‘*Candidatus* Phytoplasma vitis’

Scientific Name
‘*Candidatus* Phytoplasma vitis’ IRPCM 2004

Note: The provisional name ‘*Candidatus* Phytoplasma vitis’ has been suggested to denote a phytoplasma associated with flavescence dorée disease in Europe, but the name has yet to be formally published and accepted.

Common Name(s)
FD, flavescence dorée phytoplasma, flavescence dorée de la Vigne, flavescencia dorada de la viña, flavescenza dorata de la vite, grapevine "flavescence dorée" phytoplasma, grapevine yellows.

Some older papers refer to the disease as grapevine bois noir. It was demonstrated, however, that this disease is caused by a distinct phytoplasma ‘*Candidatus* Phytoplasma solani’ or the bois noir phytoplasma (Daire et al., 1993; Quaglino et al., 2013).

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes, **Order:** Acholeplasmatales, **Family:** Incertae sedis, **Genus:** **“***Candidatus* Phytoplasma”

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List - 2014

Background Information
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).

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 incorporating 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).

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 contain at least one phytoplasma species (Zhao et al., 2009). For example, '*Candidatus* Phytoplasma vitis' is classified in group 16SrV, subgroups C & D (16SrV-C and 16SrV-D).

A new '*Candidatus* Phytoplasma' species may be recognized if the nucleotide sequence of 1,200 bases 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).

Pest Description

'*Candidatus* Phytoplasma vitis' (herein abbreviated '*Ca*. P. vitis') infection causes flavescence dorée (FD), a very serious grapevine yellows disease in vineyards and a quarantine pest in Europe. The most common damage associated with FD is the significant loss of grape and wine production due to the progressive decline of the plants. In most cases, especially in the more sensitive varieties, the infected grapevines die within a few years. FD is caused by several strains which belong to the 16SrV-C and –D phytoplasma phylogenetic subgroups. Strains classified in 16SrV-D subgroup are most widespread (Arnaud et al. 2007; Filippin et al., 2009).

Biology and Ecology

Like other phytoplasmas, '*Ca.* P. vitis' is an obligate intracellular parasite that occurs in the phloem sieve tubes of infected plants (CABI, 2007). In general, phytoplasmas are transmitted to plants in a circulative-propagative manner by phloem-feeding insect vectors. Their ingestion of sap from diseased plants is followed by an incubation/latency phase lasting for one to several weeks during which time these bacteria circulate, multiply, and parasitize various tissues and organs of their respective vectors. Once salivary glands have been colonized, vectors are then capable of transmitting phytoplasmas during any subsequent feeding activity for their remaining lifespan (Weintraub and Beanland, 2006; Gitau et al., 2009). Phytoplasmas are also transmitted by grafting of infected material.

The confirmed vector for FD is the leafhopper *Scaphoideus titanus* Ball (*S. titanus*), commonly known as the American grapevine leafhopper (Schvester et al*.,* 1961). For FD, the incubation/latency period in *S. titanus* is 4 weeks. By feeding on an infected

grapevine and then feeding on other grapevines, the vector spreads the disease through the vineyard. *S. titanus* is responsible for the rapid spread of FD in France and in Europe (Papura et al. 2009). Long-distance dissemination is, however, largely the result of the transport of infected propagative material by humans. *S. titanus* is native to the United States and was likely introduced in Europe when American rootstocks were imported as a part of the fight against downy mildew and phylloxera in the early twentieth century (Caudwell et al., 1983; Papura et al., 2012). Recent genomic studies have demonstrated that the phytoplasma strains responsible for FD originated in Europe (Arnaud et al., 2007, Malembic-Maher et al., 2011). It was only with the recent introduction from North America of the vector *Scaphoideus titanus* that FD was able to rapidly spread among the *Vitis vinifera* grapevines (INRA, 2013).

