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Comments by scientists on the "Evaluation of Asymptomatic Citrus Fruit (*citrus* spp.) as a pathway for the Introduction of Citrus Canker Disease (*Xanthomonas axonopodis* pv. *citri*)"

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Regulatory Analysis and Development
PPD, APHIS, Station 3A-03.8
4700 River Road Unit 118
Riverdale, MD 20737-1238

Subject: Docket No. APHIS-2006-0045

The California Citrus Quality Council (CCQC) appreciates the opportunity to comment on the "Evaluation of Asymptomatic Citrus Fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv.*citri*)."

The California Citrus Quality Council, herein after referred to as the Council, represents the entire California citrus industry on matters of science including pest and disease issues. The Council views this proposal as one of the most important issues facing the California citrus industry. Since citrus from California is grown exclusively for the fresh market and more than 25% is exported to more than 40 international markets, the issues of invasive diseases are vital to the industry. Changing the California citrus industry to purely domestic markets or to processing is not an option. The Council's approach to trade access is to bring the best available science to bear on issues such as this proposal (herein after referred to as the PRA) being addressed here.

We approached a worldwide body of scientists and were able to receive comments from experts on three continents. These comments have been used in our analysis without attribution. The Council believes strongly in making the basis for an important proposal such as this PRA as transparent as possible. We applaud the Department for allowing time for comments to be received on this proposal.

General Comments: The systems approach to the control of citrus canker in this Pest Risk Assessment depends directly upon the ability of at least two defined mitigation steps to provide independent effects in mitigating pest risk associated with the movement of commodities at the level needed. Control of this disease is also dependent upon both the basic science being

sufficiently clear and the operational steps being consistently attainable. Although no mention is made of pest free sites in the proposal, we feel that the following comments by international experts on this issue should be included.

Based on the FAO International Standard for Phytosanitary Measures (ISPM) 10 "Requirement for the establishment of pest free places of production and pest free production sites" Florida cannot now be eligible for these designations. The pest does not move slowly over short distances, does have opportunity for artificial spreading, does have a high level of survival from season to season, does have a high level of reproduction and does not have sufficiently sensitive methods for detection.

Since the issue of buffer zones is not covered in this PRA we assume that Florida would not currently meet the requirements of ISPM 10. In particular we note that in the absence of buffer zones or hosts of the pest or adequate control of the pest on these hosts, the extent of the buffer zone should be determined on the basis of the distance over which the pest is likely to spread naturally during the course of the growing season.

Comments on Specific Sections

In the Biology and Epidemiology section on Page 5, 3rd Paragraph:
One should also reference Peltier and Frederich (1926, J. Agr.Res. 32, 147-164). Effects of weather on the world distribution and prevalence of citrus canker and citrus scab are also presented.

Page 5 under Biology and Epidemiology Section, 4th Paragraph, Line 5:
Koizumi (1976,1981) stated that the disease developed in the range of 13-36C with an incubation period preceding lesion development of 15-30 days at 15C, 7-15 days at 20C and 5-10 days at 25C.

Page 6, 1st Paragraph, Line 4:
There appear to be contradictory statements regarding the susceptibility of leaves to infection. Graham (1992) states that leaves are highly susceptible to infections from two thirds to full expansion stage. Serizawa, S. (1981). Recent studies on the behavior of the causal bacterium of the citrus canker are also found in the article (Proc. Int. Soc. Citriculture 395-7.) Serizawa, S. and K. Inoue (1974). Studies on citrus canker and the influence of wind on infection are cited (Bull Shizuoka Pref. Citrus Exp. Sta. 11:54-67).

Page 6, 2nd Paragraph, Line 5:
Newly developed shoots are not susceptible to infection immediately after bud burst but are highly susceptible to stomatal infection when 50-80% fully expanded (Koizumi, 1981).

Page 6, 3rd Paragraph, Line 1:
Also there are additional references about infections during the period of fruit enlargement (Stall et al., 1981) until the early stage of maturation or just before yellowing (Goto,1992) becoming resistant as fruit matures (Koizumi, 1981, Fulton and Bowman, 1929)

Page 6, Paragraph 3, Line 6:
There are additional studies that show the surrogate populations of *Xanthomonas axonopodis* survive for up to 21 days (Cooksey and Adaskaveg 2002).

