EPPO Datasheet: *'Candidatus Phytoplasma aurantifolia'*

Last updated: 2023-06-12

IDENTITY

Preferred name: *'Candidatus Phytoplasma aurantifolia'*
Authority: Zreik, Bové & Garnier
Taxonomic position: Bacteria: Tenericutes: Mollicutes: Acholeplasmatales: Acholeplasmataceae
Other scientific names: *Candidatus Phytoplasma citri*, *Lime witches' broom phytoplasma*, *Phytoplasma aurantifolia* Zreik, Bové & Garnier, *Phytoplasma citri*
Common names: Oman witches' broom disease, WBDL, witches' broom of lime
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EU Categorization: Quarantine pest ((EU) 2019/2072 Annex II A)
EPPO Code: PHYPAF



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Notes on taxonomy and nomenclature

'Candidatus Phytoplasma aurantifolia' is classified in the peanut witches' broom group, taxonomic subgroup 16SrII-B of the classification based on the 16S rRNA gene sequence. The reference strain is the lime witches' broom phytoplasma (Zreik *et al.*, 1995).

Although several '*Ca*. P. aurantifolia'-related strains have been reported in the literature on various hosts, to date they have not been determined to belong to the subgroup II-B and are therefore not covered in this datasheet.

HOSTS

The main natural host of lime witches' broom phytoplasma is *Citrus aurantiifolia*, the small-fruited acid lime which is grown mainly in India, China, Arabian Peninsula, Mexico, Southern Iran, the West Indies, Egypt, and the tropical Americas.

As shown in the host list below, some other *Citrus* species can also be a natural host of '*Ca.* P. aurantifolia' (Hemmati *et al.*, 2021a). In Iran, the disease is not frequent on *Citrus* other than *C. aurantiifolia* and Bakraee (a natural hybrid of *C. reticulata* commonly used as a rootstock in Iran), and its occurrence appears to depend on the susceptibility of the rootstock, the vector populations, disease pressure in the infected region (% incidence), and the presence of infected lime trees in the vicinity (Salehi *et al.*, 2022).

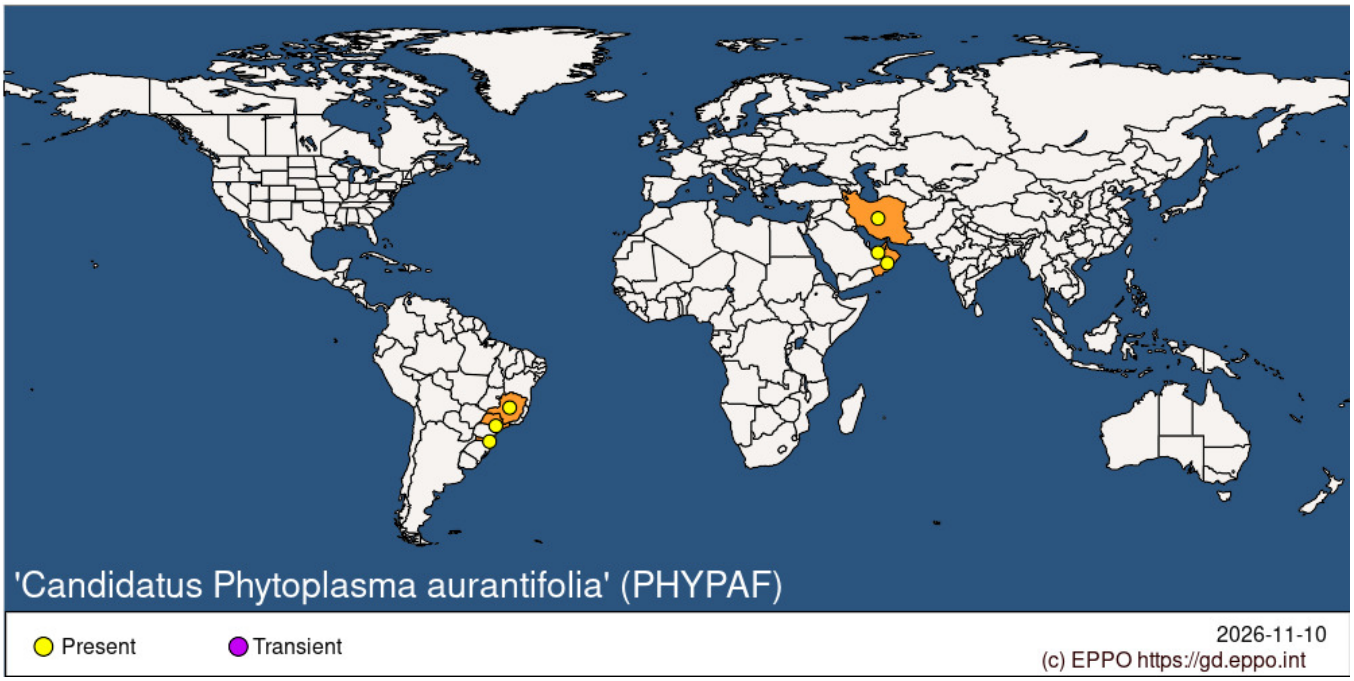
In experiments, the phytoplasma has been graft-transmitted to several *Citrus* species, including *Citroncirus webberi*, *Citrus macrophylla* and *C. trifoliata*, but at a lower frequency and with slow symptom development (Garnier *et al.*, 1991, Hassanzadeh *et al.*, 2019; Azadvar *et al.*, 2023). Persian lime (*Citrus* × *latifolia*) is known as the most resistant lime cultivar to *'Ca*. P. aurantifolia' (Hassanzadeh *et al.*, 2019). Dodder (*Cuscuta*) has been used to transmit the phytoplasma to *Catharanthus roseus*, a widely used indicator plant for phytoplasmas.

