‘*Candidatus* Phytoplasma australiense’

Scientific Name
‘*Candidatus* Phytoplasma australiense’ Davis et al., 1997

Synonyms
Phytoplasma solani australensis, Phytoplasma solani australiense

Common Name(s)
Australian grapevine yellows, Australian lucerne yellows, Australian papaya dieback, cabbage tree sudden decline, coprosma lethal disease, cordyline sudden decline, cottonbush witches’ broom, gomphocarpus yellowing, Jaunisse de la Vigne, liquidamber yellows, mung bean witches’ broom, papaya dieback, paulownia yellows, periwinkle phyllody, phormium yellow leaf, pumpkin yellow leaf curl, strawberry green petal, strawberry lethal yellows, and yellow leaf disease.

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes, **Order:** Acheloplasmatales, **Family:** Acheloplasmataceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2009 through 2011; Pests of Economic and Environmental Importance List 2012-2017

Pest Description
Mollicutes are prokaryotes that have small genomes (530 to 1350 kbp), lack a cell wall, are pleomorphic, and have a low G + C content (23-29 mol%). Phytoplasmas belong to the class Mollicutes and are the proposed causative agents of diseases in several hundred plant species (McCoy et al., 1989). Phytoplasmas reside in the phloem tissue of the infected plant host and are transmitted by insect vectors, principally leafhoppers and planthoppers (White et al., 1998). Although phytoplasmas have been detected in affected plant tissues and insects with the use of technologies based on the transmission electron microscope, antibodies, and nucleic acids, they are unable to be cultured *in vitro*. Phytoplasmas cannot be morphologically or ultrastructurally distinguished from one another using either electron or light microscopy (CABI, 2007). *Candidatus* in scientific classification is a formal word that is placed before the genus and species name of bacteria that cannot be maintained in a Bacteriology Culture Collection. *Candidatus* status may be used when a species or genus is well characterized but unculturable.

‘*Candidatus* Phytoplasma australiense’ (herein abbreviated as ‘*Ca* P. australiense’) is the phytoplasma associated with Australian grapevine yellows (AGY), papaya dieback (PD), and phormium yellow leaf diseases (PYL) in New Zealand and Australia (Davis et al., 1997). Molecular studies have shown that the phytoplasmas associated with AGY, PD, and PYL belong to the same species (Liefting et al., 1998). This phytoplasma is distinct from German grapevine yellows and stolbur. PYL is a lethal disease of New Zealand flax (*Phormium tenax*) and mountain flax (*P. cookianum*), which is only present in New Zealand (first found in 1908). It is transmitted by a planthopper, *Zeoliarus* (*Oliarus*) *atkinsoni*. PD can be a devastating disease in Queensland, Australia (first found in 1922). Australian grapevine yellows was first reported in Australia in 1975. No vectors have been identified for the latter two diseases.

Taxonomists classify phytoplasmas by directly comparing their 16S ribosomal ribonucleic acid (rRNA) sequences plus the 16S-23S rRNA intergenic spacer region (White et al., 1998). They group isolates that are ≥ 97.5% in the same *Candidatus* species, unless significant biological or genetic properties suggest that they should be classified separately (Andersen et al., 2006). Andersen et al. (2006) reveal three distinct subgroups within '*Ca.* P. australiense' by sequencing the *tuf* gene, which encodes the elongation factor Tu.

Figure 1. Symptoms associated with '*Candidatus* Phytoplasma australiense' in grape. Mild, irregular chlorosis, leaves with backward curling, overlapping of leaves, tip death (left); necrosis (center); chlorosis along the veins (right). Photos courtesy of Fiona Constable (CABI, 2007).

Biology and Ecology

The biology of '*Ca.* P. australiense' is not completely understood. Like other phytoplasmas, it is an obligate intercellular parasite that occurs in the phloem sieve tubes of infected plants and the salivary glands of insect vectors (CABI, 2007). '*Ca.* P. australiense' cells, like other phytoplasmas, are surrounded by a single-unit membrane, lack a rigid cell wall, and are pleomorphic in shape. When observed by transmission electron microscopy, they appear as rounded to filamentous, pleomorphic bodies with a mean diameter of 200 to 800 nm (IRPCM, 2004). They are sensitive to antibiotics of the tetracycline group but not to penicillin.

