cdfa CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

California Pest Rating Proposal for

Spiroplasma citri Saglio et al., 1973

Stubborn disease of citrus

Current Pest Rating: none

Proposed Pest Rating: C

Domain: Bacteria, Phylum: Firmicutes
Class: Mollicutes, Order: Entomoplasmatales
Family: Spiroplasmataceae

Comment Period: 06/21/2021 through 08/05/2021

Initiating Event:

This pathogen has not been through the pest rating process. The risk to California from stubborn disease of citrus caused by *Spiroplasma citri* is described herein and a permanent pest rating is proposed.

History & Status:

Background: Citrus stubborn disease was first observed in Washington navel orange trees in California about 1915 (Fawcett et al., 1944). For many years it was thought to be caused by a virus, but citrus pathologists at UC Riverside and in France were able to culture and describe a "mycoplasma-like organism" and complete Koch's postulates in the 1970s (Saglio et al., 1973; Fudl-Allah et al., 1972). *Spiroplasma citri*, the causal agent of stubborn disease, is a phloem-inhabiting, cell wall-less bacterium in the class Mollicutes. It is an obligate parasite, surviving in citrus or in a variety of other host plants, and in its leafhopper vectors. It was named "stubborn" because of the persistence of the symptoms even after an infected tree is top worked with healthy budwood.

Spiroplasmas are single-celled, soft-skinned bacteria that lack an outer cell wall. They are named for their spiral (helix) shape that forms during certain phases of growth. *Spiroplasma citri* was the first mollicute of plant origin to be obtained in culture and the first cultured mollicute to be shown to have a helical morphology. There are only two other *Spiroplasma* species that cause plant diseases, *S.*

kunkelii, which causes corn stunt and *S. phoeniceum*, which infects periwinkle. There are dozens of species of *Spiroplasma* that can infect insects and ticks (Bove, 1997).

Isolation and culturing of *S. citri* is technically demanding and time consuming. The pathogen is typically low in titer (concentration) and unevenly distributed in trees, making reliable detection challenging (Wang et al., 2015). The symptoms alone are non-diagnostic and variable between varieties and conditions, but typically include generalized stunting, short leaf internodes, leaf mottling, unseasonal blossoming, and lopsided fruits. The pathogen is spread in a propagative manner by leafhoppers (mostly by beet leafhoppers, *Circulifer tenellus*) and by grafting with infected propagative material. The disease is more symptomatic on younger trees and can also be transmitted to several weeds and other crop species (Dreistadt, 2012).

Stubborn disease primarily affects grapefruits, mandarins, tangelos, and sweet oranges. The disease is prevalent in temperate regions with arid or semi-arid climates where citrus is grown with irrigation. It is not known to occur in tropical or sub-tropical regions. There have been observations of stubborn disease throughout the warm inland growing areas, i.e., the Coachella Valley and the interior valleys of central and southern California. It is present in the Mediterranean region including Israel, Syria, Turkey, and Morocco. These areas have limited seasonal rainfall but enough to support rapid germination and growth of weed hosts and for the weeds to support the leafhopper vectors. Because leafhoppers remain infectious for life, this pathosystem with the weeds presumably sustains *S. citri* and only limited spread occurs in citrus. Secondary spread in citrus is not thought to be important to the epidemiology of the disease.

