'Candidatus Phytoplasma phoenicium' and related strains/subgroups

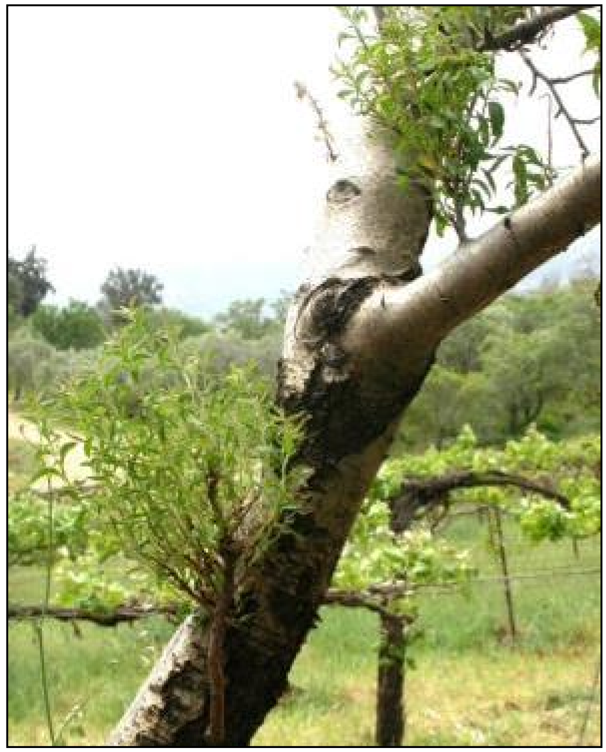

Figure 1: Witches' broom symptoms in an almond infected with '*Ca*. P. phoenicium'. Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.

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.

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).

Figure 3: An infected almond branch (left) vs. a healthy branch (right) in both images. Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.

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.

Figure 4. Left: A healthy peach tree and an infected peach tree (red arrow). Right: Shoot proliferation in an infected nectarine. Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.

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.

Figure 5: A nectarine infected with 'Ca. P. phoenicium' with early leaf development (middle). Courtesy of Yusuf Abou-Jawdah and Marina Molino-Lova.

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 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).

Figure 6: *Empoasca* spp., a leafhopper very similar in appearance to the vector *A. decedens* (syn. *Empoasca decedens*). Cybernadette, Wikimedia commons, CC-BY-SA-3.0

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* cf *cypricus* (Tedeschi et al., 2015).
- *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*, *Lylatina 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.

