PEER REVIEW REPORT FOR

EVALUATION OF ASYMPTOMATIC CITRUS FRUIT

(*CITRUS SPP.*)

AS A PATHWAY FOR THE INTRODUCTION OF

CITRUS CANKER DISEASE

(*XANTHOMONAS AXONOPODIS* PV. *CITRI*)

August, 2006

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Peer Review Report
Reviewer No. 1

August, 2006

Comments on Canker Risk Assessment Document

USDA-APHIS Document (March 20, 2006): Evaluation of asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. citri)

Summary: The overall conclusion of this document is that asymptomatic citrus fruit that has been surface disinfested is not likely to serve as a pathway for dissemination of the citrus canker pathogen, *Xanthomonas axonopodis* pv. citri (Xac). In my opinion, this is a reasonable conclusion supported by the available experimental and epidemiological evidence. However, this conclusion is weakened by two problems. First, much of the available evidence is not of very high quality, in many cases deriving from either non-peer-reviewed studies of uncertain objectivity (e.g. the 2004 Argentinian Risk Assessment) or from older publications reporting research done before the invention of sensitive detection technologies like real-time PCR (e.g. the key 1969 study by Obata et al. on efficacy of fruit disinfestation). Second, the document was not prepared with the necessary care and attention to detail. In many cases inappropriate sources are cited in support of a statement, making it difficult to find the relevant evidence. More substantially, the details of the supporting evidence are not given in the document, and there is no critical assessment of the reliability or strength of this evidence. I therefore recommend that the document be significantly revised before any regulatory action is taken. If, upon thoughtful assessment of the available evidence, it appears that there is not enough data to allow confident prediction of risk, it may be necessary to have additional experiments conducted by unbiased scientists (who should then be encouraged to publish their findings in the peer-reviewed literature).

General comments on document preparation:

Appropriate level of detail and analysis: Most of the results relevant to this document's conclusions are only summarized qualitatively. Data supporting the document's key conclusions should be presented in more detail, describing each study separately and including methods and specific results. A better approach is exemplified in the brief but complete summary of experiment and results on bacterial survival on artificially infested fruit surfaces that is given in a bullet point on p. 12. However, the cited source for this is the Risk Asessment (2005) from Argentina, which is neither peer-reviewed nor a primary source. It was not possible to get directly back to the original methods and data (which are apparently presented in a 2001 paper by Canteros). Moreover, when results are presented, they are offered without framing or critical analysis. Given that the purpose of this document is to inform regulatory decisions, it would be appropriate to assess the relative reliability of the various studies. Did the experimental conditions reflect commercial practices? Were appropriate strains and environmental conditions used? Were the methods sensitive and reliable enough to make the conclusions credible? Were appropriate experimental controls in place? Was the researcher free of obvious bias (e.g., not working for a business or government agency with a financial or political interest in

the outcome)? Were sample sizes and statistical analyses robust enough to support the conclusions drawn? Was the research peer-reviewed?

Citation practices should be more precise. For maximum credibility and ease of data traceback and evaluation, only the original papers documenting specific experiments and conclusions should be cited. Citation of subsequent review articles, risk assessments, press releases, and similar secondary or "gray literature" publications makes it difficult to determine if a critical result was actually replicated or only repeatedly cited. The original source should be cited, not a review or summary that is a step removed from the actual study that underlies the conclusion. For example, on p. 12 the statement that "Populations decline rapidly even within the lesions of infected fruit after harvest" is attributed to Civerolo 1981. However, Civerolo 1981 is a review article that makes the same assertion but gives no experimental methods or data, only a list of seven references; it appears that the actual data derive from papers by Goto (1976) and Koizumi (1972), which are not themselves cited in this document. This makes it difficult to assess the reliability of the data. Meeting proceedings papers, abstracts and personal communications without specific descriptions of methods and results cannot be considered good evidence upon which to base regulatory decisions. They may, however, be used to support other more formal scientific data if appropriately annotated.

Specific Scientific Questions. This document's conclusion is supported by two key assertions:

1. Xac survives poorly on fruit surfaces if no lesions are present, and
2. Disinfectant dips effectively kill both epiphytic and lesion-dwelling Xac cells.

Epiphytic survival of Xac on fruit:

There do not appear to be any direct data on population sizes of Xac on surfaces of asymptomatic citrus fruit grown in naturally infested orchards. However, Timmer et al (1996) found Xac population sizes on asymptomatic grapefruit leaves from naturally infected orchards ranged from undetectable to 10^5 cfu per leaf. Bacterial population sizes on artifically infested leaf surfaces dropped from 5×10^5 to 100 cfu/leaf in 24 hours even when leaves were kept humid and warm. These data suggest the bacterium may not be well adapted to epiphytic life, although results may differ between leaves and fruit. Civerolo (1981) asserts that Xac survives poorly in lesions on infected fruit, but the relevant data are apparently presented in papers by Goto (1976) and Koizumi (1972), which are uncited in this document and difficult to obtain, as described above. Belasque and Rodrigo Neto (2000), cited in Argentina 2005, apparently found that Xac survived less than 5 days on fruit following artificial inoculation with 10^6 cfu. However, the methods and specific results were not presented in the secondary source, making this evidence difficult to evaluate. The original Belasque paper appears to be an abstract in Spanish. Taken together, the evidence suggests but does not prove that Xac survives poorly on citrus fruit. Additional research is needed to determine quantitatively the survival of Xac on fruit under normal harvest and packing conditions, and to measure the

sizes of naturally occuring populations on surfaces of asymptomatic fruits from infested orchards.

Disinfestation:

The data concerning the effectiveness of disinfestation to remove Xac cells from fruits are not as robust as would be desired, but they do support the contention that surface populations of Xac are dramatically reduced by soaking fruit in either 200 ppm chlorine or in 2% sodium orthophenylphenate (SOPP). The best data appear to come from a 1969 paper by Obata. These authors determined a survival curve for pure Xac cells in various solutions of SOPP and chlorine, finding that under these conditions Xac is efficiently killed by 5 minutes' exposure to 1.6 ppm chlorine. A more representative but still artificial study measured efficacy of soaking artificially infested fruit in various concentrations of chlorine. They tested 50 fruits per experiment, with five different experiments. Fruits were sprayed with various concentrations of Xac ranging from 10^8 cfu/ml to 10^4 cfu/ml. This experiment found that 200 ppm chlorine for 2 minutes was effective against up to 10^7 cfu/ml on fruit. Obata's fruit disinfestation studies used an indirect but apparently sensitive bacteriophage method to enumerate viable Xac cells following treatment, but this method makes it difficult to compare their results to others that use a direct plating method. It is not clear if naturally infested fruits would ever carry this many Xac cells; there do not appear to be good data on this question.

