

'*Candidatus* Phytoplasma prunorum'

Scientific Name

'*Candidatus* Phytoplasma prunorum' Seemüller and Schneider, 2004

Synonyms:

Apricot chlorotic leaf roll, plum leptonecrosis, peach decline, apricot dieback, peach yellows, and peach vein clearing.

Note: Peach rosette is a distinct disease from the disease caused by the North American peach rosette phytoplasma ('*Candidatus* Phytoplasma pruni' (Davis et al., 2013)).

Common Name(s)

European Stone Fruit Yellows (ESFY)

Type of Pest

Phytoplasma

Taxonomic Position

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

Reason for Inclusion in Manual

CAPS Target: AHP Prioritized Pest List – 2010 through 2011; Stone fruit survey

Pest Description

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 after a latency period of 20-40 days to plants during feeding activity by their vectors, primarily leafhoppers, planthoppers, and psyllids (IRPCM, 2004; Weintraub and Beanland, 2006).

Although phytoplasmas 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 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; Harrison et al., 2011, 2014; 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 prunorum*' is classified in group 16SrX, subgroup B (16SrX-B).

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 \geq 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).

European stone fruit yellows (ESFY) is a severe disease of stone fruit caused by a phytoplasma in the apple proliferation group (group 16SrX) of phytoplasmas. The group/cluster also includes phytoplasmas associated with other perennial fruit tree diseases present in Europe, including apple proliferation ('*Candidatus Phytoplasma mali*') and pear decline ('*Candidatus Phytoplasma pyri*'). In contrast, phytoplasmas infecting stone fruit in North America (X-diseases, caused by *Candidatus Phytoplasma pruni*) are members of group 16SrIII (the Western-X disease group) (Poggi Pollini et al., 2001; reviewed by Marcone et al., 2010).

Diseases of stone fruits associated with phytoplasmas, including apricot chlorotic leaf roll, plum leptonecrosis, and peach decline, were found to have a common etiology and a single name of European stone fruit yellows was proposed (Lorenz et al., 1994). The disease is known to be present in Europe, North Africa, and western parts of Asia.

Nemeth (1986) described the morphological features of the ESFY phytoplasma, referred to as apricot chlorotic leafroll phytoplasma, as pleomorphic (varying in size and shape) bodies. Bacilliform (rod-shaped) particles were also found. Round-shaped or spherical intravascular bodies can be found in young and lightly infested phloem cells. Bodies in old and heavily infested cells are compressed and degenerated (Nemeth, 1986).

Biology and Ecology

ESFY is an epidemic disease, characterized by widespread movement when conditions are favorable for host-plants and vectors (Carraro and Osler, 2003). The phytoplasma is graft-transmissible to *Prunus* (Morvan 1977; Carraro et al., 2004b) and has been transmitted from apricot to *Vinca rosea* (Madagascar periwinkle) by dodder (*Cuscuta subinclusa*) (Carraro et al., 1992).

Cacopsylla pruni (plum psyllid) is the primary vector of the disease (Carraro et al., 1998b). The psyllid vector completes one generation per year and overwinters as an adult on shelter plants (conifers) (Carraro et al., 2001a). At the end of winter, *C. pruni* moves from shelter plants to *Prunus* species (stone fruit trees and wild *Prunus*) for oviposition (egg-laying). From May till the beginning of July, the new generation feeds on *Prunus* species (wild and cultivated). As soon as adult development is complete, *C. pruni* abandons the stone fruit trees (Carraro et al., 2001a). *Cacopsylla pruni* prefers blackthorn (*Prunus spinosa*) for its development (Lauterer, 1999). *Prunus mahaleb* is also found as an important host in Spain (Sabaté et al., 2014). *Cacopsylla pruni* is strictly oligophagous on *Prunus* species. Carraro et al. (2004b) demonstrated experimentally that, among the 12 *Prunus* species tested, *C. pruni* progeny was greatest on *P. domestica*, *P. salicina* and *P. spinosa*; it should be noted that apricot, peach and almond ranked among the hosts of intermediate quality for *C. pruni*.



Figure 1. *Cacopsylla pruni*. Photo courtesy of B. Jarausch. RLP Agrosience.