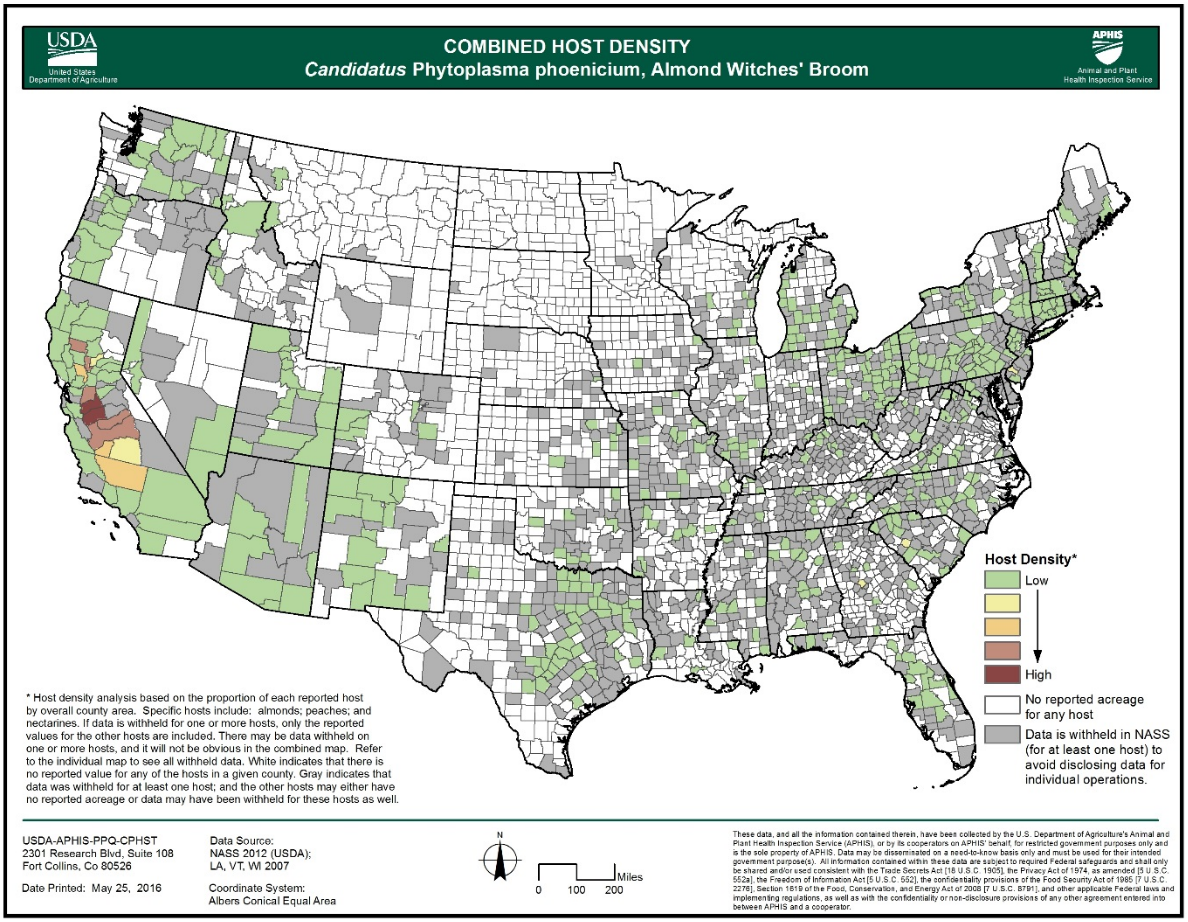

Figure 7. Combined distribution map for '*Candidatus* Phytoplasma phoenicium' within the continental United States. Values represent combined host density low to high (almond, peach, and nectarine). Map courtesy of USDA-APHIS-PPQ-CPHST.

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 phytyoplasma group 16SrIX-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).

References

Abou-Jawdah, Y. 2016. Biology of '*Candidatus* Phytoplasma phoenicium'. Personal communication to M. J. Sullivan on March 7, 2016 from Y. Abou Jawdah (American University of Beirut).

Abou-Jawdah, Y., A. Abdel Sater, M. Jawhari, H. Sobh, H. Abdul-Nour, and P. A. Bianco. 2014. *Asymmetrasca decedens* (Cicadellidae, Typhlocybinae), a natural vector of '*Candidatus* Phytoplasma phoenicium'. Annals of Applied Biology 165(3): 395-403.

Abou Jawdah, Y., E. Abou-Fakhr, H. Sobn, M. Molino Lova, A. Vercesi, and P.A. Bianco. 2010. Almond witches'-broom phytoplasma ('*Candidatus* Phytoplasma phoenicium'): a real threat to almond, peach and nectarine. 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. Julius-Kühn-Archive 427: 418-420.

Abou-Jawdah, Y., A. Karakashian, H. Sobh, M. Martini, and I.-M. Lee. 2002. An epidemic of almond witches'-broom in Lebanon: Classification and phylogenetic relationships of the associated phytoplasma. Plant Disease 86(5): 477-484.

Al-Saady N.A., A.J. Khan, A. Calari, A.M. Al-Subhi, and A. Bertaccini. 2008. '*Candidatus* Phytoplasma omanense', associated with witches'-broom of *Cassia italica* (Mill.) Spreng. Oman. Int. J. Syst. Evol. Microbiol. 58 (2): 461–466.

AQAS. 2015. Agricultural Quarantine Activity Systems. Queried December 3, 2015 from, www.aqas.aphis.usda.gov/.

BONAP. 2014. Biota of North America Program, North American Plant Atlas. Queried December 6, 2015 from, www.bonap.org/.