Host list: *Citrus hybrids, Citrus medica, Citrus x aurantiifolia, Citrus x aurantium var. paradisi, Citrus x aurantium var. sinensis, Citrus x limon var. limetta, Citrus x limon var. limettioides, Citrus x limon, Citrus x limonia var. jambhiri, Citrus x tangelo, Pyrus communis, x Citrofortunella floridana*

GEOGRAPHICAL DISTRIBUTION

The witches' broom disease of lime associated with *'Ca*. P. aurantifolia' was reported in a few countries in Asia including Oman (Bové, 1986), the United Arab Emirates (Garnier *et al.*, 1991), and Iran (Bové *et al.*, 2000).

An asymptomatic infection of lime in Brazil was observed and mentioned to belong to the subgroup II-C by Silva *et al.* (2014). However, Al-Subhi *et al.* (2021) stated that the sequences of the *imp*, 16SrRNA, and *tuf* genes from some phytoplasma isolates from Oman, Iran, Brazil, the United Arab Emirates and Saudi Arabia were identical and grouped with 16SrII-B (*'Ca.* P. aurantifolia') in phylogenetic analysis.



Asia: Iran, Islamic Republic of, Oman, United Arab Emirates
South America: Brazil (Minas Gerais, Santa Catarina, Sao Paulo)

BIOLOGY

Lime witches' broom is the first well-characterized phytoplasma disease of citrus. It is an economically important and destructive disease and a significant limiting factor on acid lime production in the Middle East and threatens its production globally. *Hishimonus phycitis* is known as the main vector of the associated phytoplasma to healthy mature lime trees and seedlings (Bagheri *et al.*, 2009; Hemmati *et al.*, 2020). The Asian psyllid *Diaphorina citri* was also able to experimentally transmit the phytoplasma at a lower efficiency in a no-choice assay (Queiroz *et al.*, 2016), and no report is available regarding the natural transmission. To date, seed transmission of *'Ca.* Phytoplasma aurantifolia' has not been proven (Faghihi *et al.*, 2011).

DETECTION AND IDENTIFICATION

Symptoms

On lime, witches' broom symptoms develop over the whole tree, accompanied by the production of small-sized leaves on proliferating shoots that tend to be yellowish-green, and reduced flower and fruit production. These are found in clusters on the branches of infected acid lime trees. The symptoms develop on branches gradually and the trees become unproductive until the whole tree collapses within 4-8 years of the first appearance of symptoms (Al-Ghaithi *et al.*, 2017). In some cases, no symptoms have been observed in infected trees (Silva *et al.*, 2014, Al-Subhi *et al.*, 2021).

Morphology

The presence of 200-800 nm-sized phytoplasma cells that are pleomorphic can be easily observed through electron

microscopy in sieve tubes (Garnier *et al.*, 1991).

Detection and inspection methods

Visual symptoms are important for diagnosis in symptomatic plants (above-mentioned symptoms). Although *'Ca*. P. aurantifolia' is usually symptomatic, it may be present in the absence of symptoms due to its long incubation period, or the reaction of some cultivars like in Brazil (Silva *et al.*, 2014). The phytoplasma has been detected in infected trees through the use of monoclonal antibodies, PCR, and real-time PCR, by using specific primers (Al-Subhi *et al.*, 2021; Hemmati *et al.*, 2021b). Mexican lime and periwinkle can be used as indicators in graft and biological transmission assays. In laboratories in the EPPO region, these methods are less used currently because of the lack of validation data, and detection using PCR followed by identification by sequence analysis is recommended, as described in the EPPO diagnostic protocol PM 7/133 (1) Generic detection of phytoplasmas (EPPO, 2018).

