'Candidatus Phytoplasma pini' 16SrXXI-A

Scientific Name
'Candidatus Phytoplasma pini' (Schneider et al., 2005)

Synonyms
Phytoplasma pini, *Phytoplasma sp. PinG*, *Phytoplasma sp. PinP*

Common Name(s)
Disease: **Pine witches' broom**, pine shoot proliferation, and *Pinus sylvestris* yellows

Phytoplasma: Pine witches' broom phytoplasma, phytoplasma PinY

Type of Pest
Phytoplasma

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

Reason for Inclusion in Manual
Pests of Economic and Environmental Importance – 2014 through 2016

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, endocelluar 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 generally cannot be 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 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; 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). Each 16S rDNA RFLP group contains at least one phytoplasma species. For example, '*Candidatus* Phytoplasma pini' is classified in group 16SrXXI, subgroup A (16SrXXI-A).

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 pini' (herein abbreviated as '*Ca.* P. pini') is associated with the 16S rRNA gene sequence accession AJ632155, with oligonucleotide sequences complementary to unique regions of the 16S rRNA gene [5'-GGAAATCTTTCGGGATTTTAGT-3' (positions 67- 88) and 5'-TCTCAGTGCTTAACGCTGTTCT-3' (positions 603-624)]. According to the phylogenetic analysis carried out by Schneider et al. (2005), the pine phytoplasma strains form a distinct branch and are only distantly related to other phytoplasmas (Firrao, 2005).

The closest relatives of '*Ca*. Phytoplasma pini', sharing 94.5% or less 16S rRNA gene sequence identity, are the phytoplasmas associated with diseases of rice ('*Ca.* P. oryzae'), coconut, and chestnut ('*Ca.* P. castaneae'), included in the 16SrIV or 16SrXI groups by Lee et al. (1998, 2000). The restriction map of the 16S rDNA gene deduced from the sequence suggests that specific RFLP patterns should result from the digestion, with the enzymes commonly used for the characterization of phytoplasmas (*Alu*I, *Rsa*I, *Mse*I, *Hha*I and *Hinf*I), of an amplification product obtained using general phytoplasma-specific primers. The reference strain is Pin127SR from *Pinus halepensis* in Spain (Firrao, 2005).

A phytoplasma strain (MDPP) closely related to the '*Candidatus* Phytoplasma pini' reference strain from Europe was recently found associated with witches'-broom of pine in the United States (Maryland). Strain MDPP shares 98.4% 16S rRNA gene sequence similarity with previously described '*Candidatus* Phytoplasma' species. This phytoplasma is currently referred to as a '*Candidatus* Phytoplasma pini' – related strain, and it represents the only member of a new subgroup, 16SrXXI-B (Costanzo et al., 2016).

Biology and Ecology

The biology of '*Ca.* P. pini' is currently not well understood. Like other phytoplasmas, it is an obligate intercellular parasite that occurs in the phloem sieve tubes of infected plants and likely the salivary glands of insect vectors. The insect vector has not yet been identified (CABI, 2011). 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 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 lifespans (Weintraub and Beanland, 2006; Gitau et al., 2009).

Environmental and host factors that influence susceptibility to '*Ca.* P pini' infection in conifers are currently unknown. There is variation in the colonization of the trees and in the concentration (titre) of the phytoplasma within the trees. A study by Kaminska et al. (2011) suggests that detectability of the phytoplasma fluctuates depending on the year or season of testing, even within the same tree. In addition, witches' broom symptomatic trees do not always test positive for the pathogen (Sliwa et al., 2008; Kaminska et al., 2011), suggesting that there are other causes of these growth abnormalities. Some of these results indicate that trees may have the ability to recover from or resist '*Ca.* P pini' infection depending on environmental conditions.

'Ca. P pini' infected evergreen trees can be propagated by grafting (Sliwa et al., 2008). Other than that, there are currently no other verified mechanisms, except transmission by insects, for the spreading of this pathogen.

Symptoms/Signs

Pine: Yellowing, dwarfing/stunting, twisted needles ("form dense ball-like structures"), and prolific branching / proliferation of small shoots/twigs (i.e., witches' broom, **Fig. 1**), and little leaves are observed (Schneider et al., 2005).

Spruce: Shoot and needle malformation and stunted growth are the main symptoms observed (Kaminska and Berniak, (2011) (**Fig. 2**).

Figure 1: Witches' broom symptoms typical of '*Ca.* P pini' infection in a *Pinus halepensis* tree in Cadiz, Spain. Photo courtesy of Juan Bibiloni. http://mundani-garden.blogspot.com.es/2011/07/candidatus-phytoplasma-pini-it-makes.html.

Fir and Hemlock: Witches' brooming and needle discoloration are the primary symptoms associated with the disease (Kaminska et al., 2011).

Pond Cypress: Necrotic little leaves, twig, and overall plant necrosis (tissue death) have been seen in China (Huang et al., 2011).

Pest Importance

The increasing number of reported hosts and expanding known host range of '*Ca.* P. pini' are cause for concern. The timber industry and the scenic beauty of forestland in each of the known host countries could be affected by this pathogen. For example, China exported a total of 26,777,117m^3 of forest products in 2003 (Sun et al., 2005). *Taxodium distichum* var. *imbricarium* is frequently used for wetland and riparian zone restoration in China (Huang et al., al., 2011). In Lithuania, timber accounts for 2% of all industrial production and engages 13% of the workforce (Valiunas et al., 2010). Germany is home to the largest timber reserves in Europe. At 25 million cubic meters, Germany's sawmill industry is the largest producer in the European economic area. Approximately 30 percent of German sawn timber production is exported worldwide. Based on the presence of 150,000 companies, annual sales of approximately €170 billion ($221 billion), and almost 1.2 million employees, the German timber and forestry industry cluster is a key global player (Wood Germany, 2013). Croatia has forests covering 43% of its landmass, and total exports of wood products were valued at over $336 million in 2004 (Motik, 2006).

