'Candidatus Phytoplasma solani' (Quaglino et al., 2013)

Synonyms
Phytoplasma solani

Common Name(s)
Disease: Bois noir, blackwood disease of grapevine, maize redness, stolbur

Phytoplasma: CaPsol, maize redness phytoplasma, potato stolbur phytoplasma, stolbur phytoplasma, tomato stolbur phytoplasma

Type of Pest
Phytoplasma

Figure 1: A 'dornfelder' grape cultivar infected with '*Candidatus* Phytoplasma solani'. Courtesy of Dr. Michael Maixner, Julius Kühn-Institut (JKI).

Taxonomic Position
Class: Mollicutes, **Order:** Acholeplasmatales, **Family:** Acholeplasmataceae
Genus: '*Candidatus* Phytoplasma'

Reason for Inclusion in Manual
OPIS A pest list, CAPS community suggestion, known host range and distribution have both expanded; 2016 AHP listing.

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). 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).

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). Phytoplasmas classified in group 16SrXII infect a wide range of plants and are transmitted by polyphagous planthoppers of the family Cixiidae. Based on 16S rRNA gene sequence identity and biological properties, group 16SrXII encompasses several species, including '*Candidatus* Phytoplasma australiense', '*Candidatus* Phytoplasma japonicum' and '*Candidatus* Phytoplasma fragariae'. Other group 16SrXII phytoplasma strains are associated with stolbur disease in wild and cultivated herbaceous and woody plants and with bois noir disease in grapevines (*Vitis vinifera* L.). Such latter strains have been proposed to represent a separate species, '*Candidatus* Phytoplasma solani'.

Pest Description

'*Candidatus* Phytoplasma solani' (herein abbreviated '*Ca*. P. solani') is classified in group 16SrXII, subgroup A (16SrXII-A), and is most closely related to '*Candidatus* Phytoplasma australiense', which shares 97.6% nucleotide sequence identity of 16S rDNA. Multiple 16S rRNA gene sequence alignments identified unique signature sequences in '*Ca*. P. solani' which differ from previously named phytoplasma species. For example, the sequence at nucleotide positions 189–221 differed by at least one base from comparable regions in 16S rRNA genes of all previously described species, and differed at three base positions from the comparable region in the 16S rRNA gene of '*Ca*. Phytoplasma australiense'. In addition, the combination of two other sequence regions in the 16S rRNA gene distinguished '*Ca*. P. solani' from all previously named species. These sequence regions are designated as distinguishing sequence blocks (DSBs). DSB1 is located in nucleotide positions 452-480, and DSB2 is found in nucleotide positions 602-627. For additional detail on the criteria for distinguishing '*Ca*. P. solani', see Quaglino et al. (2013).

'*Candidatus* Phytoplasma solani', also known as stolbur phytoplasma, affects a wide range of wild and cultivated plants including grape (Maixner, 2011), corn (Jović et al., 2009), and solanaceous hosts (EPPO, n.d.). Numerous weedy hosts act as pathogen reservoirs. In grape hosts, there are two major stolbur phytoplasma genetic clusters (genotypes) involved in the two distinct epidemiological cycles of bois noir (BN) disease, namely, tuf-a and tuf-b, which is in accordance with variants of the gene encoding the

Figure 2: a) *Urtica dioica* (stinging nettle), the common reservoir host of the tuf-a genotype of '*Ca.* P. solani', and b) *Convolvulus arvensis* (bindweed), the common reservoir host of the tuf-b genotype. Photo credits: (a) Robert Vidéki, Doronicum Kft., Bugwood.org, (b) Steve Dewey, Utah State University, Bugwood.org

elongation factor Tu (Langer & Maixner, 2004). The tuf-a and tuf-b2 genotypes are associated with *Urtica dioica* (stinging nettle) (Fig. 2), which acts as its primary host plant and pathogen reservoir, and it is the more common genotype in northwestern disease occurrences (Germany, France, Switzerland, Austria, Italy), where it is an epidemic. The tuf-b genotype is predominantly present in the southeastern and eastern range of BN disease, and *Convolvulus arvensis* (field bindweed) (Fig. 2) is generally recognized as its reservoir, although it infects a number of diverse wild host plants (Cvrković et al., 2014).