Dakhil, H.A., E.A.F. Hammad, C. El-Mohtar, and Y. Abou-Jawdah. 2011. Survey of leafhopper species in almond orchards infected with almond witches'-broom phytoplasma in Lebanon. Journal of Insect Science 11(1): 60.

Davis R., Y. Zhao, E.L. Dally, I.-M. Lee, R. Jomantiene, and S.M. Douglas. 2013. '*Candidatus* Phytoplasma pruni', a novel taxon associated with X-disease of stone fruits, *Prunus* spp.: multilocus characterization based on 16S rRNA, *secY*, and ribosomal protein genes. International Journal of Systematic and Evolutionary Microbiology 63:766-776.

EPPO. 2015. EPPO Reporting Service No. 11, November, 2015. European and Mediterranean Plant Protection Organization, Paris.

Freitas, N., and D. Aguin-Pombo. 2006. Distribution, food plants and control of *Asymmetrasca deceedens* (Paoli, 1932) (Hemiptera: Cicadellidae). Boletim do Museu Municipal do Funchal 57(316): 11-27.

Harrison, N.A., D. Gundersen-Rindal, and R.E. Davis. 2011. Genus I. "*Candidatus* Phytoplasma" gen. nov. IRPCM.

IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (IRPCM). 2004. '*Candidatus* Phytoplasma', a taxon for wall-less, non-helical prokaryotes that colonize plant phloem and insects. International Journal of Systemic and Evolutionary Microbiology 54: 1243-1255.

Jamshidi, E., B. Jafarpour, H. Rouhani, and M. Salehi. 2014. Association of members of clover proliferation (16SrVI) and pigeon pea witches 'broom (16SrIX) phytoplasma groups with tomato big bud disease in Iran. Iranian J Plant Pathol 50: 77- 89.

Jawhari, M., P. Abrahamian, A. Sater, H. Sobh, P. Tawidian, and Y. Abou-Jawdah. 2015. Specific PCR and real-time PCR assays for detection and quantitation of '*Candidatus* Phytoplasma phoenicium'. Molecular and cellular probes 29(1): 63-70.

Lee I-M., R.E. Davis, and D.E. Gundersen-Rindal. 2000. Phytoplasma: phytopathogenic mollicutes. Annual Review of Microbiology 54: 221-255.

Maixner M. 2011. Recent advances in bois noir research. Petria 21: 17–32.

McCoy, R.E., A. Caudwell, C. J. Chang, T.A. Chen, L.N. Chykowski, M.T. Cousin, J.L. Dale, G.T.N. de Leeuw, D.A. Golino, K.J. Hackett, B.C. Kirkpatrick, R. Marwitz, H. Petzold, R.C. Sinha, M. Sugiura, R.F. Whitcomb, I.L. Yang, B.M. Zhu, and E. Seemuller. 1989. Plant diseases associated with mycoplasma-like organisms. In: The Mycoplasmas, Vol. 5. Whitcomb, R.F., and J. G. Tully (ed.). Academic Press, New York.

Molino Lova, M., F. Quaglino, Y. Abou-Jawdah, E. Choueiri, H. Sobh, A. Alma, R. Tedeschi, P. Casati, and P. A. Bianco. 2011. Identification of new 16SrIX subgroups, -F and -G, among '*Candidatus* Phytoplasma phoenicium' strains infecting almond, peach and nectarine in Lebanon. Phytopathologia Mediterranea 50(2): 273-282.

Motamedi, E., M. Salehi, S. K. Sabbagh, and M. Salari. 2015. Differentiation and classification of phytoplasmas associated with almond and GF-677 witches'-broom diseases using rRNA and rp genes. J Phytopathol doi: 10.1111/jph.12448.

Picciau L., H. Hoch, M. Asche, R. Tedeschi, and A. Alma. 2016. The genus *Cixius* Latreille, 1804 (Hemiptera, Fulgoromorpha, Cixiidae) in Lebanon with the description of two new species. Zootaxa 4093 (1): 085-102.