The cixiic planthopper, *Zeoliarus* (*Oliarus) atkinsoni* and *Zeoliarus oppositus*, which occur in New Zealand, are the only known vectors of '*Ca.* P. australiense'. *Z. atkinsoni* has only been demonstrated to transmit the phytoplasma from *Phormium* plant to *Phormium* plant (Phormium yellows); while *Z. oppositus* is polyphagous and has been reported on many different plants (Cumber, 1953; Liefting et al., 1997; Beever et al., 2008). To date the insect vector responsible for the spread of '*Ca.* P. australiense' in Australia is not known.

Symptoms/Signs

<ins>Grape:</ins> Symptoms include yellow (chlorotic) and downward curled leaves that fall prematurely; reddening may be seen in red varieties (Fig. 1, 2). The chlorotic patches on affected leaves may become necrotic. Leaves of affected shoots can overlap one another. Shoots are stunted and unlignified. Abortion of flowering bunches early in the season has been observed (Constable et al., 2004). Any time from flowering, bunches may shrivel and fall (Magarey and Wachtel, 1986; CABI, 2007). Stems of affected shoots often take on a bluish hue (Constable et al., 2004). Only a few shoots on grapevine are usually affected, and inflorescence and fruit are generally only affected on symptomatic shoots. Later in the season, affected shoots tend to be green and rubbery (CABI, 2007). The symptoms associated with '*Ca.* P. australiense' can be influenced by the environment. Infected grapevines are less likely to show symptoms in summer than winter (CABI, 2007). Although the infected vines are likely to show symptoms year after year, the disease can go into remission and not express symptoms (CABI, 2007).

Figure 2. Symptoms associated with '*Candidatus* Phytoplasma australiense' in grape. Irregular reddening. Photos courtesy of Fiona Constable (CABI, 2007).

A symptomatic screening aid in grape is available at: https://caps.ceris.purdue.edu/dmm/964.

<ins>Solanaceous Hosts:</ins> In potato, upward rolling and purpling of the leaves were observed (Fig. 3a,b). The symptoms appeared similar to those of 'zebra chip', a disorder of potato

recently found to be associated with ‘*Candidatus* Liberibacter solanacearum’ in New Zealand and the United States (Liefting et al., 2009).