The review by Canteros, 2004 recomends some fruit disinfestation schemes based on experiments cited in this paper as Canteros 2001; unfortunately the reference section of Canteros 2004 lists six papers by that author in that year, and it is impossible to determine which one describes the experiment(s) in question. In any case, none are in the mainstream English scientific literature and were not available to this reviewer. There is therefore no way to assess their quality. The other papers cited on disinfestation are of questionable relevance. Graham and Gottwald (1991) offer no original data on disinfestation in their review article but cite a study by Brown and Schubert. The study by Brown and Schubert (1987) is also cited on p. 11 of this document in support of the idea that Xac populations are reduced by chlorine or SOPP disinfestation. However, the experiments of Brown and Schubert were actually done on a different bacterium, *X. campestris* pv. vesicatoria (which infects pepper), as a proxy for Xac. These data may not be relevant to Xac because bacteria differ significantly in their susceptibility to disinfectants and moreover these two species are ecologically adapted to different plant hosts. Similarly, Stapleton (1986) measured efficacy of various methods for disinfestation of citrus fruits to remove the Mexican lime bacteriosis pathogen. However, he was probably working with the species now known as *X. axonopodis* pv. aurantifolii. This organism is phylogenetically and apparently epidemiologically different from Xac, so these data also may not apply to Xac. Thus, there appear to have been two studies on disinfestation using Xac; these both support the idea that chlorine and SOPP can be effective, but the irrelevant review and papers based on other species should not be cited in support of this conclusion. Instead, a more detailed description and critical assessment of the methods and results of Canteros and Obata should be given.

If the research data on this topic are as scant as they appear to be, additional studies on survival and disinfestation of Xac cells on *Citrus* fruit surfaces should be a priority for APHIS. A further topic of potential importance is the presence and survival of the pathogen inside aymptomatic fruit and on surfaces of packing material. These potential sources of transmission were not addressed in the document. There does not appear to be a robust body of experimental data on Xac epidemiology, especially field data under conditions representative of US growing conditions. Nevertheless, it appears that some possibly relevant research was done but never published (by Ed Civerolo?). If this is the case, it would be very useful if those data could be written up formally and submitted for publication. It would save time and money if studies did not have to be conducted *de novo*.

Peer Review Report
Reviewer No. 2

Peer Review: Citrus Canker PRA for Asymptomatic Fruit

USDA-APHIS, PPQ Document: Evaluation of asymptomatic citrus fruit (*Citrus spp*.) as a pathway for the introduction of {the} citrus canker disease {pathogen} (*Xanthomonas axonopodis* pv. *citri*) [April 6, 2006]

1. Are the conclusions and inferences correctly supported by the evidence provided?

Overall, the conclusions and inferences about the likelihood of introducing *Xanthomonas axonopodis* pv. *citri* (Xac) on asymptomatic fruit produced under a systems approach as described on pages 4-5 are reasonable and are supported by the scientific evidence provided in the document. However, as noted below, there are a few peer-reviewed publications, as well as some unpublished data, that may be relevant to evaluating the risk of mature, asymptomatic citrus as an epidemiologically-significant pathway for the long-distance dissemination/transmission of Xac. Fruit inspection (page 5) for blemished, disfigured, damaged and diseased fruit, as well as fruit culling in the field and packinghouse (page 9; Figure 1), are important components of the Systems Approach to mitigate the risk of long distance dissemination of Xac on mature asymptomatic fruit. However, as pointed out on page 16 of the document, research is needed to determine that relationship between the proportion of symptomatic fruit in the field and the proportion of infected fruit after postharvest culling and the effectiveness of various field and packing procedures and equipment to remove fruit with canker lesions.

2. Are the approach and process appropriate for the analysis?

Generally, the approach and process are appropriate for the analysis. However, I think that, where possible, more emphasis should be placed on data regarding the detection, identification, treatment and survival of Xac on the surface of naturally-infested fruit. Similar scientific data for asymptomatic fruit artificially infested with Xac, although instructive, may be of limited value for extrapolation to asymptomatic fruit naturally infested with Xac. For example, of Xac microcolonies and/or (but not necessarily limited to) biofilm(s) may potentially occur on the surface(s) of naturally-infested fruit. These associations likely develop over time under natural conditions as fruit develop and are exposed to Xac at various times during development Such associations between Xac and fruit surfaces are not likely to occur on the surfaces of mature asymptomatic fruit artificially infested one time with Xac (or surrogate xanthomonads) for experiments to evaluate Xac (epiphytic) survival and treatment effects on Xac survival. The role(s) of these (and possibly other) Xac-fruit surface associations on Xac survival are not known, or at least not known very well.

3. Have any important data or considerations about the disease or pathway been omitted?

Published results of research done in Argentina (Canteros, et al, 2000; Rybak & Canteros, 2001) contain the data referred to in the paper by and entitled Canteros, B.I. 2004. Management of citrus canker in Argentina: A Review. In: Proceedings of the 10th International Conference of the International Society of Citriculture, 2004, paper 90. In addition, information presented recently in an unpublished manuscript by Verdier et al in Uruguay, might be relevant to cite in the document (e.g., fourth paragraph on page 9). See below under question #5.

While probably not critical references, the papers by Mohammadi et al (2001) and Vernière et al (1998) may, nevertheless, be relevant to 'Spread Potential' (page 7). These papers indicate that Xac was isolated from citrus (bacterial) canker-affected lemon (*C. limon*) in Southern Iran, as well as from citrus (bacterial) canker-affected Mexican lime (*C. aurantifolia*).

4. Are all important assumptions identified and uncertainties clearly stated?

I am not sure what is meant or implied by "The bacteria have been dispersed from 32 meters to a few hundred meters to several miles" (bottom of page 5). This statement might be misleading. There is little or no doubt that, based on the available scientific data, the incidence of citrus (bacterial) canker disease can increase up to '…several miles…' from an infection focus (e.g., Gottwald et al, 2001). However, this statement might also be interpreted to mean or suggest that Xac bacteria can be dispersed in or via a single environmental event (e.g., hurricane). I am not aware of good, reliable, conclusive scientific documentation to support reference to dispersal of (Xac) '…bacteria…to several miles…' in this context. It seems possible that by the time that citrus (bacterial) canker disease incidence is detected several miles from a focus that, at least some, secondary spread/dispersal of the pathogen could/may have occurred.

Assessment of research on, and conclusions drawn from results concerning, survival of Xac on citrus tissue surfaces (e.g., leaves, fruit) based on 'detection' of the pathogen that do not detect viable Xac (e.g., PCR-based methods) may be problematic. While the technology exists to use molecular methods to detect other microorganisms, I am not aware of any method other than isolation, phage assay or susceptible host tissue inoculation available for detection of viable Xac. Evaluation of the epidemiological significance of Xac that may occur on the surface of mature, asymptomatic citrus fruit should be related to the viability of the pathogen, even though molecular methods may 'detect' Xac (or at least Xac DNA).

Biology and Epidemiology section (page 7, paragraph at top of page in which Xac survival is discussed). Xac has been reported to occur epiphytically on the surfaces of citrus (e.g., 'Duncan' grapefruit, *Citrus paradisi*) naturally infested with the pathogen where citrus bacterial canker disease occurs (Gottwald et al, 1992; Timmer et al 1996; Timmer et al, 1991). In these cases, the populations ranged from undetectable to about 10^5 colony-forming units (cfu) per asymptomatic leaf when using a modified (1% peptone instead of phosphate buffer to swab leaves) leaf swab technique (Egel et al, 1991; Gottwald et al, 1992) to sample Xac leaf surface populations and plating swabates on a semi-selective KCB medium (McGuire et al, 1986). However, no evidence has been found (Timmer et al, 1996) that Xac multiplies on leaf surfaces and reaches high populations before disease develops. In addition, Xac populations in droplets of aqueous cell suspensions applied to the upper surface of mature, potted sour orange (*C. aurantium*) seedlings declined drastically (10^5 cfu/leaf to 10^1-$<10^1$ cfu/leaf) and rapidly (within about 48 hours), although $<10^1$-10^1 viable Xac cfu/leaf were still detectable after 5 days (Timmer et al, 1996). Accordingly, Xac is considered (Timmer et al, 1996) to be a casual epiphyte (*sensu* Leben, 1963) on citrus tissue (e.g., leaf) surfaces capable of survival (for short periods of time) but not capable of multiplying rather than a resident epiphyte capable of multiplying on plant leaf surfaces (*sensu* Leben, 1963). Moreover, epiphytic Xac populations were not related to disease development and do not appear to be epidemiologically significant sources of inoculum ((Timmer et al, 1996). The discussion in this paragraph in the document, as well as the text here above, does not address survival of Xac on mature asymptomatic fruit directly. However, it is

likely that any Xac populations that might occur on the surface of mature asymptomatic fruit are also not epidemiologically significant for the same reason(s) as those cited in the paragraph on page 7 of the document. But the context should be that of resident (those populations that multiply) and casual (those populations that do not multiply) populations on citrus tissue surfaces, such as leaf and fruit surfaces. There is no direct evidence that Xac is a resident of, and multiplies on, citrus leaf or mature fruit, and/or that this is an epidemiological significant source of inoculum