Biology and Ecology

The spread of BN in grape occurs via a disease cycle including herbaceous host plants as phytoplasma reservoirs and insect vectors. *Urtica dioica* (stinging nettle) and *Convolvulus arvensis* (bindweed) (Fig.2) are considered to be the main phytoplasma sources with the disease transmitted by *Fulguromorpha* insect species of the family Cixidae. *Hyalesthes obsoletus* (Fig. 3) is regarded as the main vector in many countries, and *Reptalus panzeri* had been proved as an additional important vector in Balkans (Maixner, 2001; Johannesen et al., 2012; Cvrković et al., 2014). The juvenile overwintering stages of *H. obsoletus* live in the soil, feeding on the roots of host plants from which they acquire '*Ca.* P. solani' (Maixner, 2011). The epidemiology of BN is coupled to the infection of these two herbaceous host plants (*U. dioica* and *C. arvensis*) and not to grapevine, since grapevine is not a substrate for

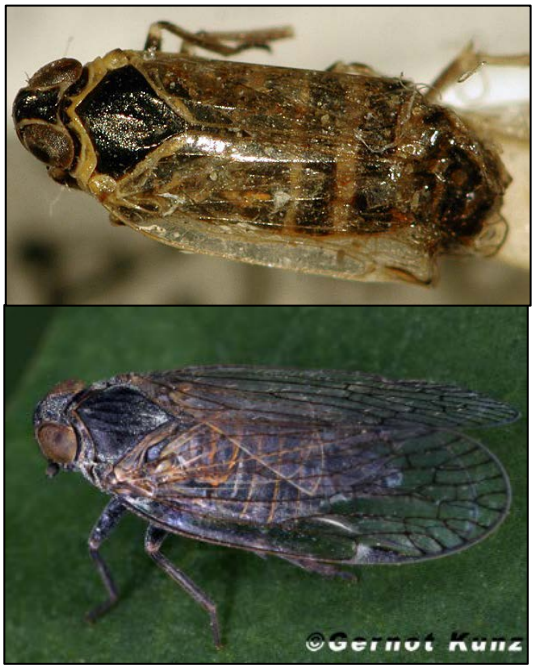

Figure 3: Top. *Hyalesthes obsoletus* (Michael F. Schönitzer, Creative Commons). Bottom. *Reptalus panzeri* (Courtesy of Gernot Kunz, Karl-Franzens University of Graz, Austria).

Figure 4: Symptoms of corn redness disease in infected maize. Photos courtesy of Dr. Jelena Jović, Institute of Plant Protection and Environment, Zemun (RS) eppo.int.

nymphal *H. obsoletus* and, consequently, it is a dead-end host for the phytoplasma (Johannesen et al., 2012). While the distribution of BN is closely associated with the presence of reservoir plant hosts, it is not closely associated with the presence of insect vectors (Maixner, 2011).

'*Candidatus* Phytoplasma solani' is also spread by the planthopper *Reptalus panzeri* (Fig. 2). *R. panzeri* is known to transmit the phytoplasma to both grape and corn (Jović et al., 2009; Cvrković et al., 2014). In the corn disease cycle, *R. panzeri* nymphs lay eggs on infected maize roots and nymphs living on these roots acquire the phytoplasma from infected maize. The nymphs overwinter on the roots of wheat planted into maize fields in the autumn, allowing emergence of phytoplasma-infected vectors the following July (Jović et al., 2009). The practice of corn-wheat crop rotation, which is common in Serbia, may be exacerbating maize redness (MR) disease (Fig. 4) problems by fostering high *R. panzeri* levels (Jović et al., 2009).

In solanaceous hosts, disease outbreaks seem to occur in cycles, being favored by hot dry summers, which stimulate vector migration. The most severe attacks appear to occur when climatic conditions force the vectors to transfer from infected wild plant host populations to cultivated solanaceous hosts which, otherwise, they tend not to favor. Furthermore, it is not clear whether vectors spread stolbur within economic host crops to any extent. In fact, in nature, the economically important host crops are not important for the survival and spread of the phytoplasma; an incomparably greater role is played by such wild plants as *Convolvulus arvensis* (bindweed), clovers, and, probably, Asteraceae and other plants (EPPO, n.d.).

In experiments, the incubation (latent) period of '*Ca*. P. solani' in the vector was about 1 to 2 months and 1 month, respectively, for *Aphrodes bicinctus* and *Euscelis plebeja*; while for *H. obsoletus*, an incubation period of 2 to 7 days has been reported (EPPO, n.d.).