The psyllid transmits the ESFY phytoplasma in a persistent-propagative manner (Thébaud et al., 2009). The minimum acquisition period is 2-4 days, the minimum latent period is 2-3 weeks, and the minimum inoculation period is 1-2 days (Carraro et al., 2001a). The retention of infectivity of *C. pruni* lasts through the winter and the following spring. When the overwintered insects reach the stone fruit trees, they are already infected and infective (Carraro et al., 2001a). The natural transmission period lasts as long as the vector is present on *Prunus* species (Carraro et al., 2004a). In areas with high infection pressure, the natural infectivity of *C. pruni* reaches mean individual transmission rates of 8.6% for the mature adults and 1.7% for the immature adults (Carraro et al., 2004a), and the annual rate of newly infected plants was 20% (Carraro et al., 1992). Similar results were obtained in Spain (around 30% of phytoplasma carriers in reimmigrant individuals and around 15% in new generations) (Laviña et al. 2004).

Thébaud et al. (2009) found that immature and mature *C. pruni* were hardly infectious (0.6%) despite effective phytoplasma acquisition and multiplication. Immature vectors born on infected plants were able to reach their maximum phytoplasma load (10^7 genomes per insect) only after



Figure 2. Leaf rolling symptom of ESFY in apricot. Photo courtesy of G. Morvan. EPPO.

migrating to conifers. After a life-long retention of phytoplasma, their transmission efficiency was very high (60%) at the end of the winter (when they migrate back to their *Prunus* host). The authors concluded that most transmissions occur only after an effective latency of 8 months, following vector migrations and overwintering in conifers in mountainous regions in France (Thébaud et al., 2009).

Carraro et al. (1998b) found that it took 4-5 months for *Prunus* plants to show typical ESFY symptoms (referred to as incubation period) after vector transmission. Seemüller et al. (1998) found that the ESFY phytoplasma can persist in the stem of *Prunus* taxa in the dormant (winter) season, which is in sharp contrast to the apple proliferation and pear decline phytoplasmas in this group.

Carraro et al. (2002) demonstrated the important role played by wild *Prunus* species, such as *P. spinosa* (blackthorn) and *P. cerasifera* (cherry plum). These plants are hosts for the vector and the ESFY phytoplasma in the epidemic cycle of the disease. The phytoplasma can, therefore, survive and persist in nature independently of the presence of cultivated plants. It should also be noted that some cultivated *Prunus* spp. are completely tolerant, and these plants can act as sources of inoculum for the spread of ESFY (Morvan et al., 1977).

Jarausch et al. (2001) detected the ESFY-phytoplasma in *Celtis australis* (European hackberry), *Fraxinus excelsior* (European ash), and *Rosa canina* (dog rose) growing in the surroundings of infected apricot orchards. Varga et al. (2000) detected the ESFY phytoplasma in grapevine in Hungary. The exact role played by these non-*Prunus* species in the epidemiology of the disease is



Figure 3: Apricot tree showing symptoms of yellowing, leaf curl, and decline. A symptomless shoot is shown in the foreground. Image from Davies and Adams (2000).



Figure 4. Reddening of Japanese plum leaves affected by ESFY (right) compared to an unaffected leaf (left). Photo courtesy of Dr. B. Schneider, BBA.

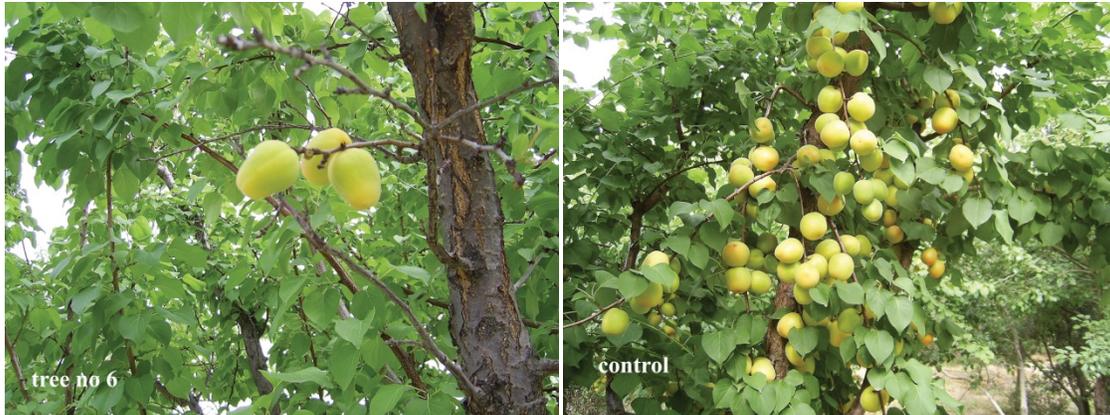


Figure 5: Fruit set of ‘*Ca. Phytoplasma prunorum*’ infected tree (left) compared with the control (non-infected) tree (right). Image from Gazel et al. (2009).

not clear, especially given that *C. pruni* is strictly oligophagous on *Prunus* species. Jarusch et al. (2001) speculated that they may be dead-end hosts of the phytoplasma.