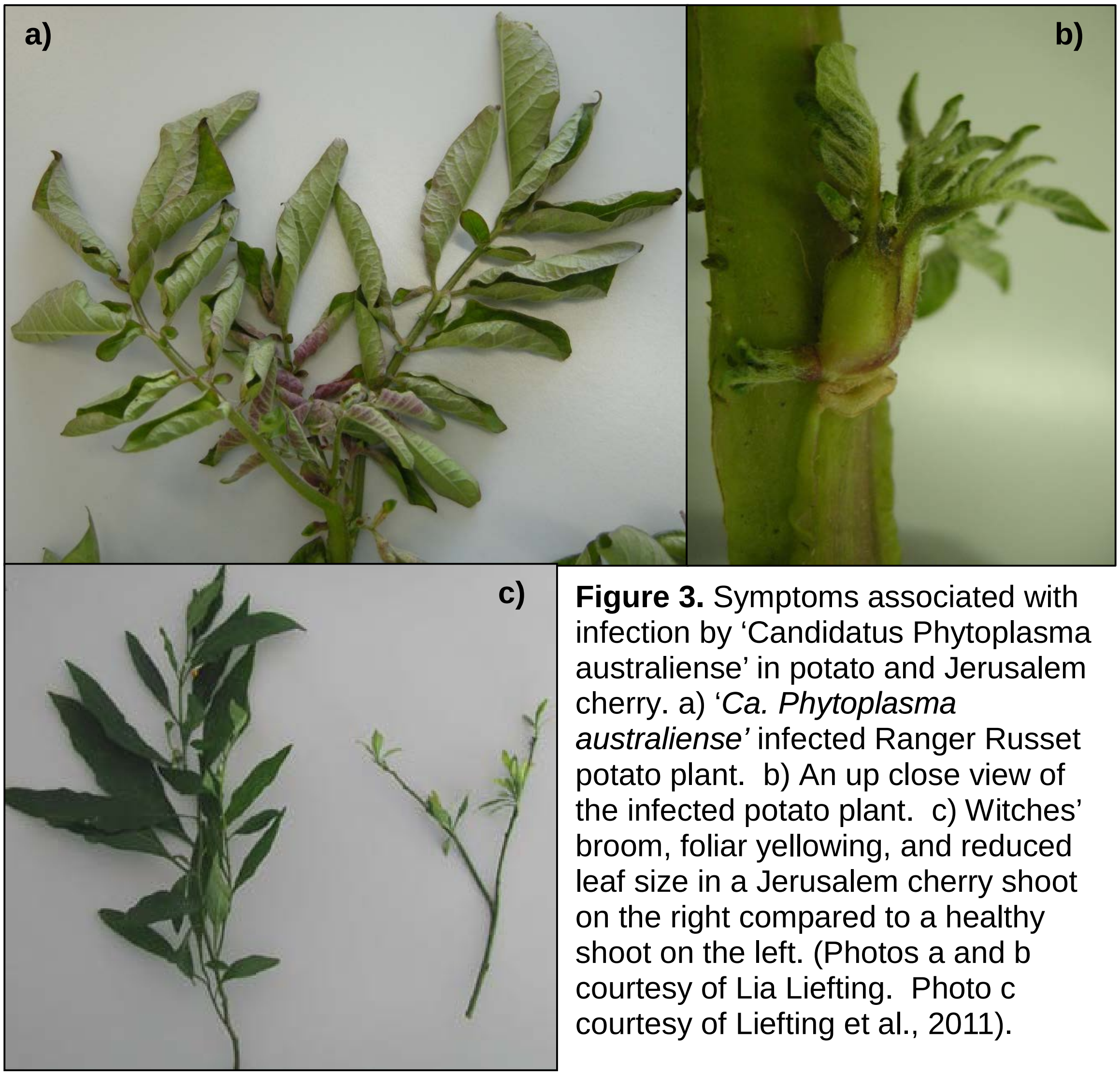

Figure 3. Symptoms associated with infection by ‘Candidatus Phytoplasma australiense’ in potato and Jerusalem cherry. a) ‘*Ca. Phytoplasma australiense’* infected Ranger Russet potato plant. b) An up close view of the infected potato plant. c) Witches’ broom, foliar yellowing, and reduced leaf size in a Jerusalem cherry shoot on the right compared to a healthy shoot on the left. (Photos a and b courtesy of Lia Liefting. Photo c courtesy of Liefting et al., 2011).

In Jerusalem cherry (*Solanum pseudocapsicum*), symptoms include: witches’ broom, foliar yellowing, and reduced leaf size (Fig. 3c) (Liefting et al., 2011).

<u>Other hosts:</u>

- In alfalfa (lucerne), symptoms range from a yellow to red discoloration of the leaves, a yellowish-brown root discoloration under the periderm, to plant death (Pilkington et al., 2003).
- In boysenberry, symptoms are obvious close to flowering when the lateral branches become stunted. Young leaves are smaller than normal and chlorotic. As the disease progresses, the older leaves become purple-bronze in color, particularly towards the margin. The fruit set as usual but remain small. New

canes fail to grow and the plant is dead by the following winter (Liefting et al., 2011).
- In celery, pink and yellow foliage and leaf deformation were observed (Liefting et al., 2011).
- Coprosma lethal decline causes leaf reddening and bronzing, heavy leaf loss, dieback, and plant death (Beever et al., 2004).
- Cordyline sudden decline may cause the death of mature cabbage trees.
- On New Zealand flax, abnormal yellowing of the leaves, stunted growth, increased root death, phloem necrosis, and xylem gummosis of the rhizome vascular system are observed (CABI, 2007).
- On papaya, symptoms include dieback, bending of the growing tip, bunching and chlorosis of the crown leaves, followed by necrosis of the young leaves and stem. Laticifer discoloration, particularly in the vicinity of the vascular tissue is evident. Plant death is observed within 2 to 3 weeks of first visible symptoms.
- Paulownia yellows stunts plant growth, causes leaves to 'yellow', and reduces internode length and leaf size (Bayliss et al., 2005).
- In pumpkin and strawberry plant growth is stunted and leaves turn yellow and curl (roll).
- In red clover, diminished leaf size, pallor, rugosity, leaf deformation shoot proliferation, and severe stunting were observed (Saqib et al., 2006).
- In swan plant (*Gomphocarpus fruticosa*), symptomatic plants exhibited abnormal foliar yellowing and slight upward rolling of the leaves. The symptoms eventually resulted in plant death (Liefting et al., 2011).
- In sweetgum, the crown may have patchy chlorosis, chlorotic shoots with comparatively few leaves, dieback of apical and lateral branches, small leaves showing tip necrosis, and reduced fruit production (Habili et al., 2006).