Symptoms/Signs

In corn: symptoms include midrib, leaf, and stalk reddening, followed by desiccation of the entire plant, abnormal ear development, and incomplete kernel set (Fig. 4). Maize redness (MR) may cause significant economic losses. Environmental factors play a role in both the intensity and incidence of MR, with more severe disease being associated with early-planted fields and hot, dry summers (Jović et al., 2009; Kovacević et al., 2014).

In grape: Typical symptoms comprise discoloration of leaves including the veins, often associated with downcurling of the leaf blade, lack of or incomplete lignification of shoots that later turn black, abortion of fruit clusters or shriveling of the ripening fruit (Fig.5). In most cultivars, the symptoms of bois noir remain restricted to parts of the infected vines for several years. Bois noir usually does not kill the infected vines, although their vigor can be significantly reduced. Remission of symptoms and even complete recovery of infected vines are common phenomena (Maixner, 2011). In red grape cultivars, leaf reddening occurs. In white cultivars, yellow, necrotic veins occur. Shriveled grape clusters occur in both red and white grape cultivars.

In the some BN-affected vineyards, bindweed (*Convolvulus arvensis* L.) plants showing stunting and leaf chromatic alteration have been found (Salem et al., 2013).

In potato: Symptoms in potato include reddening and upward rolling of leaflets, reduced size of leaves, shortened internodes, and aerial tuber formation (Holeva et al., 2014). Plants grown from infected tubers give rise to normal or spindly sprouts (hair-sprouting). Where normal sprouts arise, symptoms are first apparent about 60 to 80 days after sowing, as a yellowing and rolling of the leaves. This is followed by production of aerial stolons and tubers in different parts of the stems close to the axils (Fig. 6) (EPPO, n.d.).

In tomato: Leaves that develop before infection become greenish-yellow, especially at the margins, which may roll upward (Fig. 6). Newly formed leaves become more yellow and are smaller. Stems become thin at the apex as growth ceases, but stems enlarge at infection sites as a result of abnormal phloem formation. This abnormal phloem appears as a greenish, water-soaked band 1 to 2mm wide, which extends towards the xylem. Lateral shoots develop, giving the plant a bushy aspect. Flower buds assume an abnormally erect position; the sepals, whose veins develop a violet color, remain completely joined and the calyx is enlarged and cyst-like ("big bud").

Flowers, if already formed when infection occurs, become similarly erect and may be sterile, and petals are greenish instead of yellow. Flower distortion is common, and petals of young flowers become totally dwarfed and green (virescent). Peduncles are thicker than normal. Fruit development is arrested following infection. Green fruits already formed become solid, dry and ripen very slowly. Necrosis occurs at the embryonic center in younger fruits. Pedicels of fruits are thicker than in healthy plants, in spite of the relatively small fruit size (EPPO, n.d.).

Figure 5: Symptoms of '*Ca.* P. solani' infection in grape include leafroll, leaf blotching, stunted fruit growth, short internodal spacing, and browning of stems. Courtesy of Dr. Michael Maixner, Julius Kühn-Institut (JKI).

Cultivars shown: a) Pinot Noir, b) Kerner, c, d, e) Riesling, f) Dornfelder

Figure 6: Symptoms of '*Ca*. P. solani' infection in potato (a, b), celery (c), tomato (d), and pepper (e). Note the aerial tubers in infected potato (b) Photos a and d courtesy of Ministry of Agriculture (TR), eppo.int. Photo b courtesy of Courtesy of M.T. Cousin, INRA, Versailles, France, eppo.int. Photos c and e courtesy of Xavier Foissac, INRA.

In wheat, strawberry, and stone fruit: Symptoms in wheat infected by '*Ca*. P. solani', including reddening of upper leaves, have been recorded (Jović et al., 2009). The incidence and damage caused by '*Ca*. P. solani' in wheat, however, has not been well documented. In infected strawberry, symptoms include stunted growth, poor root development, purple leaf discoloration, leafroll, sterile flower formation, and small and deformed fruit formation (Terlizzi et al., 2006). '*Ca.* P. solani is also known to infect stone fruit, including apricot and peach (Quaglino et al., 2013), but it is unclear if this disease has a significant impact on these hosts or not. Reported symptoms are similar to those shown by peach yellow leafroll disease (Quaglino et al., 2013).