Figure 2: Shoot and needle malformation in infected *Picea pugensis* (left two branches) compared to a healthy branch (right).

Known Hosts

Major hosts: *Pinus halepensis* (Aleppo pine), and *P. sylvestris* (Scot's pine) (Schneider et al., 2005).

Other hosts: *Abies procera* (noble fir), *Picea pungens* (Colorado blue spruce), *Pinus banksiana* (Jack pine), *P. mugo* (mountain or mugo pine), *P. nigra* (European black pine), *P. tabuliformis* (Chinese pine), *Taxodium distichum* var. *imbricarium* (pond cypress), and *Tsuga canadensis* (Eastern or Canadian hemlock) (Sliwa et al., 2008; Huang et al., 2011; Kaminska and Berniak, 2011; Kaminska et al., 2011).

Known Vectors (or associated insects)

At this time, no vectors have been confirmed for '*Ca*. P. pini'.

Known Distribution

Europe: Croatia, Czech Republic, Germany, Lithuania, Poland, and Spain (Schneider et al., 2005; Sliwa et al., 2008; Valiunas et al., 2010; Kaminska et al., 2011; Jezic et al., 2012).

Asia: China (Huang et al., 2011).

Although a '*Ca.* P. pini-related strain' was found in Maryland, United States, '*Ca*. P. pini' (16SrXXI-A) is not known to occur in the United States (Costanzo et al., 2016).

Pathway

There is currently no known vector for '*Ca.* P. pini'. However, phytoplasmas can also spread from infected propagative plant material. There are multiple reports of *Abies sp.*, *Picea sp*., *Pinus* sp., *Taxodium distichum*/*Taxodium* sp., and *Tsuga* sp., propagative material being imported from countries known to have '*Ca.* P. pini'. This includes Germany (25 shipments), China (180 shipments), Czech Republic (3 shipments), Poland (1 shipment), and Spain (3 shipments) (AQAS, 2013). Based on the units used (g, kg, flask, and plant unit), it is likely that these shipments are composed of seed, cuttings, and/or plants based on the quantity. Phytoplasmas, however, are not known to be seed transmitted.

There have also been numerous shipments of lumber and logs of host species from countries known to have this phytoplasma. All wood products are subject to 7CFR319.40-5 (Logs, lumber, and other unmanufactured wood articles - importation and entry requirements for specified articles), which requires either heat or methyl bromide treatments. Heat and methyl bromide have been shown to have efficacy against phytoplasmas.

There are symptomless hosts, which make identification of this disease at the port of entry unlikely. There have also been many interceptions of plants for propagation from countries known to have '*Ca.* P. pini' including Germany (13), China (30), Czech Republic (3), and Poland (5) that could harbor the pathogen (AQAS, 2013).

Potential Distribution within the United States

The United States is home to numerous known '*Ca*. P pini' host genera and species. For example, *Pinus sylvestris* is present in the northeastern United States, and *P. halepensis* is present in parts of California and Arizona (BONAP, 2014). In addition, *Abies procera* is present in the Pacific Northwest, *Picea pungens* is widespread in the Rocky Mountains, and *Tsuga canadensis* is widespread in the eastern United States (BONAP, 2014). While there are several host species present in the United States, the potential of this phytoplasma to spread is very difficult to predict without knowledge of the vectors and their distribution. The impact of this phytoplasma on North American pine species is currently unknown.

Survey

CAPS-Approved Method*: 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

CAPS-Approved Method*:

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: PCR and RFLP analysis has been developed to detect '*Ca.* P. pini' from plant material (Sliwa et al., 2008).

Easily Confused Species

At least two other phytoplasmas ('*Candidatus* Phytoplasma asteris' and the X-disease phytoplasma group) are known to infect *Picea* spp. with witches' broom symptoms (Kaminska and Berniak, 2011).

A phytoplasma strain (MDPP) closely related to the '*Candidatus* Phytoplasma pini' reference strain from Europe was recently found associated with witches'-broom of pine in the United States (Maryland). Strain MDPP shares 98.4% 16S rRNA gene sequence similarity with previously described '*Candidatus* Phytoplasma' species. This phytoplasma is currently referred to as a '*Candidatus* Phytoplasma pini' – related strain, and it represents the only member of a new subgroup, 16SrXXI-B (Costanzo et al., 2016).

Kaminska et al. (2011) and Sliwa et al. (2008) tested several pines with typical visual symptoms of '*Ca.* P. pini' infection that ended up having no detectable presence of phytoplasma. This research suggests that there may be non-phytoplasma causes of ball-like structures and witches' broom symptoms in pine trees. Additionally, uneven colonization and concentration of phytoplasma within a given plant can make phytoplasma detection and identification more difficult.

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Update History

June 2015: Conducted literature review of all sections for new information since last datasheet update.

June 2016: Replaced link in survey and identification section with the FY2017 Phytoplasma sample submission document. Updated pest description, distribution, and

easily confused species sections to include the detection of a related phytoplasma strain in Maryland.