Hosts: There are hosts of *S. citri* in multiple plant families including Amaranthaceae, Apiaceae, Brassicaceae, Asteraceae, Liliaceae, Chenopodiaceae, Convolvulaceae and Rutaceae. Many are common orchard weeds in California. Hosts include *Allium* sp. (onion), *Apium graveolens* (celery), *Armoracia rusticana* (horseradish), *Aster amellus* (Italian aster), *Barbarea vulgaris* (common wintercress), *Brassica nigra* (black mustard), *Brassica oleracea* (cabbages, cauliflowers), *Brassica rapa* (field mustard), *Brassica rapa* subsp. *chinensis* (Chinese cabbage), *Brassica rapa* subsp. *rapa* (turnip), *Brassica tournefortii* (African mustard), *Callistephus chinensis* (China aster), *Capsella bursa-pastoris* (shepherd's purse), *Catharanthus roseus* (Madagascar periwinkle), *Chrysanthemum maximum*, *Citroncirus webberi* (citrange), *Citrus aurantium* (sour orange), *Citrus jambhiri* (rough lemon), *Citrus limettioides* (palestine sweet lime), *Citrus limon* (lemon), *Citrus limonia* (mandarin lime), *Citrus madurensis* (calamondin), *Citrus maxima* (pummelo), *Citrus reticulata* (mandarin), *Citrus reticulata* x *paradisi* (tangelo), *Citrus sinensis* (sweet orange), *Citrus unshiu* (satsuma), *Citrus x paradisi* (grapefruit), *Convolvulus arvensis* (bindweed), *Daucus carota* (carrot), *Digitalis purpurea* (foxglove), *Echium* sp., *Fortunella* (kumquats), *Helminthotheca echioides* (bristly oxtongue), *Hirschfeldia incana* (shortpod mustard), *Leucanthemum* sp., *Matthiola incana* (stock), *Oryza sativa* (rice), *Phlox drummondii* (annual phlox), *Plantago ovata* (spogel plantain), *Poncirus trifoliata* (trifoliate orange), *Prunus avium* (sweet cherry), *Prunus persica* (peach), *Pyrus communis* (European pear), *Raphanus raphanistrum* (wild radish), *Raphanus sativus* (radish), *Sesamum* sp. (sesame), *Sesamum indicum* (sesame), *Sinapis arvensis* (wild mustard), *Sisymbrium irio*, *Sorghum halepense* (Johnson grass), *Tagetes erecta* (Mexican

marigold), *Trifolium pratense* (red clover), *Vinca* sp. (periwinkle), *Viola cornuta* (horned violet), *Zinnia elegans* (zinnia).

Symptoms: Infected trees show unseasonal flushes of leaves, stems and flowers. Leaves can be smaller than normal, cupped, abnormally upright, sometimes mottled or chlorotic. In severe examples and under very hot conditions, there are multiple buds and an excessive number of shoots and internodes, with leaves on some shoots misshaped, blunted or heart-shaped, with yellow tips, and some shoots forming a witches-broom. Trees are stunted, and fruit production is reduced. Fruit from infected trees are smaller and can be lopsided, with a curved columella. This symptom could be confused with another important bacterial disease, Huanglongbing. Mainly in grapefruit and tangelos, the albedo may become blue, and the flavor of the juice may be lacking, sour or bitter. Fruit can also show color inversions where the stylar end remains green even as the peduncular end becomes orange. Roots also may be stunted, and the seeds may be partially aborted (Calavan and Oldfield, 1979; Dreistadt, 2012; Eskalen and Adaskaveg, 2019).

The disease has a long latent period of months to years after inoculation. Detection varies with season with highest titer detected in hot summer months, concomitant with most pronounced symptom expression (Yokomi, 2013). Other hosts show symptoms of stunting and deformation, which is more severe when grown at higher temperatures.

Transmission: The pathogen lives in the phloem sieve tubes of infected plants, and inside various phloem feeding leafhoppers. It can multiply in the vectors and they transmit it from plant to plant by feeding, but transovarial transmission is not known to occur. In California, these leaf hoppers in the family Cicadellidae are proven vectors of *S. citri* and are known to occur in citrus growing parts of the state: *Circulifer tenellus*, *Scaphytopius nitridus*, and *S. acutus* (Kaloostian et al., 1979; Mello et al., 2009; Oldfield, 1988; Oldfield et al., 1976, 1977).

Spatial and temporal analysis shows there is mostly primary spread from weeds to citrus with very limited or secondary spread of citrus to citrus. *Scaphytopius* leafhoppers can develop on citrus, but the populations are generally low. *Circulifer tenellus* leafhoppers have a wide host range which includes many natural hosts of *S. citri,* but citrus is generally not a host of these leafhoppers. Citrus can become infected when inoculative *Circulifer* vectors feed temporarily on citrus during their migratory flights (Yokomi, 2013).

The disease is graft-transmissible through infected budwood, but the rate of bud transmission is generally low due to usually low pathogen titer. It is not seed transmitted or mechanically transmitted by pruning (Rangel et al., 2005). *Spiroplasma citri* –free mother trees should be used for propagation and citrus nurseries should be in locations where there is very low or no spread of *S. citri*. Trees that appear diseased and showing symptoms, or are abnormally stunted, should be removed and replaced with healthy replants or more tolerant varieties. Elimination of weed hosts within and around citrus groves is a way to reduce vectors and reservoirs of inoculum. (Yokomi, 2013; Oldfield et al., 1977; Dreistadt, 2012).