Symptoms/Signs

‘*Ca. Phytoplasma prunorum*’ is associated with European stone fruit yellows (ESFY) disease, which primarily includes diseases of apricot, Japanese plum, and peach. Symptoms of ESFY are influenced by species, cultivar, rootstock, and environmental



Figure 6. Premature leaf emergence in an infected *Prunus salicina* (Japanese plum) tree (left), and phloem necrosis (right) in a *Prunus* spp. affected by ESFY. Photos courtesy of Assumpcio Batlle, IRTA, Catalunya, Spain (left), and Dr. B. Schneider, BBA (right).

factors. There are many tolerant hosts that do not show any symptoms of disease but can harbor infections.

Apricot and Japanese plum trees in general show typical 'yellows' symptoms accompanied by leaf roll (Fig. 1-3) followed by leaf reddening (Fig. 4), reduction, or suppression of dormancy with the consequent risk of frost damage, severe and progressive necrosis, decline, and eventual death of the tree (Morvan, 1977). Peaches exhibit early leaf reddening, severe upward longitudinal rolling of leaves, abnormal thickening and suberization of the midribs and primary veins, autumnal growth of latent buds which produce tiny chlorotic leaves and sometimes flowers, and early phylloptosis (leaf fall) (Poggi Pollini et al., 2001). The leaves also tend to be 'more brittle' than normal. Symptoms first appeared in late summer in Italy with latent bud production occurring in September (Poggi Pollini et al., 2001).

ESFY affects tree flowers and shoots in winter, which leads to lack of fruit production (Fig. 5) and chlorosis of the leaves later in the growing season. A common symptom in most *Prunus* species is the early break of leaf buds in late winter (Fig. 6) (Jarausch et al., 2008). The early break in dormancy increases the susceptibility of affected trees to frost, which can cause damage to the phloem (Fig. 6). Disease often starts with only a few branches affected but the whole tree may become affected as the disease progresses. Infected shoots are typically shorter and bear smaller, deformed leaves. Leaves can drop prematurely. Shoots may die back. Yield is reduced. Fruit on affected branches develops poorly and may fall prematurely.



Figure 7: Host mortality of *Prunus salicina* trees infected by 'Ca. P. prunorum'. Photos courtesy of Assumpcio Batlle, IRTA, Catalunya, Spain.

Pest Importance

The ESFY phytoplasma induces economically important disorders of apricot (Desvignes and Cornaggia, 1982), Japanese plum (Dosba et al., 1991), and peach (Marccone et al., 1996). Within the most sensitive cultivars of apricot and Japanese plum, 100% of the infected plants can die (Fig. 7) (Carraro and Osler, 2003). Production can be totally lost (Carraro and Osler, 2003). In Turkey, susceptible young apricot and plum trees infected with 'Ca. Phytoplasma prunorum' die quickly (within 1 to 2 years after infection), and the pathogen also causes yield and quality losses on trees older than five years (Gazel et al., 2009).

'*Candidatus Phytoplasma prunorum*' is listed as a harmful organism in Colombia, Japan, and Peru (USDA-PCIT, 2014). There may be trade implications with these countries if this pathogen becomes established in the United States.

Known Hosts

Major Hosts: *Prunus armeniaca* (apricot), *Prunus salicina* (Japanese plum), and *Prunus persica* (peach) (Carraro et al., 1992; Jarausch et al., 2000a; Carraro and Osler, 2003; Sabate et al., 2007).

Note: The level of susceptibility and symptom expression varies significantly among the 'other' hosts.

