‘*Candidatus* Phytoplasma mali’

Scientific Name
‘*Candidatus* Phytoplasma mali’ Seemüller & Schneider, 2004

Figure 1. Witches’ broom on apple due to ‘*Ca*. P. mali’. Image courtesy of Biologische Bundesanstalt für Land-und Forstwirtschaft, Institut für Pflanzenschutz im Obstbau Archive, www.bugwood.org.

Synonyms:
None

Common Names
Apple proliferation (AP), AP Phytoplasma, AP-MLO, apple witches’ broom

Type of Pest
Phytoplasma

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

Pest Recognition
This section describes characteristics of the organism and symptoms that will help surveyors recognize possible infestations/infections in the field, select survey sites, and collect symptomatic material. For morphological descriptions, see the Identification/Diagnostic resources on the AMPS pest page on the CAPS Resource and Collaboration website.

Apple proliferation is a severe disease associated with the presence of ‘*Candidatus* Phytoplasma mali’, a phytoplasma in the apple proliferation group (group 16SrX) (Seemüller and Schneider, 2004). Phytoplasmas of this group have occasionally been detected in stone fruit trees as well (Cieślińska and Morgaś, 2011; Lee et al., 1995; Mehle et al., 2007; Navratil et al., 2001). Molecular analysis has shown heterogeneity within the phytoplasma, and numerous subtypes of ‘*Ca.* P. mali’ have been reported (Casati et al., 2010; Jarausch et al., 1994; Jarausch et al., 2000; Martini et al., 2005; Martini et al., 2008).

Symptoms
Apple proliferation infections often occur in clusters of trees, and these clusters expand year by year (Bliefernicht and Krczal, 1994). Symptoms are unevenly distributed on the host plants. Additionally, there is considerable variability in virulence in ‘*Ca.* P. mali’ (Seemüller et al., 2010), ranging from avirulent to highly virulent. In addition, trees can be simultaneously affected by more than one strain of ‘*Ca.* P. mali’ (Seemüller and Schneider, 2007).

Apple: In general, apple trees affected by ‘*Ca*. P. mali’ lack vigor. Trunk circumference, shoots, and crown diameter are smaller in comparison to healthy trees (EPPO, 1997). Shoots are thin, and the bark may have a reddish-brown color and be fluted lengthwise. Necrotic areas may appear on the bark, and some branches may wither (EPPO, 1997).

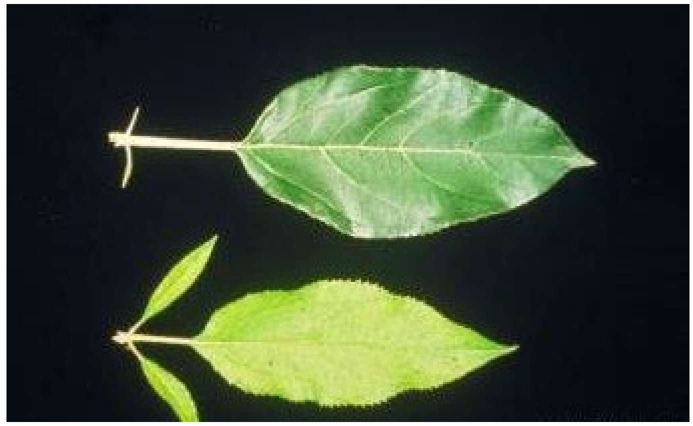

Figure 2. A healthy apple leaf (top) compared to a ‘*Ca*. P. mali’ infected leaf (bottom) showing reduced lamina size, chlorosis, and enlarged stipules. Image courtesy of Biologische Bundesanstalt für Land-und Forstwirtschaft, Institut für Pflanzenschutz im Obstbau Archive, www.bugwood.org.

Additional symptoms of apple proliferation include: witches’ brooming of shoots (Fig. 1), rosettes, small leaves with short petioles, and enlarged stipules (Fig. 2, 3) (Bovey, 1963; Seemüller et al., 2010). Additional symptoms include autumnal growth of terminal buds, enlarged flowers with many petals (Fig. 4), early bronzing and chlorosis of leaves (Fig. 2), leaf reddening, and undersized or irregular shaped fruit (Bovey, 1963). Witches’ brooming and undersized fruit are more typical in newly infected trees (Seemüller et al., 2010).