Damage Potential: The impact of citrus stubborn disease on commercially cultivated navel orange trees citrus was studied by Mello et al. (2010) in the Central Valley of California. Fruits from positive trees were smaller and often misshapen, but significant fruit number reduction occurred only in severely symptomatic trees. Trees with *S. citri* broadly distributed within the tree canopy were most impacted. There was a reduction in fruit weight, size, and quantity in severely symptomatic trees showing that stubborn is a significant constraint to production and marketability in California. Although *S. citri* naturally infects many other hosts including other crop plants, it is not reported to have any economic impact on these. Their main significance is as reservoirs of *S. citri* for infection of citrus (CABI-CPC, 2021).

Worldwide Distribution: Africa: *Algeria, Egypt, Libya, Morocco, Somalia, Sudan, Tunisia.* Asia: *Iran, Iraq, Israel, Jordan, Lebanon, Malaysia, Oman, Pakistan, Saudi Arabia, Syria, United Arab Emirates, Yemen*. Europe: *Cyprus, France, Italy, Spain, Turkey*. North America: *Mexico, United States* (Arizona, Arkansas, California, Illinois, Maryland, Ohio, Washington), Oceania: *New Zealand* (CABI-CPC, 2021).

Official Control*: Spiroplasma citri* is on the USDA PCIT's harmful organism list for: Albania, Argentina, Brazil, Cambodia, Chile, China, Colombia, Costa Rica, Ecuador, European Union, French Polynesia, Guatemala, Holy See (Vatican City State), Indonesia, Israel, Japan, Korea, Republic of, Mexico, Monaco, Namibia, New Caledonia, New Zealand, Nicaragua, Panama, Paraguay, Peru, San Marino, Serbia, South Africa, Taiwan, Thailand, Timor-Leste, Turkey, United Kingdom, Uruguay (USDA PCIT, 2021).

Spiroplasma citri is the EPPO's A1 list for Argentina, Asia and Pacific Plant Protection Commission, Brazil, Chile, East Africa, Paraguay, Southern Africa, Uruguay, the A2 list for Comite de Sandidad Vegetal del Cono Sur, Inter-African Phytosanitary Council, Turkey; a quarantine pest in Mexico and New Zealand, and a regulated non-quarantine pest in Egypt (EPPO, 2021).

Texas lists stubborn disease of citrus as a quarantine pest with a state exterior quarantine, prohibiting entry of plants and other regulated materials while exempting California-grown citrus seeds (National Plant Board, https://nationalplantboard.org/laws-and-regulations/).

California Distribution: Stubborn occurs in citrus in the Coachella Valley and interior valleys of central and southern California and Arizona. The disease is common with as many as 40% of trees testing positive in USDA research groves in Kern Co., with higher incidence in proximity to the foothills, and mostly in sweet orange. Tulare Co. also has a high prevalence of disease, although less than in Kern Co., with Fresno and Kings Co. at <5% (Dr. R. Yokomi, USDA ARS, pers. comm.; Dreistadt, 2012; Eskalen and Adaskaveg, 2019).

California Interceptions: None.

The risk *Spiroplasma citri* would pose to California is evaluated below.

Consequences of Introduction:

1) **Climate/Host Interaction:** The disease has been detected in the warmer, arid areas of the state. Symptoms have not been observed in coastal areas or Northern California

   Evaluate if the pest would have suitable hosts and climate to establish in California.

   Score: **2**
   - Low (1) Not likely to establish in California; or likely to establish in very limited areas.
   - Medium (2) may be able to establish in a larger but limited part of California.
   - High (3) likely to establish a widespread distribution in California.

2) **Known Pest Host Range:** *Spiroplasma citri* infects most citrus species and cultivars and a wide range of non-rutaceous plant species

   Evaluate the host range of the pest.

   Score: 3
   - Low (1) has a very limited host range.
   - Medium (2) has a moderate host range.
   - High (3) has a wide host range.

3) **Pest Reproductive Potential:** The pathogen needs a living host to survive and propagate. It is limited to the phloem of susceptible hosts and leaf hopper vectors. It moves locally with the vectors that can fly.

   Evaluate the natural and artificial dispersal potential of the pest.

   Score: 2
   - Low (1) does not have high reproductive or dispersal potential.
   - Medium (2) has either high reproductive or dispersal potential.
   - High (3) has both high reproduction and dispersal potential.

