

EPPO Datasheet: '*Candidatus Phytoplasma phoenicium*'

Last updated: 2021-07-29

IDENTITY

Preferred name: '*Candidatus Phytoplasma phoenicium*'

Authority: Verdin, Salar, Danet, Choueiri, Jreijiri, El Zammar, Gélie, Bové & Garnier

Taxonomic position: Bacteria: Tenericutes: Mollicutes:
Acholeplasmatales: Acholeplasmataceae

Other scientific names: *Almond witches' broom phytoplasma*,
Phytoplasma phoenicium Verdin, Salar, Danet, Choueiri, Jreijiri, El
Zammar, Gélie, Bové & Garnier

Common names: AlmWB, Almond lethal witches' broom, witches'
broom of almond

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EPPO Categorization: A1 list

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EU Categorization: A1 Quarantine pest (Annex II A)

EPPO Code: PHYPPH



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Notes on taxonomy and nomenclature

'*Candidatus Phytoplasma phoenicium*' is accommodated in the phytoplasma taxonomic group 16SrIX (group Pigeon Pea Witches' Broom) of the classification based on 16S rRNA sequences (Lee *et al.*, 2000). It has been assigned to the subgroup 16SrIX-B, according to phylogenetic analysis of 16SrRNA confirmed by that of the protein-coding genetic loci rplV-rpsC and secY (Lee *et al.*, 2012, Casati *et al.*, 2016).

The reference strain is the Lebanese strain A4 associated with a lethal almond witches'-broom (AlmWB) also known as almond lethal proliferation: it is described by its 16SrRNA sequence (GenBank accession AF515636) and possesses the AlmF1 5'-CCTTTTTCGGAAGGTATG-3' oligonucleotide sequence complementary to a unique region of 16S rRNA (Verdin *et al.*, 2003). In addition to the phytoplasma reference strain A4 (LalmWB), two other '*Ca. P. phoenicium*' strains also associated with AlmWB have been described: namely AlmWB1 from Lebanon (GenBank accession AF390136) and NalmWB from Neyriz, Iran (AF515637) (Abou-Jawdah *et al.*, 2002; Salehi *et al.*, 2006). All other phytoplasma strains possessing the AlmF1 sequence but sharing more than 97.5 % identity of 16SrRNA and less than 100% to the GenBank accession AF515636, can only be described as '*Ca. P. phoenicium*'-related strains according to the rule collectively edited and adopted (IRPCM Phytoplasma/Spiroplasma Working Team, Phytoplasma taxonomy group, 2004).

A study of the '*Ca. P. phoenicium*' genetic diversity among samples of diseased almond, peach and nectarine trees collected in diverse geographic regions of Lebanon highlighted the absence of genetic variations in their 16SrRNA, tufB and groEL while the hyper-variable gene *inmp* allowed differentiation according to plant host (Quaglino *et al.*, 2015).

HOSTS

The natural host range of '*Ca. P. phoenicium*' is mostly restricted to cultivated almond (*Prunus dulcis*) and wild almond (*Prunus orientalis*) (Choueiri *et al.*, 2001; Abou-Jawdah *et al.*, 2002; Nigro *et al.*, 2020), but '*Ca. P. phoenicium*' was also identified in association with a severe disease of peach (*Prunus persica*), nectarine (*Prunus persica* var. *nucipersica*) (Verdin *et al.*, 2003; 2004; Abou-Jawdah *et al.*, 2009) and apricot (*Prunus armeniaca*) (Salehi *et al.*, 2018). '*Ca. P. phoenicium*' was identified in the rootstock GF-677 (*Prunus amygdalus* x *Prunus persica*) and in a wild almond species (*Prunus scoparia*) (Salehi *et al.*, 2011; 2015).