5. Are there any relevant data or evidence not contained in the report?

In Argentina, Canteros et al (2001) and Rybak & Canteros (2001) assessed the level of viable Xac on the surfaces of asymptomatic citrus fruit naturally infested with Xac by (1) plating aliquots of fruit washates on a semi-selective isolation medium and (2) injection-infiltration of susceptible leaves of 'Duncan' grapefruit and Mexican (Key) lime seedlings maintained in growth chambers with fruit washates. The leaf injection-method was sensitive enough to detect as few as 10 viable Xac cells/ml of inoculum. In this work, viable Xac was either not detected (Canteros et al, 2001; Rybak, M. & B.I. Canteros, 2001) on asymptomatic fruit collected in citrus groves with low (but unspecified level(s)) incidence of citrus (bacterial) canker disease, or only detected from similar fruit collected immediately after heavy rainfall (Canteros et al, 2001).

I am aware of a manuscript (as yet unpublished) by Verdier, et al (2006) entitled 'Sobrevivencia de *Xanthomonas axonopodis* pv. *citri* en fruta citrica sometida a tratamiento de postcosecha, por detección en medio de cultivo semiselectivo y bioensayo (Survival of *Xanthomonas axonopodis* pv. *citri* on citrus fruit subjected to postharvest treatment by detection on semiselective culture medium and bioassay)' being prepared by Verdier et al in Montevideo, Uruguay regarding the effect(s) of the postharvest treatment (sodium hypochlorite and SOPP) of mature naturally- and artificially-infested asymptomatic citrus fruit on surface-borne Xac. I reviewed the manuscript during the week of June 12-16, 2006, primarily for English usage and grammar, and have returned it to Verdier. It will probably have to be reviewed again for content; however, I understand that this manuscript is intended to be submitted for consideration for publication in a postharvest biology and technology journal (possibly in electronic form online). Generally, I think that the manuscript contains additional relevant and useful information for the Pest Risk Analysis concerning the evaluation of mature asymptomatic citrus fruit as an epidemiologically significant pathway for the long-distance dissemination/transmission of Xac; however, it remains to be seen if the manuscript will be acceptable for publication in a peer-reviewed journal.

The work was carried out in five tests over two years. The primary relevant findings of the work conducted are:

1. The percent (%) of asymptomatic citrus fruit, naturally-infested with detectable Xac, was reduced from 67% (N=69) to 3% (N=72) following postharvest treatment of the fruit with NaOCl and SOPP. Actually, only 2 of 22 treated fruit in only one of the five tests had detectable Xac.

2. The population levels of Xac on the naturally-infested fruit averaged 39.4 cfu/ml (range = 10.2-93.8 cfu/ml) and 0.06 cfu/ml overall on the untreated control and treated fruit, respectively. Only an average of 0.3 Xac cfu/ml was detected on the 2 of 22 postharvest treated fruit on which viable Xac was detected. I suggested that the authors of this manuscript convert the 'cfu/ml'

data to 'cfu/fruit', as I believe that this would be more meaningful for assessing epidemiological risk of asymptomatic fruit as a pathway for long-distance dissemination of Xac. There is not sufficient information presented in the manuscript for the reader to make this determination.

The (bio)assays for Xac on the untreated control and treated fruit were isolation of the pathogen on a semi-selective medium and inoculation of leaf discs excised from 'Duncan' grapefruit leaves. Unfortunately, the authors of the manuscript did not indicate the level of sensitivity of Xac detection and identification by these bioassays. However, the inoculation of the leaf tissue discs is very specific for Xac. Our own previous research (unpublished data) with Xac bioassays based on infiltration of susceptible attached and detached citrus leaves (e.g., 'Duncan' grapefruit; Mexican lime) and inoculation of excised, wounded 'Duncan' grapefruit leaf tissue similar to that described by Verdier, et al (2006) demonstrated that these are very sensitive methods for detecting viable Xac. The sensitivity of the leaf tissue disc bioassay is probably between 100-200 Xac cells/ml of inoculum applied depending upon age of the leaves from which the leaf tissue discs were excised, number of replicated leaf tissue discs inoculated and post-inoculation handling (e.g., temperature, lighting conditions, humidity). Koizumi (1971) described a method for reliable identification and quantification viable *X. citri* (=*X. axonopodis* pv. *citri*) based on inoculation of detached, needle-prick wounded *C. natsudaidai, C. unshiu* and *C. limon* leaves. Depending upon the number and size of the wounds, the sensitivity of the detached citrus leaf bioassay was ~200-400 Xac cfu/ml of Xac-containing suspension inoculum. Thus, these bioassays provide a rough estimate of the 'inoculum potential' of any citrus surface-borne Xac to be an epidemiologically significant source of inoculum.

It may be important to note, and be aware of, a recent paper (Park et al, 2006). In this paper, the authors report that, in Korea, Xac was detected on asymptomatic leaves of *C. natsudaidai*, *C. limon* (lemon) and *C. unshiu* (Unshiu orange) naturally-exposed to Xac by PCR using new specific primers designed from the *hrpW* region (homologous to pectate lyase) of Xac. As no comparative data are presented in this paper, it is not clear if use of these new primers result in more specific or more sensitive detection of Xac (especially when applied to clinical samples of fruit naturally infested with Xac) than previously described PCR-based protocols. Nevertheless, the authors reported that using these primers with as little as 50ng of Xac DNA template a Xac-specific 561 bp DNA fragment was amplified from samples of (apparently) total DNA isolated from naturally Xac-infested. Unfortunately, this result was not related to the presence of viable Xac that was (or might have been) present on the asymptomatic leaves assayed. Nor was this method applied to assay for Xac on asymptomatic citrus fruit artificially or naturally infested with Xac No Xac-specific 561 bp amplicon was detected when healthy (i.e., presumably not exposed naturally to, or not artificially infested with, Xac) *C. unshiu* leaves were assayed. This paper lacks many details of the methodology used. However, the data presented might be used to support the conclusion that epiphytic Xac can be associated with asymptomatic citrus tissue surfaces, at least on the surfaces of asymptomatic citrus leaves naturally exposed to, and infested with, Xac.