Cherry: Symptoms of apple proliferation in cherry include wilting, dying, and floral and phloem necrosis (Fig. 5) (Mehle et al., 2007).

Apricot: Symptoms of apple proliferation in apricot include stem necrosis and leaf wilting (Mehle et al., 2007).

Plum: The primary symptom of apple proliferation in plum is late blooming (Mehle et al., 2007).

Easily Mistaken Species

Phytoplasma infection produces symptoms that may resemble other pathogens or abiotic stress and requires molecular and/or immunological confirmation. ‘*Candidatus* Phytoplasma mali’, is closely related to ‘*Ca.* P. prunorum’ (European stone fruit yellows and ‘*Ca.* Phytoplasma pyri’ (pear decline, also known as peach yellow leafroll). These three phytoplasmas belong to the 16SrX group and have nearly identical 16S rDNA sequences (Seemüller and Schneider, 2004). ‘*Candidatus* Phytoplasma pyri’ can be distinguished from ‘*Ca*. P. mali’ and ‘*Ca*. P. prunorum’ by Southern Blot hybridization with DNA fragments from ‘*Ca*. P. mali’ and by RFLP analysis of ribosomal DNA employing *Ssp*I, *Bsa*AI, and *Rsa*I restriction endonucleases (Kison et al., 1997).

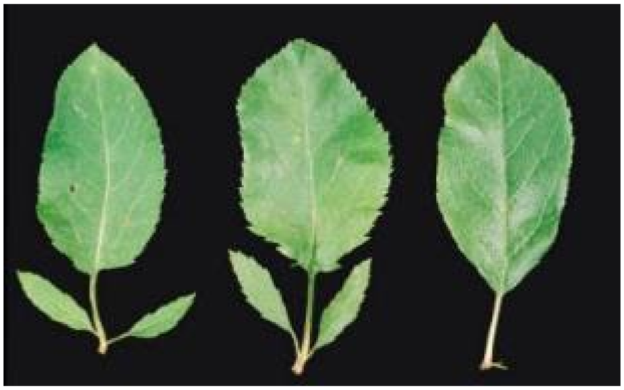

Figure 3. Healthy apple leaf (right) compared to '*Ca*. P. mali' infected leaves (left, middle showing enlarged stipules). Stipules resemble small leaflets at the base of the leaf. Image courtesy of Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenschutz im Obstbau Archive, www.bugwood.org.

Figure 4: Enlarged flower with numerous petals on apple tree infected with apple proliferation. Image courtesy of Loschi. DI4A, University of Udine, Italy. http://www.cabi.org/compendia/cpc/.

Biology and Ecology

'*Candidatus* Phytoplasma mali' is transmitted by insect vectors, including *Cacopsylla picta* and *C. melanoneura*. The spatial distribution, natural infection rate, and transmission capacity of *C. picta* and *C. melanoneura* vary among different geographic areas in relation to different associations of vector populations and phytoplasma strains (Carraro et al., 2006; Frisinghelli et al., 2000; Janik et al., 2020; Jarausch et al., 2003; Jarausch et al., 2011; Oppedisano et al., 2020). The phytoplasma is also transmitted by eggs to the new insect generations (Mittelberger et al., 2017).

C*acopsylla picta* and *C. melanoneura* have one generation per year (univoltine), and they overwinter as adults on shelter plants, mainly conifers (Čermák and Lauterer, 2008; Jarausch and Jarausch, 2014; Lauterer, 1999; Mayer et al., 2011; Pizzinat et al., 2011). Both species reproduce on apple, and the springtime generation stays on the apple host until June or July (Jarausch et al., 2011; Mattedi et al., 2006; Mayer et al., 2011; Tedeschi et al., 2002). The insects mainly transmit the phytoplasmas during the adult stage, and they are most infective from late winter until spring when they move to the apple trees from shelter plants (Carraro et al., 2006; Görg and Gross, 2021; Jarausch et al., 2011; Tedeschi and Alma, 2004). However, it was found that nymphs of *C. picta* spent more time probing the phloem than adults, and a high phytoplasma acquisition efficiency of *C. melanoneura* nymphs has been proven (Görg and Gross, 2021). A general increase of the phytoplasma level in vectors occurs proportionally after at least 15 days, whereas a plateau is reached after 60 days spent on conifers (Candian et al., 2020).