Recent studies carried out on the leafhopper *S. titanus* demonstrate that cold winter temperatures are not essential for terminating diapause although they do affect male–female ratios over time. The studies also showed that winter temperatures regulate hatching dynamics (Chuche and Thiery, 2012). These findings are very important because first hatchings can now be forecast and can be used to better control larvae before they have the chance to acquire the phytoplasma. The findings also allow predictions of the ability of the vector to colonize new wine-growing areas (INRA, 2013).

Like many other plant-eating insects, sucking insects in particular, *S. titanus* displays a strong attraction to the color yellow. It appears that the attraction is associated with young, developing leaves that are richer in nitrogen and thus of greater potential nutritional value for the insect. *S. titanus* is attracted by the color green to a lesser, but still significant, extent. Yellow and, to a larger degree, green are precisely the colors most present on grapevines. The insect's attraction to these colors is crucial for the propagation of FD. Insects are more attracted to the yellowed leaves of white varieties infected with FD, thereby spurring a vicious cycle (INRA, 2013).

Choice tests carried out with the white varietal Baco 22A, a widely planted variety in Armagnac, France that is highly susceptible to FD, confirmed that plants infected with FD attract more *S. titanus*, both at larval and adult stages. The attractiveness of infected plants may in part serve to explain the rapid spread of the disease (INRA, 2013).

There is some disagreement as to whether or not *Clematis vitalba* and *Alnu*s spp. (alder) are "natural hosts" for the FD phytoplasma. There is also disagreement as to whether or not *Dictyophara europaea* and *Oncopsis alni* are natural vectors of the FD phytoplasma. Phytoplasmas genetically very similar to the FD phytoplasmas (group 16SrV-C) have been found in both hosts and can rarely be transmitted to grapevine (Malembic-Maher/ Foissac personal communication; Maixner personal communication). Filippin et al. (2009) showed that an FD-related phytoplasma, referred to in the paper as FD, can be occasionally transmitted from infected *Clematis vitalba* plants to grapevines by the planthopper *Dictyophara europaea*, commonly known as the European lantern fly (exotic to the United States). FD/FD-related genotypes were also detected in alders (Malembic-Maher et al., 2007; Ember et al., 2011; Mehle et al., 2011), and it was shown that the leafhopper *Oncopsis alni* is able to occasionally transmit alder yellows

phytoplasmas (16SrV-C) to grapevine (Maixner et al., 2000). The FD phytoplasma by definition can be transmitted by *S. titanus*. The genetically similar phytoplasmas to FD in alder and clematis have not been demonstrated to be transmitted by *S. titanus*. Thus, these phytoplasmas will be referred to as FD-related in this document.

Symptoms/Signs

There has not been a demonstrated correlation between different symptoms and early or late infection of the plant. Some plants can express symptoms early in the season; while others express symptoms later. The cultivar, the climate, and the age of the plant are factors that can induce differences in symptoms expression. The FD phytoplasma occurs in vine tissue with an irregular distribution and at a low titre (Boudon-Padieu, 2002), which can make detection of the disease more difficult. Infection of plants by *S. titanus* typically occurs during mid-to-late summer, but the disease symptoms are usually not expressed until the following year (Boudon-Padieu, 2002).

In general, symptoms resemble those of other grapevine yellows diseases ("leaf rolling, discoloration of lamina and veins, partial or total lack of reserve accumulation (lignification) with flexuous canes, and decline of a part or the entire vine stock") (Boudon-Padieu, 2002).

Shoots of susceptible cultivars fail to ripen and are thin, rubbery, and hang down. The infected shoots become brittle and many small, black pustules develop along their length; buds may become necrotic. In more resistant cultivars, the nodes of infected shoots ripen but some of the internodes do not (Defra, 2000). During the winter, infected shoots become black. These shoots may die or survive to produce some spring growth depending upon the plant cultivar, plant age, and climatic conditions (Defra, 2000).