6. Quality and completeness of the individual components of the analysis.

The overall quality and completeness of the individual components of the analysis are generally good and adequate to justify the conclusions. However, the analysis does not, and probably cannot (at this stage anyway) assess the effectiveness, reliability and/or efficiency of fruit culling

procedures in the Systems Approach to mitigate the risk of long distance dissemination of Xac on mature asymptomatic fruit. Fruit culling steps in the 'Packing house treatments' shown in Figure 1 are not shown or indicated in Figure 4. In Figure 1, it appears that there are three fruit culling steps. However, it is not clear where these three fruit culling steps are in Figure 4. __

7. Whether/Where the document is difficult to read or understand, and recommendations or suggestions for improving the document where it is lacking.

The document is not difficult for me to read or understand. However, technically the epidemiologically significance of asymptomatic fruit is related to its being a potential pathway for the pathogen (*Xanthomonas axonopodis* pv. *citri*) not the disease. It is the pathogen not the disease that is transmitted, disseminated, spread or introduced. I realize that transmission, dissemination, spread and/or introduction of 'disease' is commonly used colloquially. But epidemiologically (and technically) it is the pathogen that is of importance or significance and, in fact, the target of risk mitigation. Also, as noted above, I believe that the epidemiologically significance of mature, asymptomatic citrus fruit as a pathway for long distance dissemination or transmission of this pathogen needs to be related to the presence of viable Xac associated with the fruit. The overall quality of the document could be improved with additional technical editing of the text throughout (e.g., use 'pathogen' spread rather than 'disease' spread, a few grammar corrections).

Peer Review Report
Reviewer No. 3

Comments on technical merit of APHIS document-2006-0045-0018:

Evaluation of asymptomatic citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis pv. Citri*)

August 3, 2006

Summary: This document (APHIS 2006-0045-0018, hereafter "the Evaluation") is well written and clear. In a systematic and stepwise fashion, it covers nearly all relevant aspects of citrus canker disease and its causative agent *Xanthomonas axonopodis* pv. *citri* (XAC) as they relate to fruit-based transmission. The logic behind the conclusions is well-presented. Although many details of the actual experimental data were not formally presented in the Evaluation, after checking key references I found the data apparently utilized by the authors to derive conclusions in the Evaluation appears valid and supportive. Discussion of specific aspects of the Evaluation follow.

1. Evidence provided. There are ~50 references that were apparently drawn upon for the conclusions. Approx. 10 of these contain the key data; most are in highly-respected refereed journals (e.g. Plant Disease, Phytopathology) and authored or co-authored by top experts on citrus canker disease (e.g. J.H. Graham and T.R. Gottwald). I obtained and carefully read these journal articles to independently view the detailed methodologies and data used to support conclusions of the Evaluation, and I concur with the conclusions and data interpretations made by the authors of the Evaluation---i.e. transmission or dissemination of citrus canker disease by packinghouse-processed asymptomatic citrus fruit is highly unlikely. Some of the other references were from more obscure or unreviewed sources [e.g. meeting reports, proceedings, bulletins, etc.] that sometimes publish less rigorously performed experiments and hence may be less reliable sources of information. Thus the authors of the Evaluation appear to have been very thorough in finding and collecting most available information relevant to the transmission of XAC by citrus fruit.

2. Methodology. This seems appropriate. The stepwise evaluation of data relevant to probabilities at each of the 5 events/stages in the hypothetical transmission/infection process by asymptomatic fruit is sound, appropriate, and easy to follow. However, it might be important to present additional scenarios for Event 5---infection of canker-free citrus trees via 'contaminated' asymptomatic fruit. I realize this may be difficult since transfer of a sufficient inoculum from XAC on asymptomatic fruit to canker-free trees is difficult to imagine. However, additional scenarios presented may emphasize the unlikelihood of such an event and thus provide a more convincing presentation. Several of the public commenters provided some additional, albeit unusual, scenarios for Event 5 that perhaps should be added to a revised Evaluation and discussed therein.

3. Completeness of data and evidence. I spent several hours searching for additional data or references relevant to the Evaluation. For the most part, I did not find much new relevant and clearly reliable information that was not already present in the document. For the search I used Google Scholar, Web of Science, PubMed, and Science Direct. I only found one additional reference for consideration: Timmer, Gottwald, and Zitko. 1991. Plant Dis.

75:192-195. The experiments in this paper show that XAC-containing lesions on citrus leaves harbor < 10^7 viable XAC cells in early disease stages (< 2 weeks), but decline to < 10^3 XAC cells per lesion after 1 month. Extrapolating this data to fruit suggests levels of XAC on any processed fruit would be below the threshold needed for infection, since Gottwald and Graham (1992) showed that concentrations <10^4 XAC cells per ml are insufficient for infection of citrus leaves even when using high pressure to mimic wind-blown transmission/infection. However, there was no data presented nor any discussion of the levels of XAC needed to infect wounded leaves. Some of the public commenters cited additional 'publications' on XAC and citrus canker, but most were not peer reviewed or were published > 60 years ago. In my opinion their additional contribution to the Evaluation would be minimal.

4. Are assumptions and uncertainties clearly stated? For the most part, Yes. However, one important assumption is that culling of blemished fruit and surface disinfection procedures at the packinghouse are performed properly and effectively, thereby reducing XAC levels on fruit to a safe level. It is unclear how compliance with these regulations is enforced and how effectiveness of the phytosanitary processing is monitored. The efficacy of chlorine as a bacteriocide decreases with time and use due to inactivation by organic matter on fruit surface (Brown and Schubert, 1987; Stapleton 1986). Although sodium orthophenylphenate (SOPP) is a much more effective biocide against XAC, and not as subject to inactivation, data concerning use of SOPP in the packinghouse and quality control on the efficacy of phytosanitary processing is not presented. This uncertainty should be clearly stated or data concerning this aspect added to a revised Evaluation and discussed.

5. Data/evidence omitted. Quantitative details of the presence and extent of survival of XAC cells on surfaces or on fruit after packinghouse processing and, more importantly, minimum concentrations/amounts of XAC potentially needed for infection of canker-free citrus trees are not given. In the absence of these details, it was somewhat more difficult to evaluate the conclusions of the document. For example, it is stated that XAC survives poorly on surfaces. This could mean a 99% loss of viability or 99.9% or 99.99%. Furthermore, without initial concentration data of XAC levels on fruit surfaces, potential inoculum size and concentration on fruit when it reaches the consumer is cannot be estimated. As pointed out by one public commenter, there are too many qualitative statements [e.g. poorly, very low, extremely low, very unlikely, etc.) that need to be backed up or defined by quantitative data [i.e. numbers]. To circumvent this insufficiency, I carefully read the key references to obtain such details and hence can provide an independent and more accurate evaluation. I found that few if any of the experimental details or quantitative results contradicted or were inconsistent with the conclusions of the authors of the Evaluation. If anything these quantitative details more strongly supported the conclusion that XAC does not survive well or for long periods of time on or in fruit, and that the amounts and concentrations of XAC expected on or in canker-free fruit before processing are likely below the levels required for infection of canker-free citrus. For example, Graham et al (1987) and Timmer et al (1996) showed leaves from symptomatic citrus have < 10^5 XAC cells per g, while active lesions contain < 10^6 XAC cells per g; these levels decline over a 30 day period by at least several logs. XAC levels and survival on infected, cankered fruit are likely similar. In fact, Graham et al (1992) artificially-inoculated XAC and a closely related citrus pathogen (*X. campestris* pv *citrumelo*) onto citrus fruit at

very high levels (10^8 cells per ml) with high pressure, and found that the fruit supports or retains only 10^4 cells/cm^2, and then only for periods less than 14 days. Stapleton (1986) also sprayed [soaked] citrus fruit with 10^8 XAC cells per ml and found "no survival" after overnight drying. Phytosanitary processes at the packinghouse should reduce XAC levels on fruit even further by several logs to likely innocuous levels. It is difficult to imagine how packinghouse processed asymptomatic fruit could carry sufficient levels of XAC to serve as a source of inocula for citrus trees, since Gottwald and Graham (1992) showed that concentrations < 10^4per ml are insufficient to infect leaves even at a high pressure mimicking high wind.