'*Candidatus* Phytoplasma mali' is also commonly transmitted by grafting (Pedrazzoli et al., 2008). Transmission of '*Ca*. P. mali' has also been occasionally reported to occur via natural root fusions/root grafts in medium-aged and old apple orchards (Baric et al., 2008; Ciccotti et al., 2007; Vindimian et al., 2002). There is no reported seed or pollen transmission (Seidl and Komarkova, 1974).

‘Candidatus Phytoplasma mali’ is found in the sieve tubes of the current season’s phloem in hosts (Seemüller, 1990). The distribution of the phytoplasma in the tree is inconsistent throughout the year (Schaper and Seemüller, 1982). During the winter months (December to March), the phytoplasma concentration decreases in aerial parts of hosts, but the phytoplasma can be detected in the roots of diseased trees throughout the year (Seemüller et al., 1984). The phytoplasma concentration in roots varies by season, with the highest populations occurring from December through May (Baric et al., 2011). According to one study by Pedrazzoli et al. (2008), ‘*Ca*. P. mali is not detectable in aerial plant parts in late spring. The phytoplasma recolonizes the aerial parts in April or May and increases to its highest level in late summer or early fall (Baric et al., 2011). Infected apple trees can enter remission and recover from infection of ‘*Ca*. P. mali’. In one study, the ‘recovered’ trees were still infected and had high populations of the phytoplasma in their roots but not in shoots (Carraro et al., 2004).

Figure 5: Wilting (top) and dying (bottom) cherry trees infected with the ‘Ca. Phytoplasma mali’. Images from Mehle et al. (2006).

Known Hosts

The host list below includes cultivated and wild plants that 1) are infected or infested by the pest under natural conditions, 2) are frequently described as major, primary, or preferred hosts, and 3) have primary evidence for feeding and damage documented in the literature. Plants are highlighted in bold if they are commercially produced and the pest causes economically significant damage.

Apple is the main host, and most cultivars are susceptible. Apple cultivars known to be affected by ‘*Ca.* Phytoplasma mali’ are included in Table 1.

Table 1: Susceptibility of apple cultivars to ‘*Ca.* P. mali’.

Rating	Cultivar	Source
Highly Susceptible	Florina	Loi et al., 1995; Osler et al., 2001
Highly Susceptible	Prima	Loi et al., 1995
Highly Susceptible	Priscilla	Loi et al., 1995
Medium Susceptibility	Idared	Németh, 1984
Medium Susceptibility	McIntosh	Nemeth, 1986; Kartte and Seemüller, 1991
Medium Susceptibility	Starking Delicious	Nemeth, 1986; Kartte and Seemüller, 1991; Rumbou et al., 2006
Medium Susceptibility	Starkrimson	Nemeth, 1986
Susceptible	Belle de Booskop	EPPO, 1997
Susceptible	Canadian Renette	Osler et al., 2001
Susceptible	Granny Smith	Osler et al., 2001
Susceptible	Gravenstein	EPPO, 1997
Susceptible	Golden Delicious	EPPO, 1997; Kartte and Seemüller, 1991; Osler et al., 2001
Susceptible	Jonathan	Kartte and Seemüller, 1991
Susceptible	Rome Beauty	Kartte and Seemüller, 1991
Susceptible	Winter Banana	EPPO, 1997
Tolerant	Roja de Benejama	EPPO, 1997
Tolerant	Antonokova	Nemeth, 1986
Tolerant	Cortland	Nemeth, 1986
Tolerant	Spartan	Nemeth, 1986
Tolerant	Yellow transparent	Nemeth, 1986
Tolerant	Wealthy	Nemeth, 1986

Other known hosts
Crataegus monogyna (Hawthorn)*, *Prunus armeniaca* (apricot)*, *Prunus avium* (cherry)*, *Prunus domestica* (plum)*, *Prunus persica* (peach/nectarine)*, *Prunus salicina* (Japanese plum)*, and *Pyrus communis* (European pear)* (CABI, 2021; Del Serrone et al., 1998; Fránová et al., 2018; Lee et al., 1995;; Mehle et al., 2007; Navratil et al., 2001; Paltrinieri, 2001).