Page 6, 4th Paragraph, Line 2:
Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* in Brazil are covered by (Chagas-MCM; Parra-JRP; Namekata-T; Hartung-JS; andYamamoto- Neotropical-Entomology. 2001, 30: 1, 55-59; 18 references.) In the same section on Page 7, it is acknowledged that asymptomatic citrus leaves are believed to harbor citrus canker bacteria for several months. Thus contaminated leaves are also a way the bacterium can survive if leaves are introduced into packed boxes.

Page 6, 4th Paragraph, added after last sentence:
However, it was found that adult citrus leafminers are not themselves efficient vectors for Xac (Belasque et al., 2005 Plant Disease 89(6), 590-4.)

Page 7, 1st Paragraph, Line 3:
Additional information on citrus weed and grass hosts on weeds is described below.

- The pathogen survived in Japan for 1-10 months on various weeds, in lesions on defoliated leaves and dropped fruit and in infested host roots (*C. natsudaidai*) and rice straw (Goto, 1970, Goto et al., 1975 a and b, 1978). However it is uncertain if the bacteria found in such places in citrus groves at rather low populations play a role as an infection agent to new shoots (Serizawa, 1981).
- X. a pv citri can be detected on the surfaces of various kinds of weeds growing in citrus groves in Japan (Goto et al., 1975 a and b, 1978). In most cases the bacterium rests on the plant surface without active multiplication after being splashed by rain. But for *Zoysia* spp. (grasses) and *Calistegia japonica* (Japanese bindweed), the longevity of the bacterium is much longer, even though they are immune to infection. The bacterium is detected from their rhizomes or root systems for at least several months.
- In Brazil, Pereira et al., (1978) found that Xa survived in the rhizosphere of guinea grass (*Panicum maximum*).
- On planting trees in 1974 in an orchard in Brazil uprooted in 1957, canker symptoms reappeared, *X. citri* was isolated from the roots and rhizosphere of *T. insularis* growing in the area. High and constant pathogenicity was found on inoculating the bacterium into orange and lime (Pereira et al., 1976)
- Civerolo (1984) cited 2 papers by Stall from his work in Argentina, where he found Xac associated with a *Cenchrus* sp., *Solanum* sp. and an unidentified broad leafed weed.

Page 7, Line 4:

- *Xanthomonas axonopodis* pv. *citri* survives and multiplies primarily in lesions (Goto, 1962, Koizumi, 1977, Stall et al., 1980) and in symptomless (discoloured) bark tissues (considered to be the old canker lesions formed on twigs) of citrus trees (Goto, 1972). Goto (1972) stated "the findings of long survival in the bark tissues … (>3 years for lemon)… imply that it may be difficult to conclude safely the eradication of the disease only by a long term disappearance of the foliage canker."
- X. a pv. citri easily persists from one growing season to the next in old lesions, especially lesions formed late in the growing season. Stem lesions can harbor viable bacteria for several years. Viable bacteria were isolated from stem lesions on 5-7 year old trunk of Mexican lime in Florida (Gottwald et al., 2002).
- Goto (1992) states it is difficult to detect the epiphytic stage of X. a pv citri on the phylloplane and infestation experiments indicate that X. a pv citri can survive on a limited number of leaves for a few months during the rainy season in Japan but it quickly declines under sunny conditions to a low level undetectable by the leaf print method.

Page 7, Line 6:

· Koizumi (1981) states that "some bacteria which drop to the soil by defoliation or dispersion can survive saprophytically for one year or more, especially in the rhizosphere of citrus trees and some kinds of herbaceous plants.

· Goto (1992) states that X. a pv citri usually dies quickly in tissues of diseased leaves which fall to the ground and become moist. The population of bacteria decreased as mesophyll rotted after infected leaves were defoliated and disappeared when the lignocellulose was rotted (Koizumi and Yamada, 1972). The bacteria survived for a longer period in leaves on the soil surface and during the winter when the leaves rotted gradually (Goto, 1981).