Another significant omission in the Evaluation are details concerning the effectiveness of the fruit dipping at the packinghouse to surface disinfest fruit from XAC contamination. According to Brown and Schubert (1987), SOPP is much more effective than chlorine and chlorine effectiveness can be compromised by organic compounds on fruit surfaces. Thus chlorine dipping can and does leave significant numbers of XAC on fruit. Finally, there is no data presented concerning the reduction in viability of XAC due to drying and waxing. Although the title of the Stapleton (1986) citation suggests this information is available, I did not find clear quantitative data in this reference. Although it is expected that drying and waxing by themselves would dramatically reduce XAC levels on fruit surfaces by at least one or two logs, quantitative data was not presented in the Evaluation. Similarly there was no data or discussion presented on the effect of chilling during shipping on viability of XAC on fruit surfaces. Viability of some phytopathogenic bacteria [e.g. Ralstonia solanacearum] that are closely related to XAC is dramatically reduced by several logs by chilling for a few days.

6. Quality and completeness of each section---Very good.

7. Ease of understanding document---This document was very easy to understand. Presentation and discussion in the format of events needed for infections made it very easy to follow.

Peer Review Report
Reviewer No. 4

Peer Review
of
Evaluation of asymptomatic citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)

I was selected for this review on the basis of my knowledge of the risk assessment process and not my expert knowledge of citrus, citrus canker or Xac. Enclosed you will find my review comments with a focus on the risk assessment process. They have been organized around the seven questions posed in your peer review plan. There is some redundancy at points that reflects the overlap in a few questions and my own uncertainty about where to best reflect a concern or observation.

1. The evidence provided and whether the conclusions and inferences are correctly supported by the evidence.

There is substantial evidence provided about the bacteria and its spread. Plant disease is not my area of expertise but I was suitably impressed by the breadth of the evidence summarized. In general I find the conclusions and inferences linked to the evidence in a credible fashion. However, I have a recurring concern that the conclusions drawn and the evidence they were based upon were at times too heavily influenced by assumptions that were made. I will take up this issue in more detail under item four below.

To illustrate this concern very briefly consider the "Event 2" discussion beginning on page 10 of the evaluation. The scientific evidence convincingly suggests that systems approaches to the management of Xac are highly effective. This argument requires an implicit assumption that such systems are functioning effectively in all points of origin. This is a critical assumption that is not supported by evidence in this document. The conclusion that "The likelihood of inoculum on infected fruit surviving the packing and treatment process is very low" is supported by the presented evidence only if the implicit assumption is valid. What, for example, would be the outcome in a grove of origin where change in management, cost cutting pressures or shortages of equipment and supplies render the systems ineffective? Does this pathway now become more viable? This analysis neither considers such alternative conditions on the pathway nor addresses why such a scenario is unlikely/impossible.

The Executive Summary conclusion states, "*that asymptomatic, commercially produced citrus fruit that has been treated with disinfectant dips and subject to other mitigations is not epidemiologically significant as a pathway for the introduction of citrus canker*." This seems a reasonable conclusion based on the stated and implied assumptions. However, there is a contestable assumption in this conclusion. Is asymptotic fruit that has not been treated as described epidemiologically significant? The evidence to suggest that treatment is widespread and reliable is not presented in the evaluation.

2. Evaluate the methodology. Is the approach and process appropriate for the analysis?

The methodology used for the pathway evaluation is consistent with the methods used by APHIS and with internationally accepted standards. In particular the evaluation is consistent with IPPC guidance found in sections 2.1 and 2.2 of ISPM 11 ***Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms ISPM No. 11*** guidelines with the exceptions noted below. I have used this international standard as a reference point for my review of the methodology. In the following paragraphs a summary of the relevant ISPM 11 steps is listed first with my own findings following.

Stage 1: Initiation
1.1 Initiation points.
1.2 Identification of PRA area
1.3 Information
1.4 Conclusion of initiation

This pathway evaluation was not a typical assessment of a specific pest and host from a specific origin. The pathway data were more broadly identified. Specific details are less applicable to an evaluation of a pest from any and all sources on a specific pathway, such as this one. The APHIS evaluation clearly identified the pest and pathway of quarantine concern. The pest risk assessment area (PRA) was the citrus growing areas of the United States. Relevant previous pest risk assessments were identified. The Stage 1 work in this assessment was appropriately sufficient.

2. Stage 2: Pest Risk Assessment
2.1 Pest categorization
2.1.1 Elements of categorization
2.1.1.1 Identity of pest
2.1.1.2 Presence or absence in PRA area
2.1.1.3 Regulatory status
2.1.1.4 Potential for establishment and spread in PRA area
2.1.1.5 Potential for economic consequences in PRA area
2.1.2 Conclusion of pest categorization

The categorization of the pest as one of potential quarantine concern was reasonably established.

The APHIS evaluation did not address the nature of the economic impact of Xac (2.1.1.5). Given the assumption of existing treatment/risk management the pathway is epidemiologically insignificant. In such a scenario the decision to overlook the economic consequences is reasonable. Under alternative conclusions the missing economic consequence would be a significant omission.

Implicit in the stated Executive Summary assumption (see above), of course, is the risk management option that would render the pathway epidemiologically insignificant. It would be appropriate to provide the evidence to support the ubiquitous nature of these risk mitigations or a more detailed explanation of how APHIS would assure the desired risk management options are in place and used diligently.

2.2 Assessment of the probability of introduction and spread
2.2.1 Probability of entry of a pest
2.2.1.1 Identification of pathways for a PRA initiated by a pest
2.2.1.2 Probability of the pest being associated with the pathway at origin
2.2.1.3 Probability of survival during transport or storage
2.2.1.4 Probability of pest surviving existing pest management procedures
2.2.1.5 Probability of transfer to a suitable host
2.2.2 Probability of establishment
2.2.2.1 Availability of suitable hosts, alternate hosts and vectors in the PRA area
2.2.2.2 Suitability of environment
2.2.2.3 Cultural practices and control measures
2.2.2.4 Other characteristics of the pest affecting the probability of establishment
2.2.3 Probability of spread after establishment
2.2.4 Conclusion on the probability of introduction and spread
2.2.4.1 Conclusion regarding endangered areas

The evaluation addressed items 2.2 through 2.2.3 adequately. Presuming the data to be complete, the methodology for gathering evidence and linking it to the findings was consistent with general practice. However, as will be discussed under item 4 below, assumptions made limit the findings to those situations in accordance with the assumptions. This is a significant limitation. Consequently, items 2.2.4 and 2.2.4.1 could be strengthened through sensitivity analysis.