Pest Importance

'*Candidatus* Phytoplasma solani' is known to infect several hosts which are important agricultural crops in the United States, including grape, corn, potato, and tomato. This phytoplasma is one of the most important diseases of grapevine in Europe (Johannesen

et al., 2008) and is viewed as a threat to the grape industry in the United States if it were to become established.

In 2013, grape was cultivated on 987,120 acres, and 8.6 million tons were harvested (USDA-NASS, 2014). One study estimates the total annual value of wine, grapes, and grape products to the U.S. economy to be $90 billion (MKF, 2006). The same year, approximately 440 million cwt (20 million tons) of potato was produced in the United States (USDA-NASS, 2014). Total U.S. potato and potato product exports reached record levels in fiscal year 2013. The value of these exports is estimated to be $1.6 billion and consists of 17% of U.S. potato production (USPB, 2013). In 2013, tomato was cultivated on 93,600 acres, and the harvest was valued at approximately $1.1 billion (USDA-NASS, 2014). In 2012, strawberry was grown on 30,149 acres (27,000 acres in California alone) and the harvest was valued at $2.4 billion (USDA-NASS, 2014).

Corn is the most widely cultivated feed grain in the United States. In 2013, corn was cultivated on over 95 million acres of U.S. land, an area roughly the size of the state of Montana. The total 2013 U.S. corn harvest was approximately 14 billion bushels, with an estimated value of $61.5 billion. Approximately 20% of the 2012 U.S. corn harvest was destined for export (USDA-ERS, 2013).

'*Candidatus* Phytoplasma solani' (and several of its prior synonyms) is listed as a harmful organism in 36 countries, including nearly every country in Europe (USDA-PCIT, 2015). If the phytoplasma were found in the United States, potential trade impacts with these countries could result.

Known Hosts

There are numerous reported plant hosts of '*Ca.* P. solani'. The literature, however, is often contradictory or does not detail the actual losses due to disease development on these hosts. Therefore, we have listed these hosts as other hosts until additional information becomes available.

Major Hosts:
Vitis vinifera (grape)

Minor Hosts:
Apium graveolense (celery), *Capsicum annuum* (pepper), *Daucus carota* (carrot), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant), *Solanum tuberosum* (potato), and *Zea mays* (corn).

Wild Hosts:
Calendula officinalis (common marigold), *Cichorium intybus* (chicory), *Convolvulus arvensis* (bindweed), *Datura stramonium* (jimson weed), *Solanum glaucophyllum* (waxy leaf nightshade), *Sorghum halepense* (johnsongrass), and *Urtica dioica* (stinging nettle),

Other Hosts:
Beta vulgaris (sugar beet), *Catharanthus roseus* (Madagascar periwinkle), *Fragaria x ananassa* (strawberry), *Helianthus annuus* (sunflower), *Hibiscus cannabinus* (kenaf), *Lavandula angustifolia* (lavender), *Macroptilium lathyroides* (bushbean); *Malus domestica* (apple), *Mespilus germanica* (common medlar), *Oenothera biennis* (evening primrose), *Paeonia suffruticosais* (tree peony), *Paeonia tenuifolia* (fernleaf peony), *Pisum sativum* (pea), *Prunus armeniaca* (apricot); *Prunus avium* (cherry), *Prunus mume* (Japanese flowering plum), *Prunus persica* (peach), *Sambucus nigra* (elder), *Rhododendron* spp. (rhododendron), *Rubus fruticosus* (blackberry), *Salvia multiorrhiza* (red sage), *Sophora alopecuroides* (sophora root), *Tagetes erecta* (Aztec marigold), *Trifolium pretense* (clover), *Triticum aestivum* (wheat), *Vaccinium corymbosum* (blueberry), *Valeriana* spp. (valerian), and *Vicia faba* (faba bean) (Gatineau et al., 2001; Filippin et al., 2008; Quaglino et al., 2010; Maixner, 2011; Admovic et al., 2013; Balakishiyeva et al., 2010; Biswas et al., 2013; Bobev et al., 2013; Gao et al., 2013; Holeva et al., 2014; Lotos et al., 2013; Quaglino et al., 2013; Starovic et al., 2013; Admovic et al., 2014; Aryan et al., 2014; Kovacević et al., 2014; Mori et al., 2014; Pavlovics et al., 2014a; Pavlovics et al., 2014b; Yang et al., 2016).