PATHWAYS FOR MOVEMENT

Lime witches' broom disease has spread naturally in Asian countries via its vector. Globally, there is a higher chance to distribute the disease with infected plants for planting. The emergence of witches' broom disease of lime in Oman, a region that has had a longstanding tradition of cultivating limes, raises the plausible assumption that the disease may have been imported from external sources. The entry of '*Ca.* Phytoplasma aurantifolia' into the EPPO region could happen via infected plants for planting or vectors. However, the import of *Citrus* plants for planting from countries where the pest occurs is prohibited in the EU (EU, 2023). The prohibition of import is likely in other *Citrus*-producing countries in the EPPO region.

Entry of the phytoplasma with infected adults and nymphs of the leafhopper vector *Hishimonus phycitis* is unlikely because they move and leap away from plants when disturbed, therefore, it is highly improbable that these mobile stages would remain on host plant materials as it is handled along a pathway. Eggs of *H. phycitis* could be present on the plants, but to date, it remains unknown if eggs can carry '*Ca*. P. aurantifolia' because it remains unknown if there is transovarial transmission of the phytoplasma to eggs.

PEST SIGNIFICANCE

Economic impact

Symptoms are severe and rapidly affect the whole tree, inhibiting flowering, and fruiting. Over 50% of the cultivated area of lime has been lost in Oman since it was reported in the 1970s and hundreds of thousands of lime trees have been destroyed in Iran due to witches' broom disease of lime. The disease occurs widely in the coastal region of the Oman Sea. The potential impact of the disease is limited to those countries that grow acid lime commercially (see Phytosanitary risk). This disease is deemed significant enough to warrant the consideration of implementing a programme to completely eliminate it.

Control

Given the lack of viable solutions for treating plants infected with phytoplasma, the primary focus is on implementing preventative measures, including agronomic techniques and vector management, to minimize the spread and impact of the disease. To effectively manage witches' broom disease of lime, it is strongly advised to utilize disease-free seedlings and planting materials and shield the nursery with a net that protects against insects. Routinely applying systemic insecticides is another strategy for controlling the vectors. Newly infected trees can be safeguarded by removing symptomatic branches, while highly infected trees should be eliminated to prevent further spread of the disease. Opting for resistant or tolerant species such as Persian lime and controlling weeds (which may serve as a reservoir for phytoplasmas) are additional measures that are highly recommended (Azadvar *et al.*, 2023).

Phytosanitary risk

Acid lime (*C. aurantifolia*), the main host of *'Ca*. P. aurantifolia', is mostly not grown commercially in the EU

because of ecoclimatic limitations (EFSA, 2017), although there is a small commercial cultivation in limited areas, such as Sicily in Italy (Gusella *et al.*, 2021). Acid lime is also produced commercially, on a smaller scale than other citrus, in the warmest parts of the EPPO region, for example in Morocco and Israel (Sammama *et al.*, 2016; EFSA, 2021a).

Other citruses, especially orange (*C. sinensis*), grapefruit (*C. paradisi*), as well as mandarins and clementines (*C. reticulata*), are the most economically important *Citrus* crops in the Mediterranean area that can be infected (Hemmati *et al.*, 2021a, Salehi *et al.*, 2022). So far, it has been observed that the incidence of infection of '*Ca.* P. aurantifolia' on such crops is relatively infrequent in nature (Salehi *et al*., 2022). In Iran, particular combinations of factors were identified as being contributory to such infection (Salehi *et al.*, 2022; see under the Hosts section), but the same conditions are currently not met within the EPPO region.

PHYTOSANITARY MEASURES

In order to prevent the introduction of the disease to the EPPO region, the importation of *Citrus* tree plants for planting, originating from areas where the disease is prevalent, can be prohibited (CABI/EPPO, 1997), as is already the case in many EPPO countries. Alternatively, measures such as pest-free areas may be appropriate, as is the case for similar pests, as well as pest-free production sites or pest-free places of production with conditions also ensuring the absence of vectors. Healthy planting material of *Citrus* can be produced in the framework of a certification scheme. A pest survey card was prepared by the European Food Safety Authority (EFSA, 2021b) to assist EU Member States in planning their annual survey activities for '*Ca.* Phytoplasma aurantifolia' and its vector *H. phycitis*.

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Datasheet history

This datasheet was first published in 1997 in the second edition of 'Quarantine Pests for Europe', and revised in 2023. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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