The key findings of this analysis are summarized as:

The evaluation concludes that asymptomatic, commercially produced citrus fruit that has been treated with disinfectant dips and subject to other mitigations is not epidemiologically significant as a pathway for the introduction of citrus canker... There is no evidence that asymptomatic fruit is epidemiologically significant as a means for spread... A strong record of empirical data from experience and interceptions further reinforce the conclusion that the likelihood of introducing citrus canker on asymptomatic fruit is extremely low. Page 1

For a reviewer who is not an epidemiologist, epidemiological (in)significance is a term of art. It lacks the specific meaning of "statistically (in)significant" at a given α for instance. The analysis repeatedly relies on terminology that lacks sufficient definition to characterize risks as clearly as possible. The lack of an objective definition of terms renders the analysis less transparent than it could be. One of the more important needs in this area is the definition of the rating scales used throughout the analysis. In fairness, it is noted that terms of art are a staple in the literature and in this kind of risk assessment. This analysis is no different in that regard.

Rating Scale

The qualitative nature of the analysis is consistent with both national and international standards and practices. Rating the five risk elements enumerated on p. 8 using qualitative means is consistent with widespread practice. My concern with this approach is not, then, directed so much at this specific analysis—which is consistent with common practice--so much as it is with that common practice.

A qualitative rating can be very useful but a vague rating limits that utility. The ratings used in this analysis are vague in the sense that that they are neither defined nor generally subject to a common understanding. Arguably the intent of the ratings is reasonably clear, but they are fundamentally subjective and unanchored and this hampers both their meaning and transparency.

Consider this excerpt from the Executive Summary (bold underline added):

Even if infected fruit were to enter a canker-free area with susceptible hosts, the establishment of citrus canker via this pathway is ***highly unlikely****. There is no evidence that asymptomatic fruit is* ***epidemiologically significant*** *as a means for spread. In the* ***unlikely event*** *that viable propagules were present, the environmental and physiological conditions necessary for disease development at the precise time that an infected citrus fruit was placed in close proximity to a susceptible host is* ***highly unlikely****. A strong record of empirical data from experience and interceptions further reinforce the conclusion that the likelihood of introducing citrus canker on asymptomatic fruit is* ***extremely low****.*

This sort of conclusion is quite representative of the information presented in this field. Its intent is also unmistakable. The risk presented by this pathway is negligible—under the conditions assumed in the analysis. The terminology used, however, is not as instructive as it could be if the rating scale was better defined. Even an informed reader is challenged to differentiate the likelihoods of unlikely, highly unlikely and extremely low events.

Usage of these terms continues in the discussion of the "events" that begins on page 8. Terms such as: likely, very low, highly unlikely, very unlikely, possible and small are used. It is not my intent by this comment to limit the usage of descriptive words when quoting or summarizing the literature or when writing expressively in a narrative. My concern relates explicitly to attempts to characterize the risk of a pathway or pest. Ratings used to characterize the risk should be consistent and at least modestly defined by the analyst.

The measurement scale could be improved. When what is measured is concrete a standardized measure is preferred. When what is measured is more complex and abstract standardized measures may neither exist nor provide a close enough fit to a particular scenario. In such case a customized scale is preferred.

Ideally, a well defined rating scale consistent across multiple uses might be defined. Even a Likert scale would be acceptable, where ordinal differences exist but may not convey an order of magnitude of difference between ratings.

Is highly unlikely a rating chosen from a well defined scale, for example? At a minimum, it would be useful to know if the analyst works from a five-, seven-, or nine-point scale. If the range for a rating can be further described, so much the better for transparency.

The failure to use such a scale or rating method is widespread and common; it is not restricted to this analysis. One unintended consequence is that such a scale can be interpreted to mean whatever the reader intends it to mean. There is a substantial literature (see for example, **Rating Scales: Numeric Values May Change the Meaning of Scale Labels** Norbert Schwarz, Barbel Knauper, Hans-J. Hippler, Elisabeth Noelle-Neumann, Leslie Clark *Public Opinion Quarterly*, Vol. 55, No. 4 (Winter, 1991) , pp. 570-582) that suggests instability in how people use these scales to convey meaning.

As a practical matter I would find it most useful if the narrative at least reported that the ratings were chosen from an x-point scale where the scale points are carefully identified and preferably defined. This would provide some useful context for the analysts' judgments. Qualitative ratings are appropriate and acceptable when they are reasonably well understood by all.

3. Are there (sic) data or other evidence complete? Have any important data or considerations about the disease or pathway been omitted?

Evidence

I am not qualified to comment on the completeness of the evidence on Xac. I did note on page 15 the analysis said (emphasis added):

*Empirical data from a **significant history** of programmatic experience and interceptions demonstrates that even with a high frequency of unauthorized citrus fruit imports, outbreaks linked to fruit have never been observed.*

None of those data are summarized or presented. A brief summary of the data may have been a useful addition for some readers.

Considerations

The major thrust of my criticism of this well done analysis is that it does not consider the entire range of possible management conditions along the pathway. These concerns are discussed at multiple points throughout this review and are not further developed here.

4. Are all important assumptions identified and uncertainties clearly stated?

Assumptions

It is my experience that this analysis has treated assumptions in a manner consistent with common practice. However, I find this common practice to be less than optimal and occasionally inadequate to support conclusions.

This analysis like all other pathway risk assessments must rely on assumptions to characterize the risk of the pathway. Some assumptions are clearly more significant to the risk characterization and subsequent decision making process than others. The most significant assumptions are not always singled out and identified as such. I suggest this is more the result of the authors' expertise than anything else. It is common practice for those with considerable expertise to over estimate what their readers already know.

This "bias" of expertise often results in a failure to explicitly identify the most important assumptions in the analysis. For purposes of clarity, I consider an explicit identification of an assumption to be one in which the author uses the word assume or assumption in close proximity to the assumption itself. I consider it a fair retort that some statements are clearly assumptions despite their failure to explicitly say so. I argue that to remove all ambiguity it would be helpful to list key assumptions in an appendix or at least to use the word assume/assumption in the narrative.

Using my own definition of terms I found five explicit assumptions as follows.

The study is not limited by the origin of the fruit but rather assumes the fruit may be from any origin where citrus canker occurs; foreign or domestic. Page 4

The first event is assumed to be likely if fruit originates in areas where the disease occurs, but the magnitude of the hazard at this stage will depend in large part on the proportion of infected fruit and the nature of the contamination. Page 8

If we assume that citrus is distributed proportionally across the United States according to population, then it is reasonable to assume that some fruit will be shipped to these States. Page 13

Assuming the inoculum is available, the following must also occur for disease development to occur:
- *A susceptible host must be available;*
- *The host must be exposed during a time when infection can occur (varies with cultivar);*
- *Free water must also be available on the surface of the citrus plants from rain; and*
- *Wind speed must reach 8 m/s*. Page 14

These points consistently argue that asymptomatic fruit is not epidemiologically significant as a pathway for introducing citrus canker if produced under the conditions of a systems approach consistent with the assumptions of this analysis. Page 15

This last point above is particularly salient. The conclusion is indeed reasonable if the assumptions are clearly articulated and then met. First, the identification of assumptions is left to the reader. Next, the epidemiological significance of the pathway if the assumptions are not met is not clear. This lack of conformity with the basic assumptions would have been a relevant focus for sensitivity analysis. A best practice sensitivity analysis most often would focus on the critical assumptions of the analysis. If they are adequately/explicitly identified this can produce a satisfactory consideration of potential changes in the outcome in response to alternative assumptions and conditions.

In this analysis the epidemiological significance of the pathway under other circumstances remains occluded in part because of the lack of a sensitivity analysis and in part because of the lack of explicitly articulated assumptions. The assumptions of this analysis are sometimes subtle and implicit rather than explicit. It is not clear what is subsumed under "*the assumptions of this analysis*" from page 15 above. For transparency those assumptions should be clearly identified somewhere in the analysis.