Red-fruited cultivars develop reddish discolorations of the leaves and leaf veins (Fig 1a-b). White-fruited cultivars develop a metallic-yellow discoloration on areas of leaf exposed to the sun (Fig 1c-d). Small, discrete, yellowish spots appear along the main veins. These spots enlarge and merge to form yellow bands which gradually spread over the leaf to produce discolored, angular areas delimited by leaf veins. The centers of the discolored areas become dry and brittle. Infected leaves often roll downward but remain on the plant longer than healthy leaves, because they are less affected by autumn frosts. They can, however, be more easily detached by the wind. Blossoms dry out and may fall from vine. Grape bunches become brown and shriveled (Fig 1e); the stalks dry out and the grapes are easily dislodged (Defra, 2000).

In *Clematis vitalba*, symptoms of FD-related phytoplasmas may include discoloration, rolling and wilting of leaves (Fig. 2), although the relationship between the presence of phytoplasma and leaf symptoms is not clear. PCR detection showed positive results only in DNA extracts for clematis plants sampled in autumn months and never in samples collected in July and August. This could reflect a low concentration or, indeed, absence of the pathogen early in the season (Angelini et al., 2004). A study by Filippin.

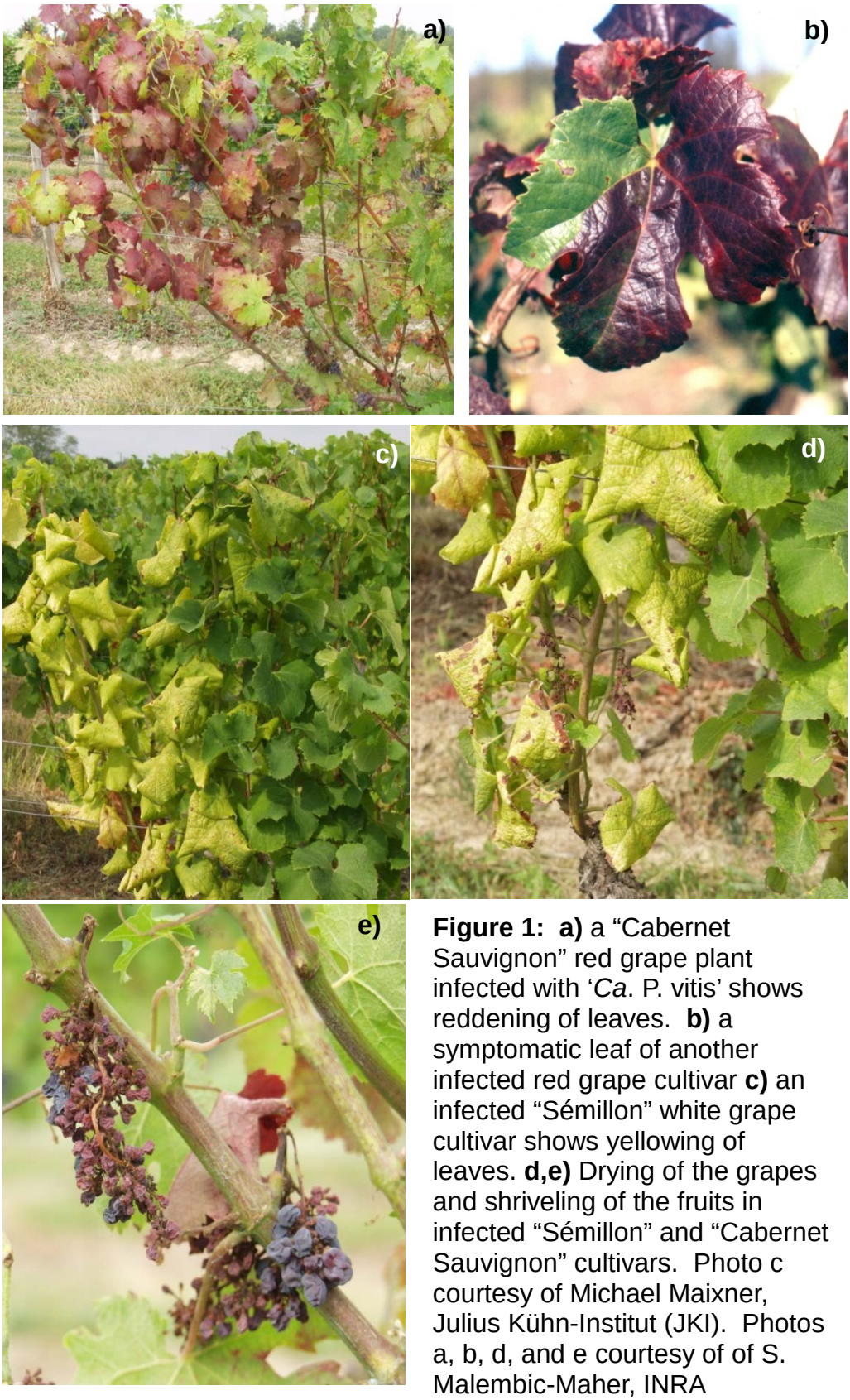


Figure 1: a) a “Cabernet Sauvignon” red grape plant infected with ‘*Ca*. P. vitis’ shows reddening of leaves. **b)** a symptomatic leaf of another infected red grape cultivar **c)** an infected “Sémillon” white grape cultivar shows yellowing of leaves. **d,e)** Drying of the grapes and shriveling of the fruits in infected “Sémillon” and “Cabernet Sauvignon” cultivars. Photo c courtesy of Michael Maixner, Julius Kühn-Institut (JKI). Photos a, b, d, and e courtesy of of S. Malembic-Maher, INRA

et al. (2009) showed that a FD-related phytoplasma is widespread in *C. vitalba*, with approximately 36% of plants surveyed throughout Europe testing positive.

Approximately 60 to 80% of alder plants (*Alnus* sp.) in Europe are infected with an FD-related phytoplasmas. Alders, however, are rarely symptomatic (Foissac, personal communication) (Fig.3).