Crataegus monogyna is a primary host of the vector *C. melanoneura* (Tedeschi et al., 2009).

Pest Importance

Apple proliferation is one of the most important insect-borne diseases of apple trees in Europe. The disease is widespread in northern Italy and in Germany, causing serious damage to several traditional apple growing areas (Bliefernicht and Krczal, 1994; Carraro et al., 2004). The disease may cause a 50% reduction in fruit size, 60% reduction in fruit weight, a reduction of sugar and acid content of fruit, an overall reduction of tree growth and vigor, and death of young trees in severe cases (Al-Jabor, 2009; Baric et al., 2008; Fialová et al., 2003; Tedeschi et al., 2002). Overall, the

* Hosts with U.S. distribution

commercial value of fruit is decreased 30 to 100% due to poor quality (Tedeschi et al., 2002). In 2020, the total value of U.S. apple production was about $2.9 billion (USDA-NASS, 2021).

Apple proliferation is classified as a quarantine organism in Europe (EPPO A2 list) and North America. Spraying insecticides to reduce vector populations and use of healthy replanting material are the primary management options (Galetto et al., 2005), though efficacy is limited. Particular attention should be paid to the management of overwintered psyllids as soon as they return to the orchards, but also to newly emerged adults, specifically in orchards with a high infection rate (Candian et al., 2020). Host resistance is also being explored to manage apple proliferation (Seemüller et al., 1984, 2008). Trees infected with apple proliferation are particularly sensitive to powdery mildew (*Podosphaera leucotricha*) (Bovey, 1963b).

'Candidatus Phytoplasma mali' is listed as a harmful organism in Brazil, Chile, China, Colombia, Ecuador, Indonesia, Israel, Japan, Madagascar, Mexico, Moldova, Monaco, Morocco, Norway, Oman, Peru, San Marino, Serbia, Turkey, United Arab Emirates, United Kingdom, and Uruguay (USDA-PCIT, 2021). There likely will be trade implications with these countries if this pathogen becomes established in the United States.

Known Vectors (or associated insects)

Primary vectors: *Cacopsylla picta* (synonym *C. costalis)*, *C. melanoneura*, and *Fieberiella florii.*

In Italy and Germany, the psyllids *C. melanoneura* and *C. picta* and the leafhopper *F. florii* are confirmed vectors of '*Ca*. P. mali' (Bliefernicht and Krczal, 1995; Carraro et al., 2008; Frisinghelli et al., 2000; Jarausch et al. 2003; Krczal et al., 1988; Tedeschi et al., 2002, 2012; Tedeschi and Alma, 2006). *Fieberiella florii* is present in North America and would likely vector the phytoplasma if it were introduced into the United States.

Known Distribution

Africa: Tunisia **Asia:** Syria, Turkey **Europe:** Albania, Austria, Belarus, Belgium, Bosnia-Hercegovina, Bulgaria, Croatia, Czech Republic, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Moldova, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, and Ukraine (Abraitienë et al., 2018; Al-Jabor, 2009; Avinent and Llacer, 1994; Bashkirova et al., 2019; Ben Khalifa and Fakhfakh, 2011; CABI, 2021; Canik and Ertunc, 2007; Del Serrone et al., 1998; Delic et al., 2007; Duduk et al., 2008; EPPO, 1997, 2021; EPPO Reporting Service, 2011; Fialová et al., 2003; Karimova et al., 2018; Križanac et al., 2010; Lemmetty et al., 2013; Myrta et al., 2003; ProMED-mail, 2013; Rumbou et al., 2006; Seljakb and Ravnikara, 2007; USDA, 2013; Valasevich and Schneider, 2016).

A "possible detection" of '*Ca*. P. mali' from Nova Scotia, Canada (USDA, 2013) could not be confirmed after further testing and analysis (CFIA, 2013). Thus, Canada has been removed from the distribution listing. There are unreliable records of this phytoplasma in Cyprus, India, and South Africa (EPPO, 2021).