An example of the more subtle assumptions, not explicitly identified, follows:

This study focuses on citrus cultivated under specific management practices that include quality control measures implemented to control the incidence of citrus canker in the field to ensure that fruit originates from groves with a low prevalence of citrus canker and that procedures are in place to prevent blemished or symptomatic fruit from being present in shipments of commercial citrus. Page 4

The study focuses exclusively on these conditions to the exclusion of all other possibilities. This is an assumption of major significance. If these specific management practices are not undertaken with due diligence the risk associated with the pathway could be different than the analysis suggests. No evidence is presented to establish the extent to which this assumption may be reasonable. Requiring, then verifying and monitoring such management practices would be a reasonable risk management plan to assure the epidemiological insignificance of the pathway. This analysis does not make it clear if this is an intent for all points of origin.

Numerous implicit assumptions are made throughout the analysis. These typically are not unreasonable because most of them imply that results found in the literature are achieved in the reality of this pathway. An example to illustrate this point follows:

Post-harvest treatments (e.g., sodium hypochlorite dips) of artificially inoculated fruit have been shown to eradicate Xanthomonas campestris pv vesicatoria, a closely related bacteria (Brown & Schubert, 1987). 200 ppm Cl applied for 2 minutes to infested fruit collected from the field showed that natural bacterial populations were lowered by 77-99%. Page 7
The implicit assumption is that these same reduction rates are being achieved in the fields of origin. This assumption is not unreasonable in many instances. However, this is a pathway analysis that is not limited by the origin of the fruit and this strains the assumption if the expected variation in the diligence with which these measures are implemented includes extremes of under performance. Very clearly, the results of this analysis suggest there is negligible risk from pathways that diligently apply the risk management measures described throughout. The concern is not with these points of origin but with the extremes of implementation that might include those on the margins of effective risk management.

This variation in management measures and their results is an inherent concern of any pathway risk assessment. The practical utility of such assessments, then, relies heavily on how risks at the margins of good risk management of the pathway will themselves be managed. In other words, what can and will be done to assure that the assumptions that lead to a finding of insignificant epidemiological risk hold in each specific case?

To further illustrate this point, consider the following excerpt:

Well-timed field treatments significantly reduce the incidence of the disease and therefore the level of inoculum and the number of symptomatic fruit in the field. Commercial growers for the fresh fruit market routinely apply copper sprays to control citrus canker (Graham and Gottwald, 1991; Gottwald et al., 2002a; Graham et al., 2004b) and as a general measure to reduce the incidence of other bacteria and fungi that adversely affect the quality and shelf-life of citrus fruit. Page 9

There would seem to be an implicit assumption in the findings of this analysis that all origins of fruit apply copper sprays and they do so in a well-timed manner. It does not strain credulity to imagine that some growers in some circumstances may be unable or unwilling to do this. The analysis provides no insight into the risks that could result from such circumstances nor does it provide any assurance of a risk management plan to assure these circumstances are met. This leaves me with some unaddressed concerns about the epidemiological significance of the pathway under a wider array of conditions than those defined for this analysis.

The analysis does a very credible job of characterizing the risks of the pathway under a specific set of assumptions. Arguably these assumptions will not always hold. When they do not we know nothing about the resulting risks or the means that will be employed to assure the assumptions do hold in all places of origin. This analysis in effect says when things are done well the risks from this pathway are negligible. We are not told the risks when things are not done well nor are we told what will be done to assure things are done well. This can be accomplished in a sensitivity analysis or possibly in a worst-case scenario analysis.

Uncertainty

Uncertainty, in a practical sense, is closely related to the assumptions made in a risk assessment. In fact, making assumptions may be the most common way to treat an uncertainty. Thus, carefully articulated assumptions go hand-in-hand with carefully articulated identification of key uncertainties in these types of qualitative risk assessments.

Uncertainty can occur at many points in a pest risk assessment. It sometimes occurs in the science as illustrated in the following, trivial to this assessment, excerpt (bold underline added):

Likewise, infected cull fruit and processed fruit pulp have also been identified as possibly facilitating long-distance spread of the pathogen. ***The likelihood of this occurring is unknown*** *(Smith et al., 1997). Page 8*

In a similar fashion there are multiple instances of admission of a lack of evidence (see example below). The analysis is exemplary in identifying these uncertainties.

There is no record *that either infected fruit with lesions or asymptomatic fruit are epidemiologically significant with respect to the initiation of new infections (Jetter et al., 2000; Canteros, 2004; Argentina, 2005; Risk Assessment,2005). Page 8*

Uncertainty can also exist within the reader of this analysis and it is unreasonable to expect the analysis to preclude all such responses. However, when uncertainty exists as a result of the terms of art used (previously discussed), the authors bear a burden of responsibility to address that uncertainty.

Perhaps the most commonly recognized uncertainty in the field is that identified by the IPPC in ISPM 11 summarized below:

2.4 Degree of uncertainty
Estimation of the probability of introduction of a pest and of its economic consequences involves many uncertainties. In particular, this estimation is an extrapolation from the situation where the pest occurs to the hypothetical situation in the PRA area. It is important to document the areas of uncertainty and the degree of uncertainty in the assessment, and to indicate where expert judgement has been used. This is necessary for transparency and may also be useful for identifying and prioritizing research needs.

Extrapolating from the literature cited to all points of origin for the fruit is a substantial challenge that could be expected to amplify uncertainty. My concern with the extent to which the stated and implied assumptions apply in all possible points of origin has been a chief example of this. In general I find the uncertainty discussion beginning on p. 15 to be very helpful in guiding future research and less helpful in understanding the key uncertainties that may affect the conclusions of this analysis. Let me illustrate the point in contrast to the uncertainty issue below.

Issue: 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, symptomatic fruit that has been treated post-harvest)?
Reason: Phytosanitary requirements are not justified if treated fruit with lesions are not a pathway. Page 16

This focuses on symptomatic fruit as do some other uncertainty issues identified. The presumption being that if treated symptomatic fruit is no risk then neither is treated asymptomatic fruit. While this may be very useful information for refining this analysis in a future iteration it fails to address those elements of this analysis that could possibly lead to an alternative conclusion. For example, it may be more critical to know the extent to which fruits are actually treated in these ways. Absent evidence of 100 percent diligent application at all points of origin then the analysis might address if or how this might affect conclusions.

The last issue identified provides an example of a more germane uncertainty issue. (Note there appears to be a typographical error in the reason narrative.)

Issue: What is the efficacy of specific packinghouse equipment and procedures in removing blemished fruit?
*Reason: Optical scanners in commercial use have been reported to accurately detect less than 1 in 100,000 *blemished* fruit (only a small proportion of the blemishes might be attributed to citrus canker). This would appear to be a highly effective mitigation. However, data have not*

been provided on the consistency, effectiveness, or other characteristics of optical scanning systems, or their relative effectiveness compared to manual systems. Page 16

A best practice consideration of uncertainty may have investigated the potential effect of using "less efficacious" equipment on the characterization of the risk of this pathway, if this had been judged a key/significant uncertainty. I consider a key/significant uncertainty to be a value that has the potential to change the findings or recommendation of a pest risk assessment.

Summary

The treatments of assumptions and uncertainty in this analysis are not inconsistent with common practice in pest risk assessments but both fall somewhat short of what I would personally consider best practice methods.