One of the difficulties in detecting the disease is that symptoms do not always appear every year and may only be present on one shoot or on a small number of shoots. In addition, *Vitis vinifera* varieties are not equally susceptible to FD and may not present symptoms with equal intensity. Rootstocks which are hybrids of various American *Vitis* sp. present very discreet symptoms or no symptoms at all. They nevertheless are carriers of the disease (Caudwell et al., 1994; INRA, 2013).

Figure 2: Infected symptomatic *Clematis vitalba* by FD-related phytoplasma in Hungary. Photo courtesy of S. Malembic-Maher, INRA Bordeaux.

Pest Importance

FD has greatly negative economic impacts among grapevine yellows causing phytoplasmas (Hren et al., 2007). FD is recognized by the European and Mediterranean Plant Protection Organization (EPPO) as a quarantine pest (EPPO, 2012). FD causes symptoms that are detrimental to infected grapevines. Depending on the intensity of infection the yields may decrease dramatically. Their vitality is affected, yields are reduced, and the quality of wine is decreased by high acid and low sugar contents of infected fruiting clusters. When no control of the vector has been undertaken, the number of infected vines may increase steadily about 10 times every year and may reach 80 to 100% within a few years. The economic viability of maintaining a vineyard ceases when the productive plants are less than 25% of the total plants in a vineyard (CABI, 2013a).

Wine production in 2006 represented 5% of the value of total European Union (EU) agricultural output. France, Spain, Italy, and Portugal, all countries known to have FD, are home to roughly 92% of all vineyard acres in the EU (Pappalardo et al., 2012).

FD is viewed as a threat to the grape industry in the United States if it were to become established. One study estimates the total 2004 value of wine, grapes, and grape

Figure 3: Infected (FD-related phytoplasma) but non-symptomatic alders next to a vineyard in Germany. Photo courtesy of S. Malembic-Maher, INRA Bordeaux.

products to the U.S. economy to be $90 billion (MKF, 2006).

FD is listed as a harmful organism by the following 16 countries: Brazil, Chile, China, Costa Rica, Israel, Japan, Mexico, Morocco, New Caledonia, Japan, Russia, Thailand, Timor-Leste, Turkey, Ukraine, and Uruguay (PCIT, queried July 18, 2013). If the pest were found in the United States potential trade impacts with these countries could result.