· The causal bacterium died within 3 weeks in lesions of grapefruit and citrange leaves that were wetted either by placing them on the soil surface or by burying them in soil at a depth of 3-6 inches (Peltier and Frederich, 1926). A rapid disintegration of the buried leaves took place so that by the end of the 3rd week the identity of the leaf was almost lost.

· However, Goto (1992) states that the bacterium can survive for 2-3 months in lesions if the diseased leaves are maintained under dry conditions either in vivo or in vitro if leaf moisture quickly decreases to <20%. Within canker lesions, the bacteria are buried in a thick matrix of extracellular polysaccharides and the bacteria can survive for fairly long periods if the extracellular polysaccharides around them dry and are left intact.

· Robbs and Deslandes (1968, cited in Rossetti, 1977) in Brazil, reported laboratory experiments in which the pathogen survived on infected fallen leaves for an average of 4 months. In a few cases, viable cells were found after 6 months and exceptionally after 8 months.

· Pathogenic X. a pv citri was detectable in leaf lesions of grapefruit and sweet orange at least 90 days after defoliation under relatively dry conditions in the spring of 1986 in Florida (Graham et al., 1987). Under similar conditions in Argentina, X. a pv citri was detected after 120 days in lesions of grapefruit leaves placed on the soil surface but only up to 85 days when leaves were buried (at this time they had decomposed beyond recognition).

Page 7, Line 7:

In soil:

· Fulton (1920), Lee (1920) and Peltier and Frederich (1926) all found that free cells of P. citri (X. a pv citri) underwent a rapid decline in numbers in all types of non-sterile soil and the vanishing point was 2 weeks.

· Fulton (1920) found that *P.citri* decreased very rapidly in various inoculated soil types: the rate of decline was most rapid for the clay soil, slightly less for leaf mold and distinctly slower for compost and garden soil. In another experiment with inoculated Florida soils (high/low humus, muck, hammock), *P.citri* could be retrieved from the muck soil at 56 days when the experiment was terminated. It was noted that watering was overlooked at one stage and the dry condition may have contributed to the long persistence. There was a very evident retardation of the rate of decline at lower temperatures and in dry soil. P. citri could be detected in inoculated dry Florida soil for 4 months but application of sterile water to the soil promptly reduced detection levels to zero.

· Jehle (1917) found red clay and glade soil inoculated with P. citri caused infection after 47 days.

· Stevens (1915) found canker bacteria alive and virulent in soil taken from the site of an infected tree destroyed 9 months previously.

· Rossetti et al., (1969, cited in Rossetti, 1977) carried out experiments where healthy nursery plants were planted in pots of soil from infected orchards destroyed 2 to 4 years earlier. Leaf symptoms of canker appeared in 12% of plants. Pereira et al., (1976) studied herbaceous weeds to determine probable alternate hosts of the pathogen. The site was an orchard in Brazil which had been destroyed by the eradication campaign in 1957. A few citrus plants were experimentally replanted in this orchard and were destroyed in 1974 because they were

infected. No citrus had been at the site for many years, indicating that the pathogen had survived in the absence of its citrus host. Pereira's research showed that X. a pv citri was living saprophytically in the roots and rhizosphere of sourgrass (*Trichachne insularis*).

- Goto et al., (1975) and Goto (1992) states that in heavily infested sites, the canker bacterium can maintain its populations in soil at levels sufficient for infection for no more than a few weeks during the citrus growing season. There is no evidence for multiplication of the bacterium in natural soil.
- Saprophytic survival of X. a pv citri in the absence of plant tissue or debris has not been conclusively established (Civerolo, 1984).
- Leite and Mohan (1984) in Brazil found that X. c. pv citri survived no more than 35 days in a naturally infested soil.
- After the removal of infested citrus plants to simulate eradication, X. a pv citri could not be recovered after 21 days by semi selective media from nursery sites in Argentina and USA (Graham et al., 1989).

Page 7, 3rd Paragraph:
Reference to studies are made in the proposal on closely related bacteria surrogates by Brown & Schubert, 1987 indicated that the use of 200 ppm Cl for two minutes reduced bacteria level by 77-99%.