'*Ca. P. phoenicium*'-related strains were found associated with the following host plants: grapevine (*Vitis vinifera*) in

Turkey (Canik *et al.*, 2011), sweet orange (*Citrus sinensis*) in Iran (Abbasi *et al.*, 2019), pomegranates (*Punica granatum* L.) in Turkey (Çaglayan *et al.*, 2019), juniper (*Juniperus occidentalis*) in the USA (Davis *et al.*, 2010), garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) in India (Goel *et al.*, 2017), chrysanthemum (*Chrysanthemum morifolium*) in Iran (Bayat *et al.*, 2013) and Madagascar periwinkle (*Catharanthus roseus*) in Brazil (Barbosa *et al.*, 2012).

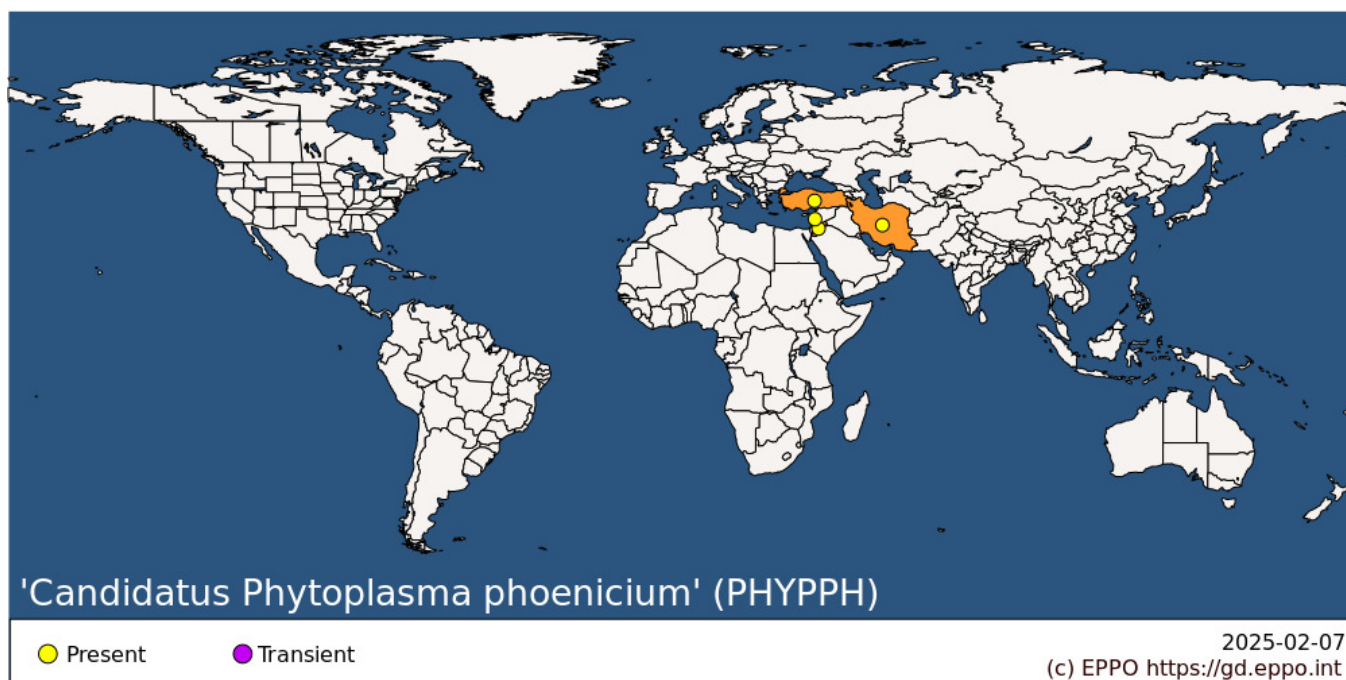
Host list: *Anthemis*, *Prunus armeniaca*, *Prunus dulcis*, *Prunus orientalis*, *Prunus persica* var. *nucipersica*, *Prunus persica*, *Prunus scoparia*, *Prunus x hybrida*, *Smilax aspera*

GEOGRAPHICAL DISTRIBUTION

In 1999, almond trees with symptoms of leaf yellowing, shoot proliferation, and dieback were observed in the Bekaa region, Lebanon, where a phytoplasma infection belonging to the pigeon pea witches' broom cluster (PPWB) was reported for the first time (Choueiri *et al.*, 2001). *Ca. P. phoenicium* was also identified in association with a severe disease of peach and nectarine in southern Lebanon (Verdin *et al.*, 2003; 2004; Abou-Jawdah *et al.*, 2009). The disease was observed throughout the country, in 16 out of 24 Lebanese districts from coastal areas to high mountainous areas (Molino Lova *et al.*, 2011a).