Quaglino, F., M. Kube, M. Jawhari, Y. Abou-Jawdah, C. Siewert, E. Choueiri, H. Sobh, P. Casati, R. Tedeschi, M. Lova, and A. Alma A. 2015. '*Candidatus* Phytoplasma phoenicium 'associated with almond witches'-broom disease: from draft genome to genetic diversity among strain populations. BMC microbiology 15(1):148.

Quaglino, F., Y. Zhao, P. Casati, D. Bulgarii, P.A. Bianco, and W. Wei. 2013. '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur and bois noir related diseases of plants. International Journal of Systematic and Evolutionary Microbiology 63: 2879-2894.

Salehi, M. 2016. Biology of '*Candidatus* Phytoplasma phoenicium'. Personal communication to M. J. Sullivan on March 7, 2016 from M. Salehi (AREEO, Zarghan, Iran).

Salehi, M., F. Haghshenas, A. Khanchezar, and Esmailzadeh-Hosseini, S. A. 2011. Association of '*Candidatus* Phytoplasma phoenicium' with GF-677 witches'-broom in Iran. Bulletin of Insectology 64:S113–S114.

Salehi, M., J. Heydarnejad, and K. Izadpanah. 2005. Molecular characterization and grouping of 35 phytoplasmas from central and southern provinces of Iran. Iran J Plant Pathol 41:62–64.

Salehi, M., K. Izadpanah, N. Nejat, and M. Siampour. 2007. Partial characterization of phytoplasmas associated with lettuce and wild lettuce phyllodies in Iran. Plant Pathol 65: 669-670.

Salehi, M., K. Izadpanah, and J. Heydarnejad. 2006. Characterization of a new almond witches'-broom phytoplasma in Iran. Journal of Phytopathology 154(7-8): 386-391.

Salehi, M., E. Salehi, M. Abbasian, and K. Izadpanah. 2015. Wild almond (*Prunus scoparia*), a potential source of almond witches'-broom phytoplasma in Iran. Journal of Plant Pathology 97: 377-381.

Seemüller, E., M. Garnier, and B. Schneider. 2002. Mycoplasmas of plants and insects. in Molecular Biology and Pathogenicity of Mycoplasmas, S. Razin and R. Herrmann eds. Kluwer Academic/Plenum Publishers, Dordrecht, Netherlands Pgs. 91-116.

Seemüller, E., and B. Schneider. 2004. '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri', and '*Candidatus* Phytoplasma prunorum', the causal agents of apple proliferation, pear decline, and European stone fruit yellows, respectively. International Journal of Systemic and Evolutionary Microbiology 54: 1217-1226.

Tedeschi, R., L. Picciau, F. Quaglino, Y. Abou-Jawdah, M. Molino Lova, M. Jawhari, P. Casati, A. Cominetti, E. Choueiri, H. Abdul-Nour, P.A. Bianco, and A. Alma. 2015. A cixiid survey for natural potential vectors of '*Candidatus* Phytoplasma phoenicium' in Lebanon and preliminary transmission trials. Annals of Applied Biology 166(3): 372-388.

UC-IPM. 2015. Bacterial spot (*Xanthomonas arboricola* pv. *pruni*)*.* University of California Agriculture & Natural Resources, Statewide Integrated Pest Management Program. http://www.ipm.ucdavis.edu/EXOTIC/bacterialspot.html.

USDA. 2015. Plants for Planting Material, updated 8/2015. Retrieved from, https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/plants_for_planting.pdf

USDA-NASS. 2015a. United States Department of Agriculture, National Agricultural Statistics Service. Noncitrus fruits and nuts, preliminary 2014 survey, ISSN: 1948-2698.

USDA-NASS. 2015b. 2015 California almond forecast. United States Department of Agriculture, National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/201505almpd.pdf.

USDA-PCIT. 2015. Phytosanitary Export Database. Queried November 3, 2015 from, https://pcit.aphis.usda.gov/pcit/.

Verdin, E., P. Salar, J.-L. Danet, E. Choueiri, F. Jreijiri, S. El Zammer, B. Gélie, J.M. Bové, and M. Garnier. 2003. '*Candidatus* Phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. International Journal of Systematic and Evolutionary Microbiology 53(3): 833-838.