4) **Economic Impact:** Citrus stubborn has been shown to be a significant constraint to production and marketability in California when spiroplasma titer is high.

   Evaluate the economic impact of the pest to California using the criteria below.

   Economic Impact: A, B, C. E
   A. The pest could lower crop yield.
   B. The pest could lower crop value (includes increasing crop production costs).
   C. The pest could trigger the loss of markets (includes quarantines).
   D. The pest could negatively change normal cultural practices.
   E. The pest can vector, or is vectored, by another pestiferous organism.
   F. The organism is injurious or poisonous to agriculturally important animals.
   G. The organism can interfere with the delivery or supply of water for agricultural uses.

   Economic Impact Score: **3**
   - Low (1) causes 0 or 1 of these impacts.

- Medium (2) causes 2 of these impacts.
- High (3) causes 3 or more of these impacts.

5) **Environmental Impact:** The pathogen lives in weeds and other naturalized plants, as do the leafhopper vectors, which has allowed it to become established outside of citrus in areas in favorable environments. However, no negative impacts have been reported.

Evaluate the environmental impact of the pest to California using the criteria below

Environmental Impact:

A. The pest could have a significant environmental impact such as lowering biodiversity, disrupting natural communities, or changing ecosystem processes.
B. The pest could directly affect threatened or endangered species.
C. The pest could impact threatened or endangered species by disrupting critical habitats.
D. The pest could trigger additional official or private treatment programs.
E. The pest significantly impacts cultural practices, home/urban gardening or ornamental plantings.

Environmental Impact Score: 1
- Low (1) causes none of the above to occur.
- Medium (2) causes one of the above to occur.
- High (3) causes two or more of the above to occur.

Consequences of Introduction to California for *Spiroplasma citri:* Medium

Add up the total score and include it here. **11**
-Low = 5-8 points
-Medium = 9-12 points
-High = 13-15 points

6) **Post Entry Distribution and Survey Information**: Evaluate the known distribution in California. Only official records identified by a taxonomic expert and supported by voucher specimens deposited in natural history collections should be considered. Pest incursions that have been eradicated, are under eradication, or have been delimited with no further detections should not be included.

With over a 100-year history in California, a mobile vector, and a host range that includes weeds and other crop plants, stubborn disease is likely fully established in the suitable climate/host areas. CDFA records indicate a “general” distribution of this disease in the state (Mayhew and Weins, 1993).

Evaluation is ‘High’.

Score: -3
-Not established (0) Pest never detected in California or known only from incursions.

-Low (-1) Pest has a localized distribution in California or is established in one suitable climate/host area (region).
-Medium (-2) Pest is widespread in California but not fully established in the endangered area, or pest established in two contiguous suitable climate/host areas.
-High (-3) Pest has fully established in the endangered area, or pest is reported in more than two contiguous or non-contiguous suitable climate/host areas.

7) **The final score is** the consequences of introduction score minus the post entry distribution and survey information score: (Score)

Final Score: *Score of Consequences of Introduction – Score of Post Entry Distribution and Survey Information* ***= 8***

Uncertainty:

None.

Conclusion and Rating Justification:

Based on the evidence provided above the proposed rating for ***Spiroplasma citri*** is **C**.

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Responsible Party:

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***Comment Period: 06/21/2021 through 08/05/2021**

***NOTE:**

You must be registered and logged in to post a comment. If you have registered and have not received the registration confirmation, please contact us at permits[@]cdfa.ca.gov.

Comment Format:

- Comments should refer to the appropriate California Pest Rating Proposal Form subsection(s) being commented on, as shown below.

  Example Comment:

  Consequences of Introduction: 1. Climate/Host Interaction: [Your comment that relates to "Climate/Host Interaction" here.]

- Posted comments will not be able to be viewed immediately.
- Comments may not be posted if they:

  Contain inappropriate language which is not germane to the pest rating proposal;

  Contains defamatory, false, inaccurate, abusive, obscene, pornographic, sexually oriented, threatening, racially offensive, discriminatory or illegal material;

  Violates agency regulations prohibiting sexual harassment or other forms of discrimination;

  Violates agency regulations prohibiting workplace violence, including threats.

- Comments may be edited prior to posting to ensure they are entirely germane.
- Posted comments shall be those which have been approved in content and posted to the website to be viewed, not just submitted.

Proposed Pest Rating: C