5. Identify any relevant data or evidence not contained in the report.

I lack the qualifications to comment on the Xac-specific scientific evidence presented. The literature review appeared very thorough to this "lay person." Noting the similarity of this question and question three I offer three points on this issue.

Problem Identification

The analysis does not include a clear, concise and complete statement of the problem in its current context. The Introduction says:

This document was …completed in response to a request by the Deputy Administrator of PPQ to evaluate scientific and other evidence associated with asymptomatic, commercially produced citrus fruit as a possible pathway for the introduction of citrus canker, a bacterial disease caused by Xanthomonas axonopodis pv. citri (Hasse) Vauterin, et al., 1995. Page 4

The reader has no specific context for the analysis or why it is being undertaken in this way at this time. Granted, an interested reader could infer much of this but each reader would likely infer something slightly different. This gives rise to a wide range of interpretations of the actual problem being investigated. For example, if this problem was framed in terms of the risks associated with asymptomatic fruits from countries with effective Xac mitigations verified and in place, many of my comments would be unnecessary. This contextual information is not provided.

Any good risk assessment model stresses the importance of clear and careful problem identification in a proper context. The Deputy Administrator made this request for a reason. Domestic producers of citrus as well as importers and exporters of citrus would all have a stake in this issue and a different view of this problem. A problem identification statement that encompasses these perspectives would be a useful addition to the analysis. This problem identification statement should include the statement of purpose for the risk assessment as well.

We live in a sophisticated and cynical time. Stakeholders and interested readers who are not provided with specific background on the events that led to the initiation of the evaluation will manufacture their own plausible explanations. It is far better to have a simple explanation of the events that triggered the evaluation included in the evaluation.

A simple and direct problem statement would enhance the transparency of the evaluation. Who cares about this issue and why? Where does the concern for asymptomatic fruit arise? Is there science behind this concern or does the concern reflect the social values of stakeholders or others? It may be advantageous to summarize known differences in points of view on this issue in the problem statement.

This is the kind of information a simple problem statement would help to convey. This information is relevant to the context in which the policy determinations that stem from the evaluation should be considered. This is surely essential information. If it has been decided that this information is not appropriate to a risk assessment then it should be made available in an alternative format.

Economic Consequences

There is no discussion of the economic consequences of an introduction or spread of citrus canker. IPPC ISPM 11 includes substantial emphasis on economic consequences.
These are not discussed in this analysis. There need not be a specific economic analysis, a simple perspective on the magnitude of potential economic effects would be sufficient. This could be part of a problem identification statement or a brief stand alone narrative. I want to stress that I do not argue the entire ISPM 11 process needs to be present for every pathway assessment but some perspective on the economic significance of the resources at risk provides useful perspective.

Intentional Introduction

Beginning on p. 13 under the discussion of Event 5 two transmission scenarios are outlined. Intentional introduction scenarios are not considered in this analysis. What are the risks associated with asymptomatic fruit in the hands of people intent on introducing the disease to the United States?

I would readily grant that such questions may be well beyond the scope of this analysis. If that is so it provides a strong argument for including a problem identification statement in the analysis that explains this. Absent such a statement it remains a relevant concern.

6. Evaluate the quality and completeness of the individual components of the analysis.

The Executive Summary is exceptional for its directness and content with the exception of concerns noted elsewhere in this review.

The Introduction is well done and could be enhanced significantly by incorporating a problem statement.

The Biology and Epidemiology appear complete to a lay reader.

The Spread Potential was adequate; its conclusions appear to have been moved to the section that followed it.

The "Phytosanitary Evidence…" section is addressed under question 7. It is well organized and logical but redundant. It would be enhanced by the addition of a summary and conclusions as noted elsewhere in this review.

The Summary is of special importance and note. It quite openly assumes the presence of a systems approach to mitigate this risk. Based on that assumption set it concludes:

These points consistently argue that asymptomatic fruit is not epidemiologically significant as a pathway for introducing citrus canker if produced under the conditions of a systems approach consistent with the assumptions of this analysis. Page 15

This presents a very convincing argument that when systems are in place and conditions are not extreme there is little risk on this pathway. What we lack is evidence on just how often these conditions exist and what the risks are when they do not exist. The summary would be more compelling if it summarized a sensitivity analysis as well as the main finding.

I find the Uncertainty section lacking substantial utility for decision making. It does not help the reader understand the limitations and weaknesses of the current analysis.

Identification of APHIS Technical Contacts and Reviewers may be adequate for APHIS purposes but they would be inadequate for any other purpose. Insufficient information is provided about roles and contact information. I make no judgment about the advisability of this, I simply observe it.

To this lay person the Literature Cited appears extensive and superior. I note that my exploratory efforts to obtain access to selected papers that were not from the publicly published scientific literature were most often unsuccessful. These efforts included an online search, a library search and the services of a research librarian. This leads me to believe there would be clear benefits to making the more difficult to obtain articles available through APHIS to the extent that confidentiality, copyright and other concerns allow. APHIS subsequently provided this reviewer with copies of the cited literature.

The Figures were exceptional and very enlightening.

7. Comment on whether/where the document is difficult to read or understand.

The document was logically organized and clearly written. The prose was readily understandable to an interested reader. The Executive Summary findings were especially clear and strong. These are not trivial accomplishments, the authors are commended for this. I have

noted concerns about the rating definitions, the unstated assumptions and the lack of sensitivity analysis on key uncertainties.

The section "Phytosanitary evidence regarding asymptomatic fruit as a pathway" beginning on page 8 is especially attractive in concept and design. It provides an effective summary for the judgments of the authors. The identification of the five necessary events on page 8 is very useful. A discussion followed by a conclusion for each event is a good idea. That said, I found it difficult to track the cumulative effects of the evidence presented on the characterization of the risk of the pathway as I read despite the clarity of the conclusions, which were well supported, predicated on the assumptions. Nevertheless, I would find a summary such as that below pulled verbatim from the report, to be a useful summary element.

The presence of canker infected fruit in an infected orchard is likely; however the prevalence of diseased fruit and of healthy fruit with ephiphytic Xac will be ***epidemiologically insignificant****.*

The likelihood of inoculum on infected fruit surviving the packing and treatment process is ***very low****.*

If bacteria survive the packing process, they will have a ***high rate of mortality*** *during shipping.*

Although shipment of imported infected fruit to a suitable habitat is ***possible****, the fraction that would be shipped to suitable habitat is* ***small****.*

It is ***unlikely*** *that viable bacteria from an infected fruit would encounter a suitable host under the conditions required for disease development.*

I would prefer such a summary table to the repetition of facts in the analysis. If there is a reasonable way to avoid presenting the facts in the early segments of the analysis and then repeating them later as evidence it would improve the readability of the document.

Documentation Enhancements

The graphics used in this analysis were exceptionally useful and well done. They go beyond the norm in aiding transparency.

A gratuitous comment is offered for future consideration in selected risk assessments, especially pathway assessments. Digital technology could be used to greatly enhance the transparency of the problem under consideration and simultaneously to improve the learning value of the risk assessment itself.

Photographs of the pests and their effects on hosts as well as GIS or other maps showing the presence of conditions to suggest the most vulnerable areas would be valuable enhancements. Showing the bacteria and examples of canker as well as mapping areas in the US that are most vulnerable to establishment would have increased transparency in this analysis.

Such changes represent substantial increases in effort that is not appropriate for all risk assessments. But the long term benefits of such thorough documentation can scarcely be overestimated.