In Iran, the disease was first detected in almond (Salehi *et al.*, 2006) and later on apricot (Salehi *et al.*, 2018; Ghayeb Zamharir & Nazari, 2019).

Recently, almond plants showing phytoplasma symptoms associated with '*Ca. Phytoplasma phoenicium*' were reported in Apulia region, Italy (Nigro *et al.*, 2020).



EPPO Region: Jordan, Türkiye

Asia: Iran, Jordan, Lebanon

BIOLOGY

Location within the plant

As is the case for other phytoplasmas, '*Ca. P. phoenicium*' is present in the phloem tissue of the stems and the roots of host plants throughout the year (Jawhari *et al.*, 2015). The phytoplasma cells are restricted to the sieve tubes and can be found in leaf petioles and midveins.

Epidemics

Epidemics of '*Ca. P. phoenicium*' are established by introduction of infected plant material followed by natural transmission due to vectors. The unintentional entry of infected plant material establishes the disease in new areas or countries and subsequent unregulated movement of plants can have disastrous consequences. There are no data on the latency period under natural conditions, which seems to vary depending on varieties and conditions. However, symptoms appeared later on *Prunus orientalis* (wild almond) than on cultivated almond (Abou-Jawdah *et al.*, 2002; E. Choueiri, unpublished data). The incubation period of '*Ca. P. phoenicium*' in stone fruits varies and may reach more than one year. In a survey in Lebanon in 2000-2002, all almond varieties were affected: some were highly susceptible and developed severe proliferation and witches'-broom leading to rapid death, whereas others were less affected (limited parts of the canopy, or only few trees in an orchard) (Verdin *et al.*, 2003). However, such differences may also be due to differences in latency in different cultivars. No specific up-to-date study on susceptibility has been carried out so far.

The phytoplasma could be experimentally transmitted by graft-inoculation of infected almond shoots onto seedlings of almond (*Prunus amygdalus*), peach (*Prunus persica* GF305) and plum (*Prunus mariana* GF8-1) and the symptoms were reproduced in 1 month (Verdin *et al.*, 2003) or 4-6 months according to other graft inoculation trials (Abou-Jawdah *et al.*, 2003). When AlmWB-infected almond trees were grafted with apricot or plum scions, their growth was symptomless for over 2 years in the field (Tawidian *et al.*, 2017; E. Choueiri, unpublished data). In addition, shoots developing from Farclo apricot grafted on AlmWB-infected trees in the field showed severe symptoms 2 months after grafting but recovered 3 months later, and remained symptomless for about 2.5 years (Tawidian *et al.*, 2017).

Transmission

The rapid spread of almond witches'-broom (AlmWB) disease, associated with '*Ca. Phytoplasma phoenicium*' over large geographical areas suggests the prevalence of efficient insect vector(s). In insect transmission trials in Lebanon, *Asymmetrasca decedens* (synonym *Empoasca decedens*) (leafhopper; Hemiptera: Cicadellidae: Typhlocibinae) was shown to transmit '*Ca. P. phoenicium*' to almond and peach (Abou-Jawdah *et al.*, 2014). In the Middle East, *A. decedens* is active throughout the year and has 4-5 generations per year. In field surveys in Lebanon in AlmWB-infected almond orchards, *A. decedens* was the most abundant Hemiptera and represented over 82% of leafhoppers trapped with the highest population levels in stone fruits in spring and summer (Dakhil *et al.*, 2011). In an infested orchard, it is therefore expected that there would be a large number of *A. decedens* carrying the pathogen. *A. decedens* has not been confirmed as a vector in Iran but it is abundant in stone fruit orchards (Salehi *et al.*, 2015).