Known Hosts

Major host: *Vitis* spp. (EPPO, 2012).

All *Vitis vinifera* varieties are sensitive to FD but may not express symptoms of the disease to the same degree (Boudon-Padieu, 2002). *Vitis* rootstocks are also susceptible to infection.

Other possible hosts: *Alnus glutinosa, Alnus incana,* and *Clematis vitalba* (Arnaud et al. 2007, Filippin et al., 2009; Mehle et al., 2011).

Recent evidence points to these hosts as possible alternate hosts of FD phytoplasma strains (Filippin et al., 2009; Mehle et al., 2011).

Experimental hosts: *Chrysanthemum carinatum, Trifolium repens,* and *Vicia faba* (EPPO, 2012).

Known Vectors (or associated insects)

Primary vector: *Scaphoideus titanus* (CABI, 2013a,b).

Other Potential Vectors: *Dictyophara europaea, Orientus ishidae,* and *Oncopis alni* (Filippin et al., 2009; Mehle et al., 2011). *Dictyophara europaea* is indicated by some authors to be a natural vector (Filippin et al., 2009). Others consider that all three of these potential vectors can harbor FD-related phytoplasmas but that they are not confirmed vectors of FD (Malembic-Maher/Foissac personal communication; Maixner personal communication).

Experimental vectors: *Anoplotettix fuscovenosus, Euscelidius variegatus, Euscelis incisus* (all leafhoppers from the family Cicadellidae) (Bressan et al., 2006).

Known Distribution

Europe: Austria, Croatia, France (including Corsica), Hungary, Italy, Macedonia, Portugal, Serbia, Slovenia, Spain (including the Balearic Islands), and Switzerland (CABI, 2013a; EPPO, 2013).

Recent studies have shown that 60 to 80% of alders (*Alnus* sp.) in Europe are infected with FD-related phytoplasma, and they are rarely symptomatic. Therefore, the range of this phytoplasma and related phytoplasmas in Europe is probably wider than currently reported (Foissac, personal communication).

A sample of *Scaphoideus titanus* collected in New York, United States was reported to carry a phytoplasma related to 'Ca. P. vitis' (Maixner et al., 1993), but *'Ca. P. vitis'* has not been confirmed there. Surveys conducted in Virginia and in different provinces of Canada led to the detection of 16SrI and 16SrIII phytoplasmas in grapevine but no 16SrV types (Beanland et al., 2006; Olivier et al., 2009).