Note: While phytoplasma infections are usually detrimental to plant growth, some plants exhibit minor symptoms or are symptomless.

Pest Importance

Diseases caused by '*Ca.* P. australiense' impact economically important food and ornamental crops. Researchers have documented vineyard losses as high as 13%. Severely affected grape vines can produce up to 54% less fruit than healthy grape vines (CABI, 2007; NPAG, 2007). Papaya dieback is responsible for annual plant losses of 10% and up to 100% during epiphytotics (epidemic among plants of a single kind over a wide area) in central and southern Queensland plantations (Glennie and Chapman, 1976; Guthrie et al., 1998). Australian lucerne yellows has caused a reduction in seed yield, which has led to the cutting or plowing-under of seed crops, resulting in estimated losses of $7 million annually (Pilkington et al., 1999).

The economic impact that '*Ca.* P. australiense' could have on new host *Solanum tuberosum* is potentially significant. The total economic value of New Zealand's potato industry in 2011 was estimated to be $382 million NZD ($300 million USD), with about a quarter of that coming from exports (Potatoes New Zealand, n.d.).

The economic impact that ‘*Ca*. P. australiense’ will have on its other newly identified hosts is potentially significant (Liefting et al., 2011). Already, phytoplasma-infected potato tubers have failed quality control checks at the processing factory. In boysenberry, fruit from infected plants are unmarketable, and the plants die within a year after symptoms are first noticeable. Although there have been no reports of the phytoplasma in commercially grown celery, the discoloration of the foliage that the phytoplasma induces in this crop would also render it unmarketable. The polyphagous feeding behavior of *Zeolarius oppositus*, the insect responsible for transmitting the phytoplasma into *Cordyline* and *Coprosma*, suggests that it may also be moving the phytoplasma into the new hosts described here. *Z. oppositus* is especially abundant in grasses and sedges that commonly grown around crops in New Zealand. These hosts may act as symptomless reservoirs of the phytoplasma, and along with weed hosts such as Jerusalem cherry, play an important role in the spread of the phytoplasma. The diversity of the new hosts described in the Liefting et al. (2011) paper emphasize the potential that ‘*Ca*. P. australiense’ has to spread into additional native plants and horticultural crops.

Some of the diseases caused by ‘*Ca.* P. australiense’ are ‘new’ diseases or recently described (see known hosts section). For example, cordyline sudden death became a concern in the late 1980s; apparently this phytoplasma jumped to other hosts, including cabbage trees. The ability to use new hosts is an important and threatening aspect of the pathogen (NPAG, 2007). Establishment of ‘*Ca.* P. australiense’ in the United States could impact trade because some countries, like Morocco, classify the pathogen as a dangerous quarantine pest (NPAG, 2007). It is also on the harmful organism listing for China, Japan, and Peru.

Known Hosts

Research implicates ‘*Ca.* P. australiense’ as the cause of disease on the following hosts:

Asclepias physocarpa (balloon plant), *Apium graveolens* (celery), *Carica papaya (*papaya), *Catharanthus roseus* (periwinkle), *Cicer arietinum* (chickpea), *Coprosma macrocarpa (coprosma), Coprosma robusta* (coprosma), *Cordyline australis* (cabbage tree), *Cordyline banksii* (cabbage tree), *Cucumis myriocarpus* (paddy melon), *Cucurbita maxima* (pumpkin), *Cucurbita moschata* (pumpkin), *Exocarpos (Exocarpus) cupressiformis* (cherry ballart), *Fragaria ananassa* (strawberry), *Fragaria virginiana* (strawberry), *Gomphocarpus fruticosa* (also cited as *G. fructicosus*) (cottonbush; swan plant), *Jacksonia scoparia* (winged broom pea), *Liquidambar (Liquidamber) styraciflua* (sweetgum), *Medicago sativa* (alfalfa), *Melilotus indicus* (hexham scent), *Paulownia fortunei* (paulownia), *Phaseolus vulgaris* (bean), *Phormium cookianum* (mountain flax), *Phormium tenax* (New Zealand flax), *Rubus loganobaccus* (logan berry), *Rubus ursinus* (boysenberry), *Solanum pseudocapsicum* (Jerusalem cherry), *Solanum tuberosum* (potato), *Trifolium pratense* (red clover), *Vigna radiata* (mung bean), and *Vitis vinifera* (grape) (EPPO, 1998; Liefting et al., 1998; Padovan et al., 1998, White et al., 1998; Schneider et al., 1999; Wood et al., 1999; Padovan et al., 2000; Andersen et al., 2001;