Moreover, the results of molecular analyzes of the insect collection of cixiids carried out during surveys in Lebanon and the preliminary transmission trials gave interesting information on the potential role of different cixiid genera in the transmission of phytoplasmas in Lebanon. Leafhopper species of the genera *Tachycixius*, *Cixius*, *Eumecurus* and *Hyalesthes* were found to be able to acquire '*Ca. Phytoplasma phoenicium*', whereas the species *Tachycixius* cf. *cypricus* and *Tachycixius viperinus* were able to transmit AlmWB phytoplasma to healthy GF305 potted peach seedlings. Such results should be further verified, because the two specimens were part of batches which included individuals of different species with potential role in disease transmission in orchards. So far, it was proven that at least leafhopper species of the genus *Tachycixius* can transmit '*Ca. Phytoplasma phoenicium*' (Tedeschi *et al.*, 2015). *Tachycixius* cf. *cypricus* and *Tachycixius viperinus* carrying Phytoplasmas were also collected from weed hosts *Smilax aspera* and *Anthemis* spp., both found infected with '*Ca. P. phoenicium*' (Tedeschi *et al.*, 2015). Mackesy & Sullivan (2016) and Casati *et al.* (2016) suggested that weeds may act as a reservoir for the spread of AlmWB to stone fruit hosts, but this has not been demonstrated so far.

DETECTION AND IDENTIFICATION

Symptoms

According to field inspections, it seems that symptoms on infected plants are expressed after a variable period of time after infection and depend on several factors (e.g. initial phytoplasma inoculum, incubation period, tree age, species/cultivar, phytosanitary status of the tree).

The symptoms on almond trees include proliferation of slender shoots at several points on the main trunk of affected trees, or from the roots, with an occasional appearance of witches'-broom. Proliferation symptoms are always observed, but witches'-broom symptoms may appear only on some trees.

Proliferation and witches' broom are also observed on branches. However, perpendicular development of many auxiliary buds on the branches was also encountered. Trees also suffer from leaf size reduction and yellowing (pale green), stunted growth with short internodes, off-season growth, leaf rosetting, early flowering, general decline of affected trees and die-back (Choueiri *et al.*, 2001; Abou-Jawdah *et al.*, 2002; Salehi *et al.*, 2006). The symptoms appeared exacerbated when trees were heavily pruned, affected branches or trees produce few or no fruits and in dry weather, the leaves may appear brownish-red. In the first symptomatic year, only some branches show symptoms, whereas the entire canopy is affected starting in the second year. Trees decline rapidly and some die within 3-4 years following the appearance of the first symptoms, whereas others may survive longer. On *Prunus scoparia*, a wild almond species, *Ca. P. phoenicium* caused severe witches'-broom, yellowing and decline (Salehi *et al.*, 2015).

In the case of peach and nectarine trees infected with '*Ca. P. phoenicium*', the first symptom observed is the early flowering (15 to 20 days earlier than normal), followed by the early development of all the buds of the infected branches. In addition, some months after the normal flowering period, phyllody and serrate, slim, light green leaves develop on the plant branches and proliferation of shoots and witches'-brooms develop from the trunks and crowns of affected trees (Molino Lova *et al.*, 2011b; Salehi *et al.*, 2019). Symptoms initially appear only on some branches, and all branches in subsequent years. The disease does not lead to dieback in peach as quickly as in almond. Field monitoring of affected peach and nectarine orchards showed that no mortality was observed after 3-4 years whereas mortality occurred within this time frame in almond groves (Choueiri, personal communication). On infected GF-677 trees (*Prunus amygdalus* x *Prunus persica*), characteristic symptoms of the disease were internode shortening, chlorosis, reduced size of leaves especially in the broom, proliferation of slender upright shoots, witches' broom, stunting and dieback (Salehi *et al.*, 2011).