Studies reported in 2002 in Cooksey and Adaskaveg on the same canker surrogate (*Xanthomonas axonopodis* pv. *vesicatoria)* showed survival after drying for up to 21 days. Even after 21 days some strains survived at levels as high as 3.5×10^3. The Florida studies on the same surrogate bacterium showed survival at only 1×10^2 to 1×10^3 after 6-12 hours.

Page 7, 2nd Paragraph, 3 separate comments in Line 3:
Papers by Serizawa and Inoue in Bulletin of the Shizuoka Prefectural Citrus Exp. Sta, 1978, 1982 (various) should be referenced.

Evaluation of bactericides for control of citrus canker in Argentina showed that good control was obtained with tribasic Cu sulphate at 0.5%, sprayed every 60 days. (Timmer-LW, Proceedings-of-the-Florida-State-Horticultural-Society. 1988, published. 1989, 101: 6-9; No.321-324, 11-14; 2 tab.; 17 ref.)

Page 7, Line 5, 2nd Paragraph:
Up to 5 sprays per season have been used in Japan (Koizumi, 1981, Citrus Diseases in Japan).

Page 7, 4th Paragraph under Spread Potential section, Line 6:
Canker spread approx 8 km from infested property at Emerald in Queensland, presumably due to summer storm activity.

Page 7, 4th Paragraph under Spread Potential section, Line 7:
As Peltier and Frederich (1926) pointed out, citrus canker is severe in regions where temperature and rainfall ascend and descend together during the season.

Page 7, 4th Paragraph under Spread Potential section, following "1980" at the end of the page:
These are all assumptions. Has any climate modeling been done with canker for these areas?

Page 8, Paragraph beginning with Long Distance (1st Paragraph) Line 4:
Stevens (1915) wetted pieces of sterilized cloth with suspensions of the canker bacterium, and then allowed them to dry. A very large number of the bacteria were alive after a drying period of 5 weeks, giving credence to canker being spread by personnel on clothing.

Page 8, 3rd Paragraph Line 4:
The likelihood of infected cull fruit is dismissed as a source of transmission. Symptomatic cull fruit transported to disease free areas should be considered a possible source of transmission.

Page 9, 2nd Paragraph:
There has to be a time period between bacterial penetration (Serizawa papers) of the host (through stomata) and visual symptoms (latent period) - the latent period in disease development. Quiescent infections are probably what the authors intended to say when they said 'that do not occur'. Latent periods for *Xanthomonas* diseases have been described from 3 to 14 (-18) days. If fruit are picked in an orchard down wind immediately after a rain storm with 18 mph winds (infection period), then there would be symptomless infected fruit.

Page 9, 3rd Paragraph, Line 2:
Fruit is susceptible during the period of fruit enlargement (Stall et al., 1981) until the early stage of maturation or just before yellowing (Goto, 1992), becoming resistant as fruit matures (Koizumi, 1981, Fulton and Bowman, 1929)

Page 9, 4th Paragraph, Line 6:
The bacterium probably survives epiphytically at lower population levels on citrus hosts without symptom development (Goto, 1972)

Page 10, under "the systems approach"…Evidence section, Bullet 1:
Their efficacy will depend on when spraying occurs relative to infection and the residual copper on the fruit at the time of infection and on the presence of recent injuries during an infection period.

Page 10, under "infected fruit express"….Evidence section, Bullet 2:
Fruit is susceptible until the early stage of maturation or just before yellowing (Goto, 1992)