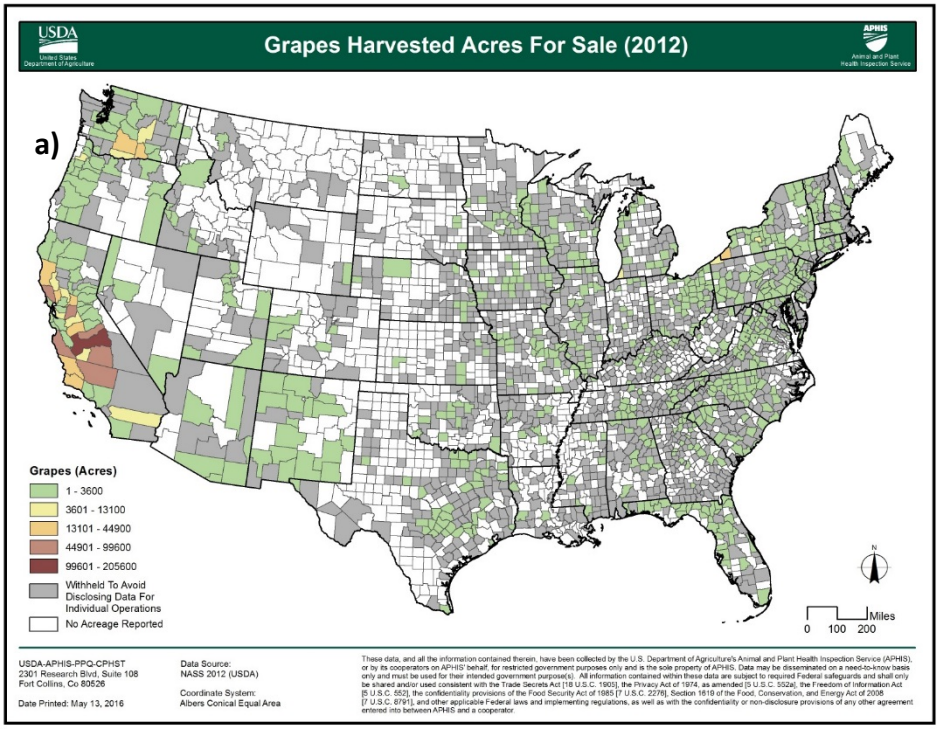
Pathway

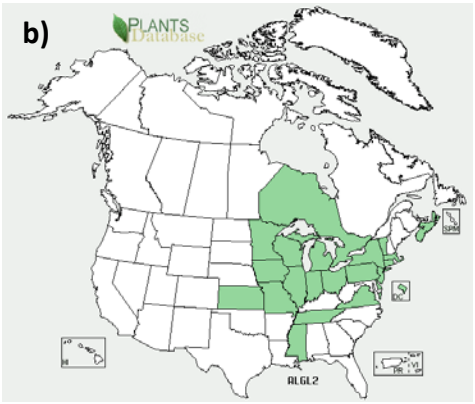
There have been 82 shipments of *Vitis* sp. propagative material from known host countries since 2003 (AQAS, queried July 12, 2013). Shipment sizes ranged from 1 gram to 1797 plant units. These shipments are likely comprised of a mixture of seed, plants, and cuttings based on the units of measure used. *Vitis* sp. (except seeds), however, are currently prohibited for import into the United States except from Canada (USDA, 2012). '*Ca.* P. vitis', like other phytoplasmas, is not known to be transported through seed. Thus, this pathway appears to be unlikely. In contrast, *Vitis* sp. plant material destined for propagation has been intercepted from known host countries 33 times in the past ten years, indicating that people are bringing plant material into the United States from areas known to have FD (AQAS, 2013).

Dormant perennials of *Clematis* sp. are allowed entrance into the United States (USDA, 2012). There have been 57 shipments of *Clematis* sp. propagative material from known host countries since 2003. Shipment sizes ranged from 1 gram to 121,481 plant units. This appears to be a potential open pathway for FD-related phytoplasmas.

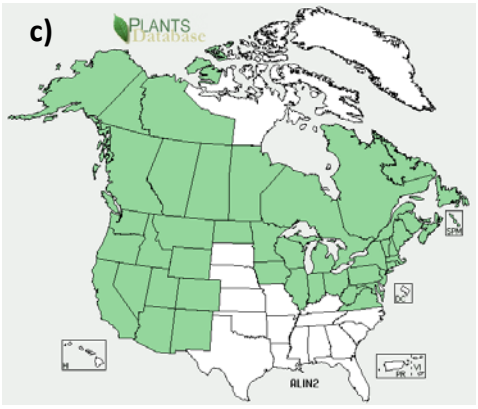
There have been shipments of *Alnus* sp. propagative material from FD containing countries (Hungary (17) and Italy (11since 2003). These were likely shipments of seed. Shipment sizes ranged from 2 kilograms to 139 kilograms with one indicating 25 plant units. There have been no reported interceptions of *Alnus* sp. propagative material since 2003 from known host countries (AQAS, queried July 17, 2013). Effective July 6, 2009, the entry of *Alnus* sp. plants for planting (except seed) was prohibited to prevent the introduction and dissemination of *Phytophthora alni* into the United States (USDA, 2012).