Experimental Hosts:
Cardaria draba (hoary cress, whitetop), *Prunus* spp., *Prunus domestica* (plum), *Syringa vulgaris* (lilac), *Ficus carica* (fig), and *Ulmus* spp. (elm).

Known Vectors (or associated insects)

'*Candidatus* Phytoplasma solani' is vectored by numerous planthoppers in the Cixiid and Cicadellid families (EPPO, n.d.; Březíková, and Linhartová, 2007; Maixner, 2011; EFSA, 2014) (Table 1). There is a wide range of preferred hosts among these vectors, therefore, transmission between plant hosts varies greatly depending on the vector. The most important vector is *Hyalesthes obsoletus*, which transmits '*Ca*. P. solani' to grape. Another important vector is *Reptalus panzeri*, which is now known to transmit '*Ca*. P. solani' to both grape and corn (Cvrković et al., 2014).

Table 1: A list of insects known to carry '*Ca.* P. solani. Important vectors in bold.

Anaceratagallia ribauti	*Lygus pratensis*
Anoscopus albifrons	*Lygus rugulipennis*
Aphrodes bicinctus	*Macrosteles cristatus*
Dictyophara europaea	*Macrosteles incisus*
Euscelis lineolatus	*Macrosteles laevis*
Euscelis incisus	*Macrosteles quadripunctulatus*
Euscelis plebeja	*Macrosteles viridigriseus*
Exitianus capicola	*Pentastiridius leporinus*
Hyalesthes obsoletus	***Reptalus panzeri***
Hyalesthes phytoplasmakosiewiczi	*Reptalus quinquecostatus*
Lygus gemellatus	*Speudotettix subfusculus*

Known (Reported) Distribution

Africa: Niger (EPPO, 2014). **Asia**: China, India, Iran, Israel, Jordan, Kyrgyzstan, Lebanon, Saudi Arabia, (Choueiri et al., 2002; Biswas et al., 2013; Gao et al., 2013; Quaglino et al., 2013; EPPO, 2014; Salem et al., 2014). **Europe**: Albania, Armenia, Austria, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, France, Georgia, Germany, Greece, Hungary, Italy, Macedonia, Montenegro, Poland, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Turkey, Ukraine (Mitrev et al., 2007; Balakishiyeva et al., 2010; Bobev et al., 2013; Kovacević et al., 2014; Lotos et al., 2013, Quaglino et al., 2013; Starovic et al., 2013; EPPO, 2014; Quaglino et al., 2014). **Caribbean**: Cuba (Quaglino et al., 2013). **South America**: Chile (Gajardo et al., 2009).

In 2006, BN was found in British Columbia, Canada in grapevines, which were imported from Europe the same year (Rott et al., 2007). Every plant from this lot was destroyed, and there have been no additional detections of BN in Canada.

In 2014, '*Ca*. P. solani' was found in symptomatic strawberry plants in Norfolk, United Kingdom (UK) (Hodgetts et al., 2015). The infected plants, which were imported from Spain, were destroyed. Containment and eradication measures are currently in place. The phytoplasma is not considered established in the UK at this time.

Pathway

'*Candidatus* Phytoplasma solani' is most likely to enter the United Stated on infected rootstock or inside an infected insect vector. This phytoplasma is not known to be transmitted by true seed.

The wide host range of '*Ca*. P. solani' increases the number of possible pathways of this phytoplasma into the United States on infected host material. There are many host plants that can be imported into the United States with no or little regulation. Some host plants that can be imported include: *Beta vulgaris, Capsicum annum*, *Lavandula* spp., *Trifolium pratense,* and *Vaccinium corymbosum* (USDA, 2015). *Hibiscus* spp. propagative material from France is also allowed (USDA, 2015), and there have been 17 shipments of it since 2005 (AQAS, 2015).

The import of *Fragaria* spp. (strawberry) plant material is allowed from Israel (USDA, 2015), which is known to have '*Ca.* P. solani'. Since 2005, there have been 12 shipments of *Fragaria* spp. plant material from Israel, containing a total of over 25,000 plant units (AQAS, 2015). The recent find of '*Ca*. P. solani' in the UK on strawberry plants, which were imported from Spain (Hodgetts et al., 2015) demonstrates that the phytoplasma can be transported in this manner.