Weintraub, P.G., and L. Beanland. 2006. Insect vectors of phytoplasmas. Annual Review of Entomology 51: 91-111.

Zhao, Y., W. Wei, R.E. Davis, and I.M. Lee. 2009. Recent advances in 16S rRNA gene-based phytoplasma differentiation, classification and taxonomy. *In:* Weintraub, P.G. and Jones, P. (eds.) Phytoplasmas: genomes, plant hosts and vectors. Pages: 64-92.

This datasheet was developed by USDA-APHIS-PPQ-CPHST staff. Cite this document as:

Mackesy, D.Z., and M. Sullivan. 2016. CPHST Pest Datasheet for '*Candidatus* Phytoplasma phoenicium'*.* USDA-APHISPPQ-CPHST.

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Last updated: 6/3/2016

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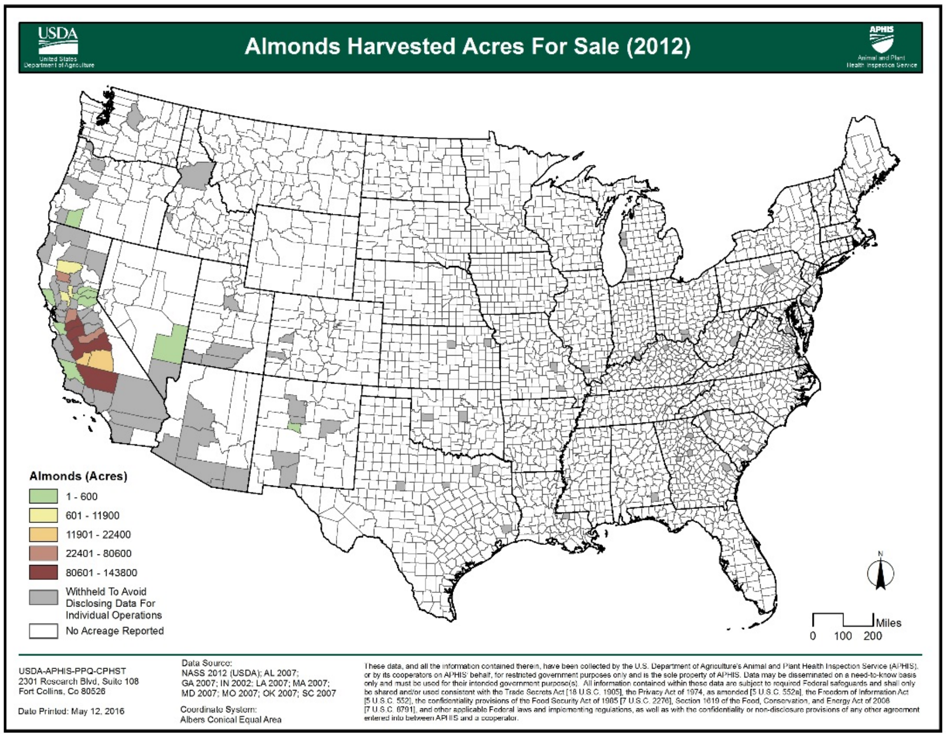
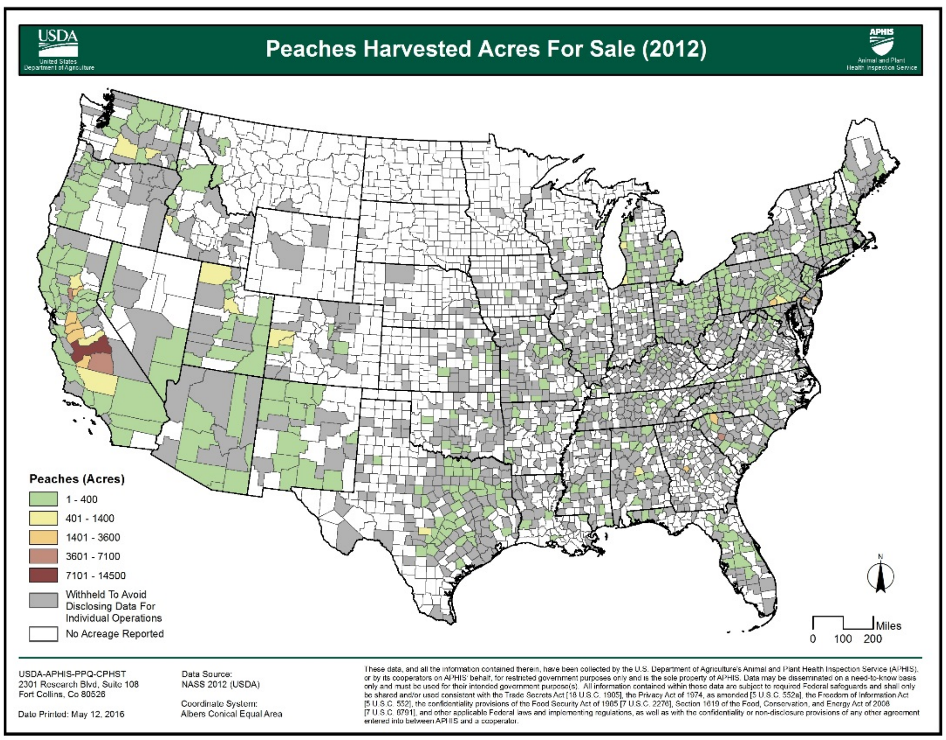
Update History:
<u>April, 2016:</u> Draft version written and sent for subject matter expert review.