Page 11, 2nd Paragraph, comments for Lines 1 and 2:
Effects of postharvest chlorine and wax treatments on surface microflora of lime fruit in relation to citrus bacteriosis disease are described by (Stapleton-JJ, Plant-Disease. 1986, 70: 11, 1046-1048; 11 ref.) in the evaluation. Citrus bacteriosis (CB), a suspected form of citrus canker (*Xanthomonas campestris* pv. *citri*) is expressed as lesions on leaves and twigs of Mexican lime (Citrus aurantiifolia) as well as on other citrus plants in Colima, Mexico. Immersion of Mexican and Persian lime [*C. latifolia*] fruit in 200 ppm. Cl, as NaOCl, for 2 min is a prerequisite for movement of fruit out of CB quarantine areas even though no bacteriosis symptoms have been observed on fruit. In addition, most Mexican citrus packers spray fruit with a protective wax coating before shipping. The effects of these treatments on lime surface microflora were evaluated. Total bacteria were reduced by 77-99+%, and fungi by 81-100% in assays of fruit washings from limes treated with 50-900 ppm. Cl as NaOCl. Nevertheless, total bacterial populations of 2.7×10^2-2.9×10^3 cfu/cm^2 of fruit surface survived chlorine concentrations above the mandated 200 ppm level. No naturally occurring *Xanthomonas* spp. were recovered from fruit washings, although bacteria artificially inoculated in high concentrations were recovered at least 2 weeks later on lime surfaces. Presumptive *X. c.* pv. *citri* was not eradicated when intact or wounded fruit were artificially inoculated with high concentrations of cells, then

immersed in 200 ppm. Cl for 2 min. The protective wax used in Colima did not increase the efficacy of Cl treatment.

Page 12 under conclusions for the second event under evidence:
It states that "fruit culling would be expected to have removed a high percentage of symptomatic and blemished fruit prior to arrival". Citrus grown in humid areas typically has blemishes because of other diseases and insects. The PRA however has no requirements for the studies to show the efficacy of this step. Based on the fact that "in commercial operations, diseased, damaged and disfigure and blemished fruit are culled in the field and at the packing house" one would expect to observe clean unmarked commercial citrus fruit from Florida. Observations this season in California for commercial fruit purchased at retail outlets would argue that this cullage is not only inefficient for blemishes but that clean symptomless fruit is exceedingly rare. The efficacy of the culling by trained inspectors as presented does not appear to be documented. This deficiency would argue that mitigation steps directed at assuring "asymptomatic fruit without injuries or blemishes" for packing is a critical step missing in the systems approach presented in this PRA.

Page 13 under conclusions for the 4th Event, second bullet:
It references personal communications that states the likelihood of canker establishment in arid regions.

Page 13, comment at the end of Event 4: Surely a lot of backyards contain citrus trees?

Page 13, comment to the conclusions for the 4th event:
Has any climate modeling been done to see what regions constitute a "suitable habitat"?

Page 13, comment to the conclusions for the 4th event, Bullet 1:
This bullet needs verification by some climatic modeling

Page 13, comment to the Conclusions for the 4th Event, Bullet 2:
As Peltier and Frederich (1926) pointed out, citrus canker is severe in regions where temperature and rainfall ascend and descend together during the season and citrus canker could develop in all of the citrus regions of the world sometime in the growing season.
(Peltier and Frederich (1926), J. Agr. Res. 32,147-164) Effects of weather on the world distribution and prevalence of citrus canker and citrus scab are discussed.

Page 13, Event 5, #2, Line 2 following host:
This scenario was claimed to be the reason for more infections of canker observed along pathways and roads in Hunan Province where peasants threw infected peel.

Page 14, top of page first line:
What about splash from infected peel to the skirt of the tree?

Page 14, under the development of disease, Bullet 4:
Should splash be added?

Page 14 under Conclusions for 5th Event, Bullet 1:
Survival short term in the rhizosphere of certain weeds should be added?

Page 14 under Conclusions for 5th Event, Bullet 2:
Stomatal infection occurs when leaves are 50-80% fully expanded (Koizumi, 1981), but much longer when leaf miner is present.

Page 14 under Conclusions for 5th Event, Bullet 3:
A clarification regarding infection during the period of fruit enlargement (Stall et al., 1981) give a range of from the early stage of maturation to just before yellowing (Goto,1992) with the fruit becoming resistant as fruit matures (Koizumi, 1981, Fulton and Bowman, 1929).

Page 14 under Conclusions for 5th Event, Bullet 5:
Leaf miner changes this. What about fruit peel miner?