The potential vector *D. europaea* has been intercepted 7 times from Italy and Turkey on imported tile since January, 2003 (AQAS, 2013). *Oncopis alni* is currently listed in the AQAS database and no interceptions have been reported to date. These potential vectors are not known to be present in the United States. *Orientus ishidae* (Japanese leaf hopper) is listed in AQAS as non-reportable, because it occurs in the United States (as of 1955 in New Jersey, New York, Maryland, Pennsylvania, New Hampshire, Washington D.C., Ohio, and Connecticut). This insect and potential vector could be allowed entry and contain FD-related phytoplasmas . There are also 26 native *Oncopis* spp. in the United States (McKamey personal communication). It is possible that one of these natives could vector FD or FD-related phytoplasmas if the pathogen were introduced.

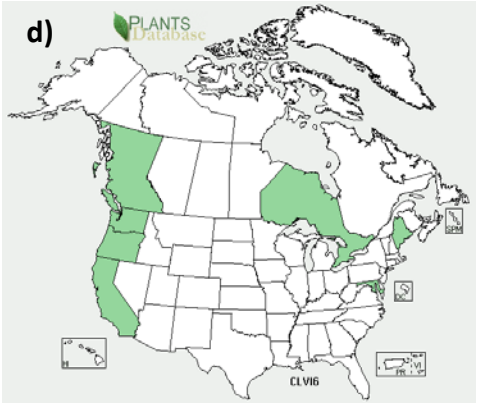




Alnus glutinosa



Alnus incana



Clematis vitalba

Present

Absent

Figure 4: Distribution of known and potential hosts of '*Ca. P.* vitis', **a)** *Vitis* sp. (values from low to high indicate host acreage by county), **b)** *Alnus glutinosa,* **c)** *Alnus incana,* and **d)** *Clematis vitalba* distribution in the United States. (**b-d** courtesy of USDA, http://www.plants.usda.gov. **a** courtesy USDA-APHIS-PPQ-CPHST.

Potential Distribution within the United States

The potential for spreading of '*Ca*. P. vitis' if established in the United States is considerable. There is a wide range of known hosts (Fig. 4). In addition, the confirmed vector *S. titanus* is native to North America and present in at least 35 states and five Canadian Provinces (CABI, 2013a). While the potential vector *Dictyophara europaea* is not known to be present in North America, it has been intercepted in shipments of ceramic and marble tile from Italy, a country known to have *'Ca. P.* vitis*'* (AQAS, 2013).

Survey

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

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

Key Diagnostics

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:

Serological: According to EPPO (2007): ELISA with polyclonal and monoclonal antibodies has been used for detection of flavescence dorée in the vector and grapevine (Boudon-Padieu et al., 1989; Caudwell and Kuszala, 1992; Osler et al., 1992; Irimia et al., 2010). According to EPPO (2007), this method relies on "availability of antibodies which are not sold commercially and has been replaced in practice by PCR, which is versatile, specific and sensitive." Irimia et al. (2010), however, state that the antibodies are available commercially. Neogen Europe and Sediag indicate that

they have ELISA materials available commercially. This ELISA procedure has not been tested for sensitivity, specificity, or for regulatory use.

Molecular: Potentially infected samples can be analyzed for the presence of FD with a real-time PCR procedure as described in Hren et al. (2007), Angelini et al. (2007), or Pelletier et al. (2009). The Pelletier et al. (2009) detection method is the French official method validated by their Plant Protection Services. It is also now used in Croatia, Hungary, and Spain.

Further molecular characterization may then be performed on the positive samples by PCR followed by RFLP or sequencing of the *secY* gene or other non- ribosomal markers as described in Angelini et al. (2001, 2003) and Arnaud et al. (2007). Recently, Kogovšek et al. (2014) describe the use of isothermic real-time loop-mediated isothermal amplification (LAMP) technology for detection of FD.

Easily Confused Species

Flavescence dorée resembles Australian grapevine yellows (AGY, '*Candidatus* Phytoplasma australiense'), bois noir, leaf curl, berry shrivel and other grapevine diseases (North American grapevine yellows, 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). There is no way to visually distinguish FD from bois noir ('*Candidatus* Phytoplasma solani') and other grapevine yellows diseases (Foissac, personal communication). Molecular methods are used to distinguish various grapevine yellows diseases.

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