The possible import of infected insect vectors into the United States is cause for concern. Since 2008, there have been interceptions of *Dictyophara europaea* from Italy (7) and Turkey (1), all on imported tile (AQAS, 2015). Both of these countries are known to have '*Ca*. P. solani'. Since 2001, there have been interceptions of

Macrosteles spp. insects from Israel (7), and Spain (1) on imported cargo (AQAS, 2015). At least five *Macrosteles* spp are known to vector '*Ca*. P. solani'. Since 2005, there have been interceptions of *Lygus* spp. insects from Italy (3), all on imported tile. At least three *Lygus* spp. are known to vector '*Ca*. P. solani'. In 2013, there was an interception of a *Euscelis* spp. adult from Italy on imported tile (AQAS, 2015). At least three *Euscelis* spp. are known to vector '*Ca*. P. solani'.

Potential Distribution within the United States

Both '*Ca.* P. solani' and an insect vector would both have to be introduced into the United States for the phytoplasma to spread here, assuming that no indigenous insect could vector this phytoplasma. Currently, neither *H. obsoletus* nor *R. panzeri* are known to be present in the United States or the western hemisphere. It is possible that some leafhopper(s) native to North America could transmit the phytoplasma if it were to be introduced in the United States. While transmission through infected planting material is possible, it is likely to be contained by phytosanitary measures, if appropriately applied (EPPO, n.d.; Maixner, 2011).

The main reservoir hosts of '*Ca*. P. solani', *Convolvulus arvensis* and *Urtica dioica* are both widespread throughout the United States and are present in every state (BONAP, 2015). Agricultural hosts including corn, grape, tomato, potato, and wheat are all widely grown in the United States (USDA-ERS, 2013; USDA-NASS, 2014). If '*Ca*. P. solani' and/or the pathogen with one of the known exotic insect vectors become established in the United States, the potential for this phytoplasma to spread is significant.

Survey

Approved Method for Pest Surveillance (AMPS)*: The 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

Literature-Based Methods

Visual surveys for symptomatic host material and for insect vectors are both used. Potential insect vectors can be collected using sweep nets and mouth aspirators (Cvrković et al., 2014). Symptomatic host material can be collected in and around vineyards (Cvrković et al., 2014) or other known host crops such as corn and solanaceous plants (Březíková, and Linhartová, 2007; Jović et al., 2009).

Key Diagnostics

Approved Method for Pest Surveillance (AMPS)*:

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:

The routine detection of bois noir and other grapevine yellows diseases depends on DNA based techniques (Maixner, 2011). Nested PCRs targeting the 16S rDNA, carried out using the primer pairs P1/P7 followed by R16F2n/R16R2 (Lee et al., 1998), have been used to detect '*Ca.* P. solani' in grape and other hosts (Quaglino et al., 2014). The Stol11 nested PCR protocol with primers F2/R1 and F3/R2 (Clair et al., 2003) can be used to detect '*Ca.* P. solani' in plant and insect material (Jović et al., 2009; Cvrković et al., 2014). Methods for DNA based identification of this phytoplasma are reviewed and described in numerous additional publications, including: Maixner et al. (2011); Lotos et al. (2013); Aryan et al. (2014); Mori et al. (2014).

Easily Confused Pests

Bois noir (BN) may be confused with other yellowing diseases caused by different phytoplasmas or other pathogens. BN resembles Australian grapevine yellows (AGY, AUSGY, '*Candidatus* Phytoplasma australiense'), Flavescence dorée (FD, '*Candidatus* Phytoplasma vitis'), leaf curl, berry shrivel, and other grapevine diseases (*e.g*., North American grapevine yellows), tomato big bud, and an uncharacterized grapevine disease also believed to be caused by a phytoplasma (Magarey and Wachtel, 1985; Davis et al., 1997, 2015; Lee et al., 1998; Constable et al., 1998; Gibb et al., 1999). There is no way to visually distinguish BN from FD and other grapevine yellows diseases (Clair et al., 2003; Belli et al., 2010; Davis et al., 2015). Molecular methods for identifying the associated phytoplasma are used to distinguish the various grapevine yellows diseases. Symptoms of '*Ca.* P. solani' infection in solanaceous hosts also vary greatly; and may be absent or hardly distinguishable (EPPO, n.d.).

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