Other Hosts: *Celtis australis* (European hackberry), *Fraxinus excelsior* (European ash), *Prunus americana* (American plum), *P. amygdalus* (sweet almond), *P. avium* (sweet cherry), *P. bokhariensis* (Indian, Persian gum), *P. brigantina* (Briançon apricot), *P. cerasifera* (cherry plum), *P. cerasus* (sour cherry), *P. coccomilia* (coccomilia), *P. consociiflora* (Chinese wild peach), *P. dasycarpa* (purple, black apricot), *Prunus domestica* (European plum), *P. hollywood* (Hollywood cherry plum), *Prunus serrulata* (flowering cherry), *P. laurocerasus* (cherry laurel), *P. mahaleb* (Mahaleb cherry), *P. maritime* (beach plum), *P. mexicana* (Mexican plum), *P. mume* (Japanese apricot), *Prunus orthosepal*, *P. padus* (European bird cherry), *P. salicina* x *cerasifera* (methley, cherry plum), *P. serrulata* (Japanese flowering cherry), *P. simonii* (apricot plum), *P. spinosa* (blackthorn), *P. subcordata* (Klamath plum), *P. tomentosa* (Nanking cherry), *Rosa canina* (dog rose), and *Vitis vinifera* (grape) (Morvan and Castelain, 1972; Giunchedi et al., 1982; Poggi Pollini et al., 1995; Jarausch et al., 1998; Jarausch et al., 1999a; Jarausch et al., 2000a; Jarausch et al., 2000b; Varga et al., 2000; Jarausch et al., 2001; Kison and Seemüller, 2001; Carraro et al., 2002; Carraro et al., 2004b; Fiavola et al., 2004; Pignatta et al., 2008).

The ESFY phytoplasma also causes infection of *Prunus* rootstocks: *Prunus besseyi* x *P. hortulana*, *P. cerasifera*, *P. domestica*, *P. domestica* x *P. cerasifera*, *P. mariana*, *P. persica* x *P. cerasifera*, and *P. salicina* x *P. spinosa* (Jarausch et al., 1998; Jarausch et al., 2000b; Kison and Seemüller, 2001). The susceptibility and sensitivity of the rootstocks to ESFY varies according to the different genotypes: some are highly sensitive, *i.e.* apricot seedlings and Rubira peach; others, such as Bromptom, are tolerant (Kison and Seemüller, 2001).

Japanese plum (*P. salicina*) and apricot (*P. armeniaca*) are the most susceptible and sensitive hosts. European plum (*P. domestica*) is susceptible but generally tolerant to ESFY (Carraro et al., 1998a). Despite a healthy appearance, European plum can be infected with ESFY phytoplasma and be important reservoirs of the pathogen. Some cultivars, however, can show weak symptoms but low mortality (Jarausch et al., 2000a).

Carraro et al. (2004b) demonstrated that all twelve *Prunus* species evaluated were hosts for the ESFY phytoplasma. *Prunus armeniaca* (apricot) and *P. salicina* (Japanese plum) showed high susceptibility and high sensitiveness (severe symptoms/leaf roll,

small chlorotic leaves); *P. persica* (peach/nectarine) and *P. tomentosa* (Nanking cherry) showed high susceptibility and low sensitiveness (mild symptoms/yellowing only); *P. cerasifera* (cherry plum), *P. domestica* (European plum), and *P. spinosa* (blackthorn) showed high susceptibility and tolerance (no symptoms); *P. amygdalus* (sweet almond), *P. avium* (sweet cherry), *P. laurocerasus* (cherry laurel), *P. mahaleb* (Mahaleb cherry), and *P. padus* (European bird cherry) showed low susceptibility and are tolerant (no symptoms).

Ferrini et al. (2002) observed similar results to the Carraro et al. (2004b) study with the exception of the reaction of *P. tomentosa* (Nanking cherry), which ranged from being tolerant to showing mild symptoms in the two studies, respectively. The authors showed that *P. cerasifera* (cherry plum), *P. mahaleb* (Mahaleb cherry), *P. padus* (European bird cherry), *P. spinosa* (blackthorn), and *P. tomentosa* (Nanking cherry) were highly tolerant to the disease and the presence of specific symptoms is the exception. Jarausch et al. (1999a) also showed that *P. avium* (sweet cherry) demonstrated a high level of resistance to ESFY. Morvan and Castelain (1972) showed that *P. americana* (American plum) and *P. coccinifera* (coccinifera) were symptomless carriers.