Davis et al., 2003; Beever et al., 2004; Bayliss et al., 2005; Lucas, 2005; Saqib et al., 2005; Streten and Gibb, 2005; Streten et al., 2005; Habili et al., 2006; Saqib et al., 2006; CABI, 2007; Getachew et al., 2007; Liefting et al., 2009; CABI, 2011; Liefting et al., 2011).

Jones et al. (2005) identified '*Ca*. P. australiense' on peach in Bolivia. This report is now thought to be a related-species and not '*Ca*. P. australiense' (USDA-APHIS-PPQ-CPHST-PERAL, 2012; Davis, 2013). A '*Ca.* P. australiense'- related strain was found in China on *Senna surattensis* (sunshine tree) (Wu et al., 2012).

Known Vectors (or associated insects)

The only known vectors of '*Ca.* Phytoplasma australiense' are *Zeoliarus* (*Oliarus) atkinsoni* and *Zeoliarus oppositus* (not known in the United States) (Liefting et al., 1997; Beever et al., 2008). Other vectors are suspected but not currently confirmed (NPAG, 2007, CABI, 2011). The vector responsible for spread of '*Ca.* P. australiense' in Australia is unknown.

Known Distribution

'Ca P. australiense' is present in New Zealand and Australia.

A '*Ca.* P. australiense'- related strain was found in China on *Senna surattensis* (sunshine tree) (Wu et al., 2012).

Note: Two incorrect reports of '*Ca*. P. australiense' occur in the literature: Nivum Haamir dieback of papaya in Israel (Gera et al., 2005) and yellow leaf roll of peach in Bolivia (Jones et al., 2005). In both cases, the 16S rRNA gene sequence did not fulfill the criteria to be classified as '*Ca*. P. australiense' (USDA-APHIS-PPQ-CPHST-PERAL, 2012).

Pathway

The only confirmed vectors of '*Ca.* Phytoplasma australiense' are *Zeoliarus* (*Oliarus) atkinsoni* and *Zeoliarus oppositus* (Liefting et al., 1997; Beever et al., 2008). Neither of these pests has been found in the United States. It is possible for 'Ca. Phytoplasma australiense' to be moved within infected vectors, particularly the polyphagous vector *Zeoliarus oppositus.* There are no interceptions of these two leafhoppers at the species level to date in the United States (PestID, 2013). *Zeoliarus* is not currently listed in Pest ID (PestID, 2013).

The current pathway of '*Ca.* P. australiense' infection in *Solanum* spp. remains unknown. One possible pathway is through *Zeoliarus oppositus.* Infected *Z. oppositus* was found in the same field as the infected potato and Jerusalem cherry (Liefting et al., 2011). Transmission of the phytoplasma into potato by *Z. oppositus* has not been demonstrated experimentally, but these planthoppers were observed on the potato foliage and in one instance exhibited mating behavior (Liefting et al., 2011). It is possible that Jerusalem cherry could harbor the phytoplasma, which is then spread into the potato crop by the *Zeoliarus* planthopper.

Spread through infected plants for planting, such as nursery stock and cuttings, is also possible (CABI, 2011). This phytoplasma pathogen is not known to be seedborne. *Vitis* sp. and *Solanum* sp. (all propagules except seeds) are prohibited from all countries except for Canada when meeting the required import conditions (Plants for Planting Manual, 2012). There have been nine interception records (two destined for propagation) of *Vitis* sp. leaves, cutting, and fruit in baggage and permit cargo from Australia. There were an additional five interception records (two destined for propagation) of *Vitis* sp. stems and fruit in permit cargo, baggage, and quarters from New Zealand. There has also been four interception records (one destined for propagation) of *Solanum* sp. from New Zealand. There have been multiple interceptions (351 from Australia and 778 from New Zealand) of all currently known hosts of '*Ca.* Phytoplasma australiense' with a total of 69 destined for propagation (PestID, 2013).