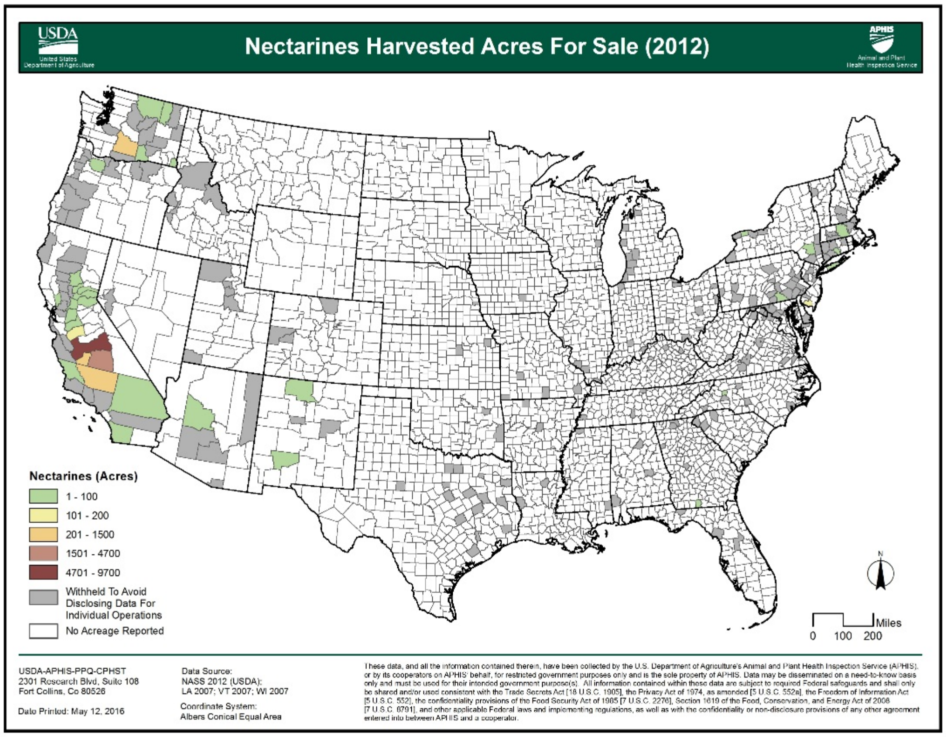
<u>June 2016:</u> Datasheet posted to CAPS Resource and Collaboration site.

Appendix:





Last updated: 6/3/2016



Last updated: 6/3/2016