Page 16, end of 2nd Paragraph:
Effects of postharvest chlorine and wax treatments on surface microflora of lime fruit in relation to citrus bacteriosis disease should be noted. (Stapleton-JJ, Plant-Disease. 1986, 70: 11, 1046-1048; 11 ref.) Citrus bacteriosis (CB), a suspected form of citrus canker (*Xanthomonas campestris* pv. *citri*) is expressed as lesions on leaves and twigs of Mexican lime (Citrus aurantiifolia) as well as on other citrus plants in Colima, Mexico. Immersion of Mexican and Persian lime [*C. latifolia*] fruit in 200 ppm. Cl, as NaOCl, for 2 min is a prerequisite for movement of fruit out of CB quarantine areas even though no bacteriosis symptoms have been observed on fruit. In addition, most Mexican citrus packers spray fruit with a protective wax coating before shipping. The effects of these treatments on lime surface microflora were evaluated. Total bacteria were reduced by 77-99+%, and fungi by 81-100% in assays of fruit washings from limes treated with 50-900 ppm. Cl as NaOCl. Nevertheless, total bacterial populations of 2.7 X 10^2-2.9 X 10^3 cfu/cm^2 of fruit surface survived Cl concentration above the mandated 200 ppm. level. No naturally occurring Xanthomonas spp. were recovered from fruit washings, although bacteria artificially inoculated in high concentrations were recovered at least 2 weeks later on lime surfaces. Presumptive *X. c.* pv. *citri* was not eradicated when intact or wounded fruit were artificially inoculated with high concentration of cells, then immersed in 200 ppm. Cl for 2 min. The protective wax used in Colima did not increase the efficacy of chlorine treatment.

Summary:
Although this proposal has included no attempt to provide a quantitative estimate of the likelihood of this occurring in a probabilistic risk assessment or probabilistic scenario analysis as was done for Argentine fruit into the U.S., we ask why not.

There is agreement among the contributors that the proposal has a number of missing elements including missed references and the need for new studies. Information on the efficacy of mitigation measures is also deficient in this proposal.

As the editor of this information received from scientists, the Council believes that no definitive decision on the shipment of asymptomatic citrus fruit from Florida to U.S. citrus producing states should be based on the existing proposal.

Since the California citrus industry produces citrus solely for the fresh market, the experience of other nations meeting the requirements for fresh citrus exports indicate that living with canker in California is not an option.

Again, we appreciate the opportunity to comment on this docket.

Sincerely,

Hugh W. Ewart, Ph.D.
President
California Citrus Quality Council

Literature Cited:

Belasque et al., 2005 Plant Disease 89(6), 590-4

Chagas-MCM; Parra-JRP; Namekata-T; Hartung-JS; and Yamamoto-Neotropical-Entomology. 2001, 30: 1, 55-59; 18 references.

Civerolo (1984)

Cooksey and Adaskaveg 2002

Fulton (1920)

Goto, 1962, Koizumi, 1977, Stall et al., 1980

Goto, 1970, Goto et al., 1975 a and b, 1978

Goto,1972

Goto, 1981

Goto,1992

Gottwald et al., 2002

Graham et al., 1987

Graham et al., 1989

Graham (1992)

Jehle (1917)

Koizumi (1976,1981)

Koizumi, 1981, Fulton and Bowman, 1929

Koizumi, 1981, Citrus Diseases in Japan

Koizumi and Yamada, 1972

K. Inoue (1974) Bull Shizuoka Pref. Citrus Exp. Sta. 11:54-67

Lee (1920)

Leite and Mohan (1984)

Pereira et al., (1976)

Pereira et al., (1978)

Peltier and Frederich (1926, J. Agr.Res. 32, 147-164). Effects of weather on the world distribution and prevalence of citrus canker and citrus scab.

Robbs and Deslandes (1968, cited in Rossetti, 1977)
Rossetti et al., (1969, cited in Rossetti, 1977)

Serizawa, S. (1981).

Serizawa and Inoue in Bulletin of the Shizuoka Prefectural Citrus Exp. Sta, 1978, 1982 (various)

Stall et al., 1981

Stapleton-JJ, Plant-Disease. 1986, 70: 11, 1046-1048; 11 ref.

Stevens (1915)

Timmer-LW, Proceedings-of-the-Florida-State-Horticultural-Society. 1988, published. 1989, 101: 6-9; No.321-324, 11-14; 2 tab.; 17 ref.)