Coprosma, Cordyline, Paulownia, and *Phormium* spp. propagative material (PM) does not appear to be subject to regulations, and there appears to be an open pathway for this PM (Plants for Planting Manual, 2012). Shipments appear to be plant units, seed, and flasks. There were 3 and 34 shipments of shipments of *Coprosma* sp. PM from Australia and New Zealand, respectively, since January of 2003. There were an additional 53 and 115 shipments of shipments of *Cordyline* sp. PM and 43 and 249 shipments of *Phormium* sp. from Australia and New Zealand respectively during the same time period. There were 15 shipments of *Paulownia* sp. PM from Australia over the past ten years as well (Plants for Planting Manual, 2012).

Potential Distribution within the United States

'Ca. P. australiense' is considered an imminent threat that could be introduced into the United States with imported leaves, stems, and roots of infected plants (NPAG, 2007). This phytoplasma has a relatively wide host range. Particular emphasis should be placed in areas that grow alfalfa, grapes, papaya, pawpaw, potato, strawberry, and sweet gum (*Liquidamber*) trees.

Survey

Approved Method for Pest Surveillance*: Visual survey is the method to survey for '*Ca.* P. australiense' by collecting symptomatic plant material. **Be sure that each plant that is sampled exhibits shriveling of the fruiting cluster when sampling grape.** For 2017 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 phytoplasma samples. **Note:** You will still have to follow the protocol in the linked document for confirmations.

A symptomatic screening aid in grape is available at: https://caps.ceris.purdue.edu/dmm/964.

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

Literature-Based Methods:
Visual survey: Conduct visual inspection for symptoms associated with the phytoplasma. Several of the known symptoms should be found together before suspecting a phytoplasma infection on grape (CABI, 2007). Symptoms begin to appear in late spring and increase in incidence until January/February in Australia (their summer). Beyond this time, AGY symptoms begin to disappear as symptomatic leaves and shoots fall from the vine (CABI, 2007). Phytoplasma DNA was detected from most plants when samples were collected in January and February (summer) compared to those sampled in Oct-Dec. (spring to early summer) and March-May (fall) in Australia (Gibb et al., 1999). The disease appears most often in Chardonnay and Riesling grapes but has also been reported in other cultivars (Magarey and Watchel, 1986).

Key Diagnostics/Identification

Approved Method for Pest Surveillance*:
Molecular: For 2017 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 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 https://caps.ceris.purdue.edu/approved-methods

Literature-Based Methods:
A 'universal' PCR assay has been developed that enables amplification of the 16S rRNA genes of phytoplasmas. Digestion of these PCR products with selected restriction enzymes provides a DNA fingerprint in the form of 16S rDNA fragment patterns that can be used to determine phytoplasma identity (Ahrens and Seemuller, 1992; Deng and Hiruki, 1991; Lee et al., 1993; Smart et al., 1996; Gibb et al., 1999). PCR assays have been developed to detect only the AGY phytoplasma in grape (Davis et al., 1997; Gibb et al., 1999).

The chromosome sequence of '*Ca.* P. australiense' isolates from papaya and grapevine in Australia is available (Tran-Nguyen et al., 2008).

Easily Confused Pests

AGY resembles flavéscence dorée, bois noir, Goldgelbe Vergilbung, leaf curl, berry shrivel and other grapevine diseases (tomato big bud and an uncharacterized disease also believed to be caused by a phytoplasma) (Magarey and Wachtel, 1985; Davis et al., 1997; Lee et al., 1998; Constable et al., 1998; Gibb et al., 1999). Detection of flavéscence dorée by cloned DNA probes was accomplished by Daire et al. (1992).

Mechanical disruption to the phloem of grape shoots can cause symptoms similar to those associated with '*Ca.* P. australiense' infection (CABI, 2007). It is important to inspect symptomatic shoots for damage to the vascular tissue due to breakage, restrictions of the vascular tissue due to tendrils or string wrapping tightly around shoots, and damage to the vascular tissue by boring insects.

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Update History:

June, 2013: Added Solanaceous host information.

June, 2014: Added link to CAPS symptomatic screening aid in Symptoms/Signs and Survey section.

July, 2014: Fixed link in document to the phytoplasma sample submission document.

November, 2014: Fixed a few links in the document.

June, 2016: Added new phytoplasma sample submission information to survey and diagnostic sections.