Pathway

Long-distance spread of phytoplasmas occurs via transport of infected planting material or infected vectors. According to Federal Order DA-2013-18, the import of *Malus* spp. and *Prunus* spp. propagative material is currently restricted to prevent the spread of *Anoplophora chinensis* (Citrus Longhorned Beetle, CLB) and *A. glabripennis* (Asian Longhorned Beetle, ALB). *Prunus* spp. may be imported from Canada and the Netherlands, and *Malus* spp. may be imported from Belgium, Canada, France, Germany, and the Netherlands. Propagative material grown in Canada is not regulated for CLB or ALB and does not have specific import requirements, but propagative material from other approved countries (Belgium, France, Germany, and the Netherlands) are required to be declared free from pathogens and enter post-entry quarantine following import.

Since 2012, there have been shipments of *Malus* spp. plant material from numerous countries where *'Ca.* P. mali' is located, including: Belgium, Czech Republic, France, Germany, Italy, and the Netherlands (ARM, 2022). The Netherlands alone has shipped over 52.2 million plant units of *Malus* spp. during this time (ARM, 2022). One shipment from the Netherlands contained over 8.1 million plant units, and a shipment from Germany contained 1.5 million plant units (ARM, 2022). Since 2012, there have also been shipments of *Prunus* spp. plant material from the following countries where *'Ca.* P. mali' is present: Czech Republic, France, Germany, Italy, Netherlands, Spain, and Turkey (ARM, 2022).

There have been recorded interceptions of *Cacopsylla* spp. from the Netherlands (15), Italy (10), Canada (4), China (1), and Mexico (1) (ARM, 2022). It is not known whether these interceptions were of a species known to vector '*Ca*. P. mali'.

Use the PPQ Commodity Import and Export manuals listed below to determine 1) if host plants or material are allowed to enter the United States from countries where the organism is present and 2) what phytosanitary measures (e.g., inspections, phytosanitary certificates, post entry quarantines, mandatory treatments) are in use. These manuals are updated regularly.

Fruits and Vegetables Import Requirements (FAVIR) Online Database: The FAVIR database lists all import requirements for fruits and vegetables. To search by commodity, select 'Approved Name' at the top left of the page. Select the commodity from the drop-down menu and then click 'Search'. Click on the 'Commodity Summary' tab for details. https://epermits.aphis.usda.gov/manual/index.cfm?action=pubHome

Plants for Planting Manual: This manual is a resource for regulating imported plants or plant parts for propagation, including buds, bulbs, corms, cuttings, layers, pollen, scions, seeds, tissue, tubers, and like structures.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/plants_for_planting.pdf

Cut Flowers and Greenery Import Manual: This manual is a resource for regulating imported fresh, cut plants used for decoration and for protecting plants from extinction due to trade.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/cut_flower_imports.pdf

Miscellaneous and Processed Products Import Manual: This manual is a resource for regulating imported processed plant and non-plant that may introduce exotic pests.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/miscellaneous.pdf

Treatment Manual: This manual provides information about treatments applied to imported and domestic commodities to limit the movement of agricultural pests into or within the United States.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Potential Distribution within the United States

Based on the known distribution of '*Ca.* P. mali', it may establish in plant hardiness zones 4-11, which encompass most of the continental United States (Takeuchi et al., 2018). Commercial apple orchards are the most vulnerable to infection and spread of '*Ca*. P. mali'. The top apple producing states in 2020 were Washington, New York, Michigan, Pennsylvania, California, Oregon, and Virginia. Washington accounted for over 70% of all U.S. apple production (USDA-NASS, 2021). These states, particularly on the west coast, are also home to commercial production of stone fruit hosts of '*Ca*. P. mali', such as cherry, peach, and plum (USDA-NASS, 2021).

Survey and Key Diagnostics

Approved Method for Pest Surveillance:

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

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2022 Reviewers

Dr. Assunta Bertaccini, University of Bologna, Italy
Dr. Wolfgang Jarausch, AlPlanta - Institute for Plant Research Germany
Dr. Marta Martini, University of Udine, Italy
Dr. Rosemarie Tedeschi, Università degli Studi di Torino, Italy

Previous reviewers

Dr. Erich Seemüller, Julius Kuehn Institute (JKI), Germany