Known Vectors (or associated insects)

Cacopsylla pruni is the only confirmed vector of the disease (Carraro et al., 1998b), although there are in fact two non-hybridizing species with a similar morphology hiding behind the same name (Peccoud et al., 2013). Poggi Pollini et al. (1996) found that the leafhoppers *Anaceratogallia* and *Euscelis* were infected by 16SrX-B phytoplasma. Two preliminary reports indicated the detection of the ESFY phytoplasma in *Empoasca decedens* (Pastore et al., 2002) and the ability of these leafhoppers to transmit ESFY was shown on two apricot plants (Pastore et al., 2004).

Known Distribution

Africa: Egypt, Tunisia. **Asia:** Azerbaijan, Turkey **Europe:** Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, England, France (including Corsica), Germany, Greece, Hungary, Italy (including Sardinia), Netherlands, Poland, Romania, Serbia, Slovakia, Slovenia, Spain, Switzerland, and the United Kingdom (England and Wales) (Jarausch et al., 1998; Carraro et al., 1998a; Davies and Adams, 2000; Jarausch et al., 2000b; Topchiiska et al., 2000; Machado et al., 2001; Navratil et al., 2001; Carraro and Osler, 2003; Myrta et al., 2003; Ramel and Gugerli, 2004; Sertkaya et al., 2005; Delic et al., 2007; Fialova et al., 2007; Mehle et al., 2007; Polak et al., 2007; Ambrozic Turk et al., 2008; Jarausch et al., 2008; CABI, 2009; Gazel et al., 2009; Verbeek, 2009; Balkishiyeva et al., 2010; Khalifa and Fakhfakh, 2011; EPPO, 2014).

The incidence of disease is different in each country. ESFY is a serious problem in countries bordering the Mediterranean Sea (Spain, France, Italy, Balkans), where the cultivation of susceptible and sensitive *Prunus* species (apricot and Japanese plum) is widespread. This phytoplasma is considered no longer present in Cyprus, and records from South Africa and Ukraine are considered invalid (EPPO, 2014).

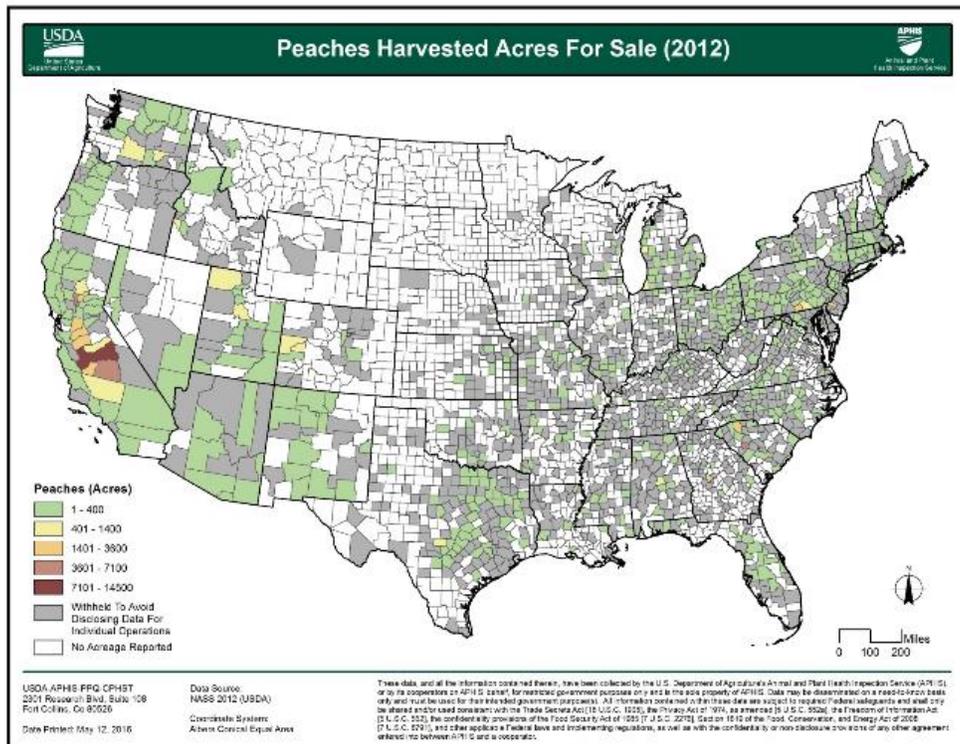