On apricot, yellows symptoms including leaf yellowing, size reduction and inward leaf curl, scorch of leaf margins, shortened internodes, rosette at the tips of branches, die back and plant death were observed on apricot trees at MeshKan, Iran (Salehi *et al.*, 2018). Affected branches either bore no fruits or fruits were small and abnormal in shape and taste. In Lebanon, a recovery phenomenon (remission of symptoms) was observed in apricot grafted on infected almond (Tawidian *et al.*, 2017).

Morphology

'*Candidatus* Phytoplasma phoenicium' is a pleiomorphic cell wall-less bacterium. Cells are predominantly filamentous and branched, 0.1–0.2 mm in diameter and are surrounded by the triple-layered cytoplasmic membrane characteristic of the class *Mollicutes* (Verdin *et al.*, 2003).

Detection and inspection methods

Visual inspection and sampling

Visual symptoms are important for diagnosis in symptomatic plants and visual inspection is a routine method to support eradication in countries where the disease is present, as well as for its surveillance in countries or areas where it is not present. Generally, '*Ca. P. phoenicium*' is symptomatic, but it may be present in the absence of symptoms because of its long incubation period. This should be taken into account for sampling. Proliferation of slender shoots and witches'-broom arising mainly from the main trunks with the appearance of small and yellowing leaves, followed by general decline of affected trees are considered the most typical symptoms and can be used in field surveys as part of an initial diagnosis.

Sampling and extraction techniques are important as phytoplasmas may be distributed unevenly in the plant. On symptomatic trees, samples should be taken from symptomatic roots and/or stems excluding necrotic tissues. It is recommended to take representative samples (at least 10 cm long), from at least three different parts of the tree, with composite sampling. Testing of asymptomatic plants may not be reliable because they would not detect very low titres, which would result in false negatives. Thus, for plants showing suspicious symptoms or asymptomatic plants, it

is recommended to collect samples from the four cardinal points in particular from the middle to high parts of the canopy (E. Choueiri, personal communication). The highest concentration of ‘*Ca. P. phoenicium*’ is usually found in the phloem tissue of leaf midrib and stems (E. Choueiri, personal communication).

Detection in plants

For more details regarding sampling, detection and identification of ‘*Ca. P. phoenicium*’ in plant material, see the EPPO Diagnostic Standard on this pest (EPPO Standard PM 7/150).

PATHWAYS FOR MOVEMENT

As ‘*Ca. P. phoenicium*’ is limited to the plant phloem, movement of infected host plant material (seedlings, pot plants, rooted or unrooted cuttings, tissue culture, scions, and rootstocks) is the main pathway for entry and spread over short and long distances (primary introduction). No transmission through pollen, seeds or fruits has been demonstrated yet (EPPO, 2017).

The potential vectors which are thought to be leafhoppers or planthoppers such as *A. decedens*, *Tachycixius cypricus* and *Tachycixius viperinus* (Abou-Jawdah *et al.*, 2014; Tedeschi *et al.*, 2015) seem to be able to transmit AlmWB phytoplasma and can be responsible for disease spread over short and long distances (secondary spread), because they can both spread the disease between plants and also travel with the plants.

PEST SIGNIFICANCE

Economic impact

‘*Candidatus* Phytoplasma phoenicium’ is an economically important and destructive phytoplasma in Lebanon where it caused the death of more than 100,000 almond trees (Choueiri *et al.*, 2001; Abou Jawdah *et al.*, 2002; Verdin *et al.*, 2003) as well as in several provinces of Iran (Salehi *et al.*, 2006; 2011; 2018).

‘*Ca. P. phoenicium*’ has had devastating effects on the production of almond, peach and nectarine in Lebanon and Iran with corresponding social and economic impacts (EPPO, 2017). In diseased orchards, fruit quality is affected, and yield reduction can reach 70 to 100 %, depending on the proportion of infected canopy and may render affected orchards unprofitable, thus leading to the death of the almond infected trees 3-4 years after the appearance of initial symptoms, which led to the loss of a large number of trees in these countries. In many districts and provinces, the economic impact has also been dramatic and difficulties in maintaining economically viable orchards have forced many growers out of business. In this regard, in North Lebanon, where almonds were grown on a large scale in rain-fed areas, farmers could not easily replace almond by another crop with profitable economic return, and consequently constituted a great economic burden on farmers. Thus, the rapid spread of this devastating disease is alarming not only for Iran and Lebanon but also for other stone fruit growing countries worldwide.

Control

Large-scale surveys are of utmost importance to detect newly established foci of ‘*Ca. P. phoenicium*’ in an area as early as possible. Once detected, a rapid elimination of phytoplasma reservoirs either from individual trees or entire planted areas is the most effective practice to avoid the inoculum dispersal and to delay epidemic spread. In fact, promising preliminary results on eradication were already obtained in the South of Lebanon, where farmers responded rapidly by eradicating and burning diseased trees and no symptoms of infection were observed later in the region where these actions had been applied (Abou Jawdah *et al.*, 2011; EPPO, 2017).

There are no curative treatments. Effective control measures against ‘*Ca. P. phoenicium*’ can be taken such as (i) promoting large-scale production of healthy nursery plants; (ii) use of certified plants from tested mother plants and healthy buds; (iii) avoiding grafting scions from infected trees; (iv) vector management applying insecticide sprays at critical flushing periods and weed management. Unfortunately, information on natural vectors is still incomplete; (v) continuous monitoring and surveillance even in regions where infected trees were eradicated; (vi) replacement of infected trees with non-hosts. This measure was adopted in Lebanon. Currently, there are no known almond cultivars

that are resistant to ‘*Ca. P. phoenicium*’.

Elimination of ‘*Ca. P. phoenicium*’ from infected trees using tissue culture techniques with or without thermotherapy has been used to produce healthy seedlings (Chalak *et al.*, 2005).

Phytosanitary risk

‘*Candidatus P. phoenicium*’ is a serious threat for the stone fruit trees growing countries in the EPPO region (EPPO, 2017). Its recent detection on almond in Southern Italy (Nigro *et al.*, 2020) raises questions on the potential movement of infested host plants from infested areas. Its impact on stone fruit and other hosts production can be destructive (Abou-Jawdah *et al.* 2009; Salehi *et al.* 2018). Damage to stone fruits crops caused by ‘*Ca. P. phoenicium*’ is expressed in the reduction and possibly complete loss of fruits and the subsequent death of trees (Abou-Jawdah *et al.*, 2002; 2011; Salehi *et al.*, 2006). ‘*Ca. P. phoenicium*’ has a high likelihood of establishing, spreading and causing impacts especially where the known vectors occur.

Among the most significant phytosanitary risks, is the difficulty or inability to secure costly eradication and nursery certification programs and vector control. Accordingly, quarantine restrictions for imports are essential. The exchange of plant propagation material of unknown sanitary status poses a high risk for the establishment and spread of ‘*Ca. P. phoenicium*’ in particular in countries with a limited plant pest diagnostic capacity. Furthermore, there is a high uncertainty of information about the probable presence or distribution of phytoplasmas in several countries.

PHYTOSANITARY MEASURES

Considering the severity of ‘*Ca. P. phoenicium*’ and in the absence of curative treatments against this pathogen, prophylactic measures are still the only effective way to avoid infection of hosts or dissemination to ‘*Ca. P. phoenicium*’-free areas. EPPO (2017) has recommended that *Prunus* host plants for planting (except seed) should either come from a pest-free area, or should be grown under complete physical isolation (in both case with measures to prevent infestation by vectors for plants with foliage) and that in-vitro plants should be tested to ensure absence of *Ca. P. phoenicium*.

It is desirable to warn NPPOs about the severe impacts of this pest on almond, peach, nectarine and apricot production.

Conventional strategies for phytoplasma containment can be adopted by: (i) prohibiting imports of plant material from high risk countries, (ii) developing a coherent legislative framework in the field of production of certified propagation material, (iii) improving phytosanitary inspection of imports to meet quarantine regulations, (iv) improve nurseries certification system (formal and/or informal), and (v) strengthening human capacity in pathogens diagnosis and controlling ‘*Ca. P. phoenicium*’ insect vectors (Choueiri, 2018).

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