‘Candidatus Phytoplasma phoenicium’ and related strains/subgroups

Scientific Name
‘Candidatus Phytoplasma phoenicium’ Verdin et al., 2003

Synonyms
Phytoplasma phoenicium

Common Name(s)
Almond witches' broom, AlmWB

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes,
Order: Acholeplasmatales,
Family: Acholeplasmataceae

Reason for Inclusion in Manual
Pest of Economic and Environmental Concern Listing 2017

Background Information
Beginning in the 1990s, an epidemic of witches'-broom disease (AlmWB) devastated almond production in Lebanon, spreading rapidly and killing over 150,000 trees in less than two decades (Abou-Jawdah et al., 2010, 2014). The causal organism was identified as a phytoplasma closely related to, but distinct from members of the pigeon pea witches' broom phytoplasma group (16SrIX), with a proposed name of ‘Candidatus Phytoplasma phoenicium’ (Verdin et al. 2003). This phytoplasma was subsequently found to infect peach and nectarine as well, and recent surveys identified over 40,000 new almond, peach, and nectarine trees infected with AlmWB (Molino Lova et al., 2011).

Phytoplasmas, formerly known as mycoplasma-like organisms (MLOs), are pleomorphic, cell wall-less bacteria with small genomes (530 to 1350 kbp) of low G + C content (23-29%). They belong to the class Mollicutes and are the putative causal agents of yellows diseases that affect at least 1,000 plant species worldwide (McCoy et al., 1989; Seemüller et al., 2002). These minute, endocellular prokaryotes colonize the phloem of their infected plant hosts as well as various tissues and organs of their

respective insect vectors. Phytoplasmas are transmitted to plants during feeding activity by their vectors, primarily leafhoppers, planthoppers, and psyllids (IRPCM, 2004; Weintraub and Beanland, 2006).

![Figure 2: Dieback Symptoms of 'Ca. P. phoenicium' infection in almond. Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.](image)

Although phytoplasmas cannot be routinely grown by laboratory culture in cell free media, they may be observed in infected plant or insect tissues by use of electron microscopy or detected by molecular assays implementing antibodies or nucleic acids. Since biological and phenotypic properties in pure culture are unavailable as aids in their identification, analysis of 16S rRNA genes has been adopted instead as the major basis for phytoplasma taxonomy. The provisional taxonomic status of ‘Candidatus’, used for incompletely described microorganisms, has been adopted for describing and naming distinct phytoplasmas (i.e., ‘Candidatus Phytoplasma’). Several species (i.e., ‘Ca. Phytoplasma’ species) have been named following established guidelines (IRPCM, 2004; Zhao et al., 2009; Harrison et al., 2011; Davis et al., 2013; Quaglino et al., 2013). A new ‘Candidatus Phytoplasma’ species may be recognized if the nucleotide sequence of the 1.2 kb F2n-R2 segment of its 16S rRNA gene shares < 97.5% identity with that of all previously named ‘Candidatus Phytoplasma’ species (IRPCM, 2004). If a phytoplasma shares >97.5% nucleotide sequence identity of 16S rDNA with any previously named species, the subject phytoplasma may be named as a distinct new species if significant biological or genetic properties distinguish the phytoplasma from already named species (IRPCM, 2004).

Phytoplasmas are classified in a system of groups and subgroups based upon DNA fingerprints (RFLP patterns) of 16S rRNA genes (16S rDNA) (Lee et al., 1998, 2000; Wei et al., 2008). Most of the 16S rDNA RFLP groups each contain at least one phytoplasma species (Zhao et al., 2009).

Pest Description

‘Candidatus Phytoplasma phoenicium’ is classified in the pigeon pea witches’ broom (PPWB) phytoplasma group 16SrIX (Verdin et al., 2003). The closest phytoplasma relatives were other members of the PPWB group, Picris echioides yellows (PEY) and Knautia arvensis phyllody (KAP), with which they share 99% 16S rDNA identity, and the PPWB phytoplasma, with which they share 98.5% identity (Verdin et al., 2003). ‘Candidatus Phytoplasma phoenicium’ was found to be different from phytoplasmas infecting other Prunus species in Europe or the United States, such as European Stone Fruit Yellows (‘Ca. P. prunorum’, 90.4% identity) and Western X (‘Ca. P. pruni’, 94.3% identity) phytoplasmas. ‘Candidatus Phytoplasma phoenicium’ is also different from phytoplasmas infecting other fruit trees, such as the apple proliferation (‘Ca. P mali’) and pear decline (‘Ca. P. pyri’) phytoplasmas (with 90.35 and 90.84% identity, respectively) (Verdin et al., 2003).

Several different strains of ‘Candidatus Phytoplasma phoenicium’ have been identified. These strains belong to phytoplasma subgroups 16SrIX-B, -D, -F, and –G. (Molino Lova et al., 2011). The original strain described was in subgroup B (Abou Jawdah et al., 2002). Subgroup B is now also designated as subgroup D (Quaglino et al., 2015). The strain from subgroup B (D) is present in Iran (Verdin et al., 2003), and every known subgroup is found in Lebanon (Molino Lova et al., 2011). In Lebanon, the presence of each subgroup appears to vary based on geographical location (Molino Lova et al., 2011). Strains from subgroups D and G are known to infect peach, nectarine, and almond, while the subgroup F strain has only been found in nectarine (Molino Lova et al., 2011). It is currently unclear whether different strains of this phytoplasma vary in their host symptom severity, insect vectors, or host range.

A study of the genome sequences of numerous ‘Ca. P. phoenicium’ strains suggests that strains originating from different hosts can be distinguished by variations in the inmp (integral membrane protein) gene sequences (Quaglino et al., 2015).

**Biology and Ecology**

Phytoplasmas live exclusively in the phloem tissue of plants and are normally transmitted by vegetative propagation or grafting (seedlings, scions, rootstocks) and by insect vectors (Lee et al., 2000). Some phytoplasmas, such as ‘Ca. P. solani’ (causal agent of bois noir/stolbur disease of grapevine), are transmitted from weeds, which act as reservoir hosts of the pathogen, to economically important hosts (Maixner, 2011). The insect vector can acquire the pathogen from the weedy hosts and transmit to more economically important host plants. The specific biology of ‘Ca. P. phoenicium’ is currently not well known, but new research has recently led to a better understanding of this pathogen.

The rapid spread of ‘Ca. P. phoenicium’ over large geographical areas in North Lebanon suggested the presence of an efficient vector (Abou-Jawdah et al., 2010), and at least three insect vectors in Lebanon have been identified (Asymmetrasca decedens, Tachycixius. cf cypricus, and Tachycixius viperinus) (Abou-Jawdah et al., 2014; Tedeschi et al., 2015). Tachycixius. cf cypricus, and Tachycixius viperinus are known to transmit the phytoplasma to peach, and A. decedens transmits the phytoplasma to both almond and peach (Abou-Jawdah et al., 2015). Several other insects have also been identified as carriers of ‘Ca. P. phoenicium’ in Lebanon (Allygus spp., Annoplotettix danutae, Balclutha spp., Cixius bifidispinus, Empoasca decipiens, Eumecurus spp., Euscelidius mundus, Fieberiella macchiae, Lylatina inexpectata, Tachycixius bidentifer, T. cf. creticus, and Thamnottetix seclusis) but are currently not confirmed to be vectors (Dakhil et al., 2011; Tedeschi et al., 2015; Picciau et al., 2016) (See vector section). Currently, the insect vectors in Iran have not been confirmed.

In an insect trapping survey of two different ‘Ca. P. phoenicium’ infected almond orchards in Lebanon, *A. decedens* was by far the most prevalent and represented 82% of all leafhoppers sampled (Dakhil et al., 2011). In this study, *Asymmetrasca decedens* and other leafhoppers were most abundant between the months of March-May, although a small number of insects were also found from November-February (Dakhil et al., 2011). In another insect survey by Tedeschi et al. (2015), *Tachycixius* spp. were most abundant in ‘Ca. P. phoenicium’ infected peach orchards from September-November followed by April-May, corresponding with two separate flight peaks.

Two weed hosts of ‘Ca. P. phoenicium’, *Smilax aspera* and *Anthemis* spp., have been identified (Tedeschi et al., 2015). *Tachycixius. cf cypricus* and *Tachycixius viperinus* were both collected from these weed hosts and later found to be infected with ‘Ca. P. phoenicium’ and capable of transmitting the phytoplasma to peach (Tedeschi et al., 2015). This study suggests that weeds may indeed prove to be reservoir hosts which contribute to the spread of AlmWB disease to stone fruit hosts by leafhoppers.

During an inoculation study by Abou-Jawdah et al. (2014), ‘Ca. P. phoenicium’ was detected in infected almond and peach seedlings that did not show characteristic witches’-broom symptoms for one year post-inoculation. That study suggests the incubation period of ‘Ca. P. phoenicium’ may be longer than one year.

**Symptoms/Signs**

**In almond**: Symptoms include early flowering, stunted growth, leaf rosetting (a disease symptom characterized by short, bunchy growth habit due to shortened internodes and

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Figure 5: A nectarine infected with ‘Ca. P. phoenicium’ with early leaf development (middle). Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.
reduction in leaf size), dieback, off-season growth, proliferation of slender shoots, witches'-broom, and smaller pale green leaves (Fig. 1, 2, 3) (Abou-Jawdah et al., 2002; Abou Jawdah, personal communication, 2016). Witches'-broom symptoms arise mainly from the trunk or roots (Fig. 1) (Abou-Jawdah et al., 2002). The phytoplasma has also been detected in symptomless almond hosts (Abou-Jawdah et al., 2002).

Some almond cultivars are more susceptible to 'Ca. P. phoenicium' than other cultivars. In Lebanon, the Alwani and Awja cultivars are highly susceptible and developed severe symptoms, and the Kachabi cultivar is less severely affected (Verdin et al., 2003). The susceptibility of almond cultivars grown in the United States is not currently known.

**In peach/nectarine:** Symptoms early in the growing season include premature flowering followed by the early development of buds (Molino-Lova et al., 2011) (Fig. 5). Symptoms later in the growing season include: shoot proliferation, smaller leaves with a pale green color (Fig. 3, 4), abnormal flowers (phyllody), and witches'-broom symptoms in rare cases (Abou-Jawdah et al., 2010). Most infected trees do not set any fruit, but some trees bore a limited number of deformed fruits (Abou-Jawdah et al., 2010).

**Pest Importance**

In the past two decades, 'Ca. P. phoenicium' has killed over 150,000 almond trees in Lebanon (Abou-Jawdah et al., 2014). Recent surveys have also identified thousands of peach and nectarine trees infected with the phytoplasma (Molino Lova et al., 2011). In highly susceptible almond cultivars, dieback from AlmWB may occur in all trees of an affected orchard (Verdin et al., 2003). Total loss of commercial yield in infected almond or peach trees can occur one year after the initial observation of symptoms (Abou Jawdah, personal communication, 2016).

Almonds are an important specialty crop in the United States, and California alone produces about 80% of the world’s almonds. Almond production in California has increased substantially over the last decade (Pierson, 2014). In 2014, almonds were grown on 860,000 bearing acres in the state (USDA-NASS, 2015a). The 2014 almond harvest was 1.87 billion pounds and a total estimated value of $6.46 billion (USDA-NASS, 2015a). The 2015 forecast for almond production is 1.85 billion pounds grown on 890,000 bearing acres (USDA-NASS, 2015b).

Peach and nectarine are also important specialty crops in the United States. In 2014, peach and nectarine was grown on over 140,000 bearing acres in the United States, and the total combined value of peach and nectarine production in was nearly $800 million (USDA-NASS, 2015a).

'**Candidatus** Phytoplasma phoenicium' was first added to the European and Mediterranean Plant Protection Organization (EPPO) Alert List in 2001 and removed from the list in 2006. Since then, due to rapid spread of AlmWB in Lebanon and the discovery of peach and nectarine as additional hosts, 'Ca. Phytoplasma phoenicium' was again added to the EPPO Alert List in 2015 (EPPO, 2015).
At the genus level, *Phytoplasma* spp. is listed as a harmful organism in Australia, Israel, and Nauru (USDA-PCIT, 2015). There may be trade implications with these countries if ‘Ca. P. phoenicium’ becomes established in the United States.

**Known Hosts**

**Major hosts:** *Prunus dulcis* (almond), *Prunus dulcis* x *Prunus persica* (GF-677 almond/peach hybrid), *Prunus persica* (peach), and *Prunus persica* var. *nucipersica* (nectarine) (Verdin et al., 2003; Salehi et al., 2006; Abou-Jawdah et al., 2010).

**Other hosts:** *Anthemis* spp. (chamomile), *Prunus scoparia* (wild almond), and *Smilax aspera* (rough bindweed), (Salehi et al., 2015; Tedeschi et al., 2015).

**Experimental hosts:** *Solanum lycopersicum* (tomato) and *Solanum melongena* (eggplant) (Salehi et al., 2006).

In Iran, association of 16SrIX-related phytoplasmas are reported from herbaceous plants including: *Sesamum indicum* (sesame) (Salehi et al., 2005), *Catharanthus* spp. (periwinkle) (Salehi, personal communication, 2016), *Solanum lycopersicum* (tomato) (Jamshidi et al., 2014), *Solanum melongena* (eggplant) (Salehi, personal communication, 2016), *Lactuca sativa* (lettuce), and *Lactuca virosa* (wild lettuce) (Salehi et al., 2006).

**Known Vectors (or associated insects)**

There are three confirmed vectors of ‘Ca. P. phoenicium’:

- *Asymmetrasca decedens* (syn. *Empoasca decedens*) (Fig. 6) (Abou-Jawdah et al., 2014).
- *Tachycixius viperinus* (Tedeschi et al., 2015).

Other known insect carriers of ‘Ca. P. phoenicium’ which are not confirmed vectors are: *Allygus* spp., *Annoplotettix danutae*, *Balclutha* spp., *Cixius* bifidispinus, *Empoasca decipiens*, *Eumecurus* spp., *Euscelidius mundus*, *Fieberiella macchiae*, *Lylalina inexpectata*, *Tachycixius bidentifer*, *T. cf. creticus*, and *Thamnottetix seclusis* (Dakhil et al., 2011; Tedeschi et al., 2015; Picciau et al., 2016). To date, none of these vectors or potential vectors have been reported to occur in the United States.

**Known Distribution**

**Asia:** Lebanon (subgroups B (D), F, and G), Iran (subgroup B (D)) (Verdin et al., 2003; Salehi et al., 2006).

**Pathway**

Long distance transport of phytoplasmas is most likely to occur via movement of infected host plant material. A recent study showed that the incubation period of ‘Ca. P. phoenicium’ in plants may be longer than one year before visual symptoms become apparent (Abou Jawdah, 2014). Currently, the import of *Prunus* spp. propagative material is prohibited from Lebanon and Iran, but import of *Anthemis* spp. is allowed (USDA, 2015).

Since 2005, there have been nine shipments of *Prunus* spp. plant material intended for propagation from Israel (AQAS, 2015), which borders Lebanon. These shipments contained a total of 364 plant units (PU). Since 2005, there have also been interceptions of *Prunus* spp. plant material intended for propagation from Iran (9), Israel (2), and Lebanon (1) (AQAS, 2015). There were also interceptions of *Anthemis* spp. from Iran (4) and Lebanon (3), but they were all intended for personal consumption (AQAS, 2015).

Another possible pathway of introduction is the movement of infected insect vectors. Since 2005, there have been 39 interceptions of *Empoasca* spp. from Israel and one interception from Lebanon (AQAS, 2015). Some of these interceptions may have been *Empoasca decedens* (syn. *Asymmetrasca decedens*), which is present in both of these countries.

**Potential Distribution within the United States**

California is by far the state most vulnerable to infection by ‘Ca. P. phoenicium’ due to the large amount of almond cultivation there (USDA-NASS, 2015ab). In addition, California is the top peach producing state and accounts for over 40% of the nation’s peach production (USDA-NASS, 2015a). The other top ten peach producing states in 2014 were, in order: South Carolina, Georgia, New Jersey, Pennsylvania, Michigan, Texas, Colorado, Washington, and Alabama (USDA-NASS, 2015a). In addition, *Anthemis* spp. are also widespread throughout the United States, including throughout California (BONAP, 2014).

The insect vector *Asymmetrasca decedens* is present throughout the Mediterranean region in Europe (Freitas and Aguin-Pombo, 2006), so it may also be able to survive in California, which has a similar climate.

A recent combined host distribution map for ‘Ca. P. phoenicium’ developed by USDA-APHIS-PPQ-CPHST (Fig. 7) identifies areas of high host acreage based on the combined acreage of almond, nectarine, and peach. This map illustrates that though there are counties in nearly all states with a low level of risk, California is the most at risk for ‘Ca. P. phoenicium’ based on host density. The host distribution maps are based on county level data. To combine host data for pest-specific analyses, CPHST normalizes the data by dividing the total host present in a county by overall county area (acres of host in county/ total acres of county). This yields host by county area and allows CPHST to properly combine host distributions without the skewing effects of overall county size. For example, 500 acres of broccoli grown in Tulare County, CA can...
now be compared to 500 acres of broccoli grown in Scott County, AR. The individual host acreage maps for almond, nectarine, and peach are provided in the Appendix at the end of the document.

**Survey**

**Approved Method for Pest Surveillance**: The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey.

For FY2017 surveys: Follow instructions in [Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2017](#).

If you have taken the hands-on phytoplasma specific training at CPHST Beltsville, you can screen your own FY2017 phytoplasma samples. **Note:** You will still have to follow the protocol in the linked document for confirmations.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

**Key Diagnostics**

**Approved Method for Pest Surveillance***:
The approved method for phytoplasmas is molecular diagnosis.

For FY2017 surveys: Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2017.

If you have taken the hands-on phytoplasma specific training at CPHST Beltsville, you can screen your own FY2017 phytoplasma samples. **Note:** You will still have to follow the protocol in the linked document for confirmations.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

**Literature-Based Methods:**
*Candidatus* Phytoplasma phoenicium’ can be identified by nested PCR and RFLP analysis (Abou-Jawdah et al., 2002, 2014; Tedeschi et al., 2015) or sequencing of 16S rDNA (Verdin et al., 2003; Abou-Jawdah et al., 2014). Jawhari et al. (2015) also developed a specific PCR and a quantitative real-time PCR (qPCR) which can detect ‘Ca. P. phoenicium’ in host phloem tissue or in insect vectors. Quaglino et al. (2015) published a draft genome sequence of ‘Ca. P. phoenicium’.

**Easily Confused Species**
Phytoplasma infection cannot be diagnosed by visual inspection alone and must be confirmed using molecular analysis. ‘Candidatus Phytoplasma phoenicium’ infection may be confused with infection of other phytoplasmas. For example, ‘Ca. P. prunorum’ and ‘Ca. P. pruni’ are two other phytoplasmas that infect stone fruit (Seemüller and Schneider, 2004; Davis et al., 2013), and ‘Ca. P. pruni’ is present in the United States. Verdin et al., (2003) identified a closely related but distinct phytoplasma that infects almonds in Iran and was found to be in phytoplasma group 16SrlX-C. This phytoplasma is tentatively named Khafr almond witches’-broom (KAlmWB) and is closely related to *Knautia arvensis phyllody* (KAP) phytoplasma (Salehi et al., 2006).

Bacterial spot (*Xanthomonas arboricola* pv. *pruni*) is a relatively new disease of almond in California. This disease also infects other stone fruit, including peach and cherry. Symptoms include leaf spotting, lesions, and cankers, however, which are not associated with ‘Ca. P. phoenicium’ infection (UC-IPM, 2015).

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