California Pest Rating Proposal for

Candidatus Phytoplasma trifolii Hiruki & Wang 2004

clover proliferation phytoplasma

Current Pest Rating: None

Proposed Pest Rating: C

Kingdom: Bacteria, Phylum: Tenericutes,
Class: Mollicutes, Order: Acholeplasmatales,
Family: Acholeplasmataceae

Comment Period: 02/19/2026 through 04/05/2026

Initiating Event:

This pathogen has not been through the pest rating process. The risk to California from *Ca.* Phytoplasma trifolii is described herein, and a permanent rating is proposed.

History & Status:

Background:

In 1967, Doi et al. observed mollicutes in the phloem of plants exhibiting yellows and witch's broom symptoms. Initially called mycoplasma-like organisms, their name was later changed to phytoplasmas. Phytoplasmas are phloem-limited pleomorphic bacteria lacking a cell wall, mainly transmitted by leafhoppers, but also by plant propagative materials. They cause yellowing symptoms by clogging sieve tubes of the phloem tissue and interfering with the transportation of photosynthate out of the leaves. They can also produce biologically active toxic substances, causing death of the leaves, inflorescences, and vegetative buds of their hosts. Species descriptions of bacteria belonging to the class Mollicutes typically require an accompanying culture of the organism. However, because phytoplasmas are very difficult to isolate in culture and maintain in vitro, lineages within this group are generally referred to as '*Candidatus* Phytoplasma species' (Davis and Sinclair, 1998).

Phytoplasmas are classified into subgroups based on RFLP analysis of their 16S rDNA segment (F2nR2 fragment) using a defined set of 17 restriction enzymes (Lee et al., 1998). The phytoplasma groups delineated using this classification scheme are consistent with 16S rRNA gene phylogeny (Zhao et al., 2009). The 16S rDNA RFLP markers further separate phytoplasma strains into subgroup lineages. The

reference strain of *Ca*. Phytoplasma trifolii is the initial member of the clover proliferation subgroup A (16SrVI-A) (Lee et al., 2000; Hiruki and Wang, 2004). Presently, *Ca.* Phytoplasma trifolii is the only species recognized in group 16SrVI-A, and any strain that shares >97.5% similarity with the type strain are considered *Ca.* Phytoplasma trifolii.

Clover proliferation disease was initially observed in Canada in the early 1960s, when the causative agent was initially thought to be a virus (Chiykowski, 1965). The symptoms were later associated with infection by a 'mycoplasma-like' organism (Chen and Hiruki, 1975). A beet leafhopper-transmitted 'virescence agent' was first described in infected Madagascar periwinkle (Oldfield, 1982). In 1983, this agent was seen by infecting periwinkles with beet leafhoppers collected in Kern County, CA (Golino et al., 1989). They were able to inoculate experimental hosts and found that 43 developed symptoms of disease. Beet leafhopper-transmitted virescence has been classified as subgroup 16SrVI-A. Phytoplasmas belonging to this lineage have now been described in association with disease symptoms in the United States, Mexico, and numerous European and Asian nations (EPPO, 2026). The disease affects a variety of vegetable and leguminous crops, resulting in notable reductions in yield and quality.

Hosts: Aegle marmelos (Indian bael), *Brassica oleracea* var. *capitata* (cabbage), *Brassica oleracea* var. *gemmifera* (Brussels sprout), *Cydonia oblonga* (quince), *Medicago sativa* (alfalfa), *Melilotus albus* (white sweetclover), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), *Trifolium hybridum* (clover) (CABI, 2026).

Symptoms: Infection of plants by *Ca.* Phytoplasma trifolii elicits a broad spectrum of host responses that vary with the species and growth stage of the host plant. During the vegetative stages of disease development, typical manifestations include excessive proliferation of shoots, formation of witches'-broom, reduction in leaf size ("little leaf"), yellowing of foliage, and overall growth inhibition. Among reproductive structures, infected plants often exhibit floral abnormalities such as green-colored flowers (virescence), leafy flowers (phyllody), enlargement or malformation of buds (big bud), and the appearance of multiple inflorescences (Hiruki and Wang, 2004).

Research on the potato purple top (PPT) phytoplasma, a strain within subgroup 16SrVI-A, has shown that infection can profoundly disrupt hormonal balance in susceptible hosts such as tomato. These disruptions include altered homeostasis of key growth regulators like gibberellins, contributing to dramatic changes in growth patterns and morphology (Ding et al., 2013). Under controlled inoculation, a single PPT infection can sequentially induce multiple symptom types on the same plant, such as witches'-broom, altered sympodial growth, cauliflower-like inflorescence, big bud, and other abnormalities, each corresponding to specific developmental shifts in the apical meristem and associated transcriptional reprogramming.

Beyond morphological symptoms, phytoplasma infection also reprograms host physiology. For instance, infections of pepper by *Ca.* P. trifolii strains alter primary and secondary metabolism, leading to increased synthesis of phenolic compounds and other secondary metabolites, while concurrently reducing photosynthetic carbon fixation, modifying carbohydrate-metabolizing enzyme activities, and shifting sugar and amino acid profiles. Such metabolic changes are consistent with broader effects on carbon and energy metabolism during phytoplasma disease development (Reveles-Torres et al., 2018).

Transmission: Phytoplasmas reside in phloem sieve cells of infected plants and can be transmitted from infected plants to healthy ones through phloem-feeding insects, mainly leafhoppers, planthoppers, and psyllids. They are not seed-transmitted. Phytoplasmas are fastidious: there is no indication that any phytoplasma can survive outside of its host, and no pure phytoplasma culture has been established in any medium thus far. (CABI, 2026). *Ca.* Phytoplasma trifolii is transmitted by *Macrosteles fascifrons*, aster leafhopper, and *Circulifer tenellus*, beet leaf hopper in California and other vectors around the world. Both the aster and the beet leaf hopper are confirmed to be major vectors and are established in California (Golino et al., 1989).

Damage Potential: Economic impacts vary year by year and location depending largely on population dynamics of the transmission vectors. Modest yield losses and quality reductions have been reported for plants infected with *Ca.* Phytoplasma trifolii, including leguminous, solanaceous, and brassica crops. Experimental work showed that even one infected beet leaf hopper per plant could cause a 12% yield reduction in potatoes due to potato purple top disease (Murphy et al, 2014). However, despite a presence in California at least from the 1980s, disease reports are very sporadic for any of the diseases now known to be caused by *Ca.* Phytoplasma trifolii.

Worldwide Distribution: North America: *Canada, Mexico, United States of America* (California, Illinois, North Dakota, Ohio, Oregon). Asia: *Bangladesh, China, India, Iran, Korea, Lebanon, Syrian Arab Republic, Uzbekistan*. Europe: *Italy, Russian Federation, Spain, Türkiye* (EPPO, 2026).

Official Control*: Ca.* Phytoplasma trifolii is on the EPPO's A1 list for Argentina and the Inter-African Phytosanitary Council, and is a quarantine pest in China, Israel, and Mexico (EPPO, 2026). It is on the USDA PCIT's harmful organisms list for Antarctica, Argentina, Ecuador, Indonesia, and Nicaragua (USDA-PCIT, 2026).

California Distribution: There are historical records of the diseases associated with individual strains, such as tomato big bud phytoplasma and potato purple top phytoplasma. These have been reported in multiple counties but at a low frequency, and there are no records since the adoption of modern molecular diagnostics (French, 1989).

California Interceptions: none

The risk that *Ca.* Phytoplasma trifolii would pose to California is evaluated below.

Consequences of Introduction:

1) **Climate/Host Interaction:** Phytoplasmas survive within their hosts and their vectors and can be found wherever the two co-occur.

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

   Score: **3**

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

2) **Known Pest Host Range:** The host range includes plants in multiple families

Evaluate the host range of the pest.

Score: 2
- 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 multiplies within host plants and vectors. The vectors can fly.

Evaluate the natural and artificial dispersal potential of the pest.

Score: 3
- 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:** Economic impacts are difficult to predict, and the populations of infectious vectors present at susceptible growth stages are key to the development of epidemics. Historically, a very low incidence of disease has been reported in California, despite the vectors being widely established.

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

Economic Impact: A, B

A. The pest could lower crop yield.
B. The pest could lower crop value (including increasing crop production costs).
C. The pest could trigger the loss of markets (including 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: **2**
- 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:** This is a quarantine pest for some trading partners.

Evaluate the environmental impact of the pest on California using the criteria below.

Environmental Impact: D

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: 2

- 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 *Ca.* Phytoplasma trifolii*:* Medium

Add up the total score and include it here. **12**
-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.

This disease has been present in California and not under official control for decades, although there are no recent detections.

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 consequence of the 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* ***= 9***

Uncertainty:

Phytoplasma diagnosis should be made by an expert diagnostician. Some older records are likely based on symptoms rather than the confirmed presence of a phytoplasma

Conclusion and Rating Justification:

Based on the evidence provided above the proposed rating for ***Ca.* Phytoplasma trifolii is C.**

References:

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Responsible Party:

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***Comment Period: 02/19/2026 through 04/05/2026**

***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
