) and Canteros, *et al.* (2000) all report that there has never been an outbreak of citrus canker traced back to commercially imported fruit anywhere in the world. The same is true for the bacterial disease of pome fruits, fire blight (Roberts, *et al.* 1998), which has many parallels to the citrus canker fresh fruit export problem.
RECOMMENDATION

Based on the preceding considerations, the Florida Citrus Canker Risk Assessment Group recommends that commercial fresh fruit movement for domestic markets or export be permitted from citrus canker quarantine areas under the following conditions:

1. Orchards to be considered for certification must be inspected tree by tree on a 60 day cycle and be found free of citrus canker. There cannot have been any canker infections in a certified grove in the previous 12 months.

2. Fruit harvested from certified orchards must be transported in a covered conveyance to the nearest citrus packing house facility. The compartment of the conveyance must be swept and disinfected after each trip, and the residue from the compartment incinerated or buried in a landfill.

3. Fruit harvested from a certified grove must be treated in the initial wash process with either:
   
   a) sodium hypochlorite at 200 ppm Cl for 2 minutes at a solution pH of 6.0 to 7.5; or
   
   b) sodium ortho phenyl phenate (SOPP) at 1.86-2.00% AI for 45 seconds if the solution has sufficient soap/detergent to produce a visible foaming action, or 1 minute if the solution does not contain sufficient soap/detergent to produce a visible foaming action.

Both these operations must be used in conjunction with brushes that provide mechanical assistance to the cleaning process and spray applicators that supply fresh rather than recycled disinfectant to the fruit as it moves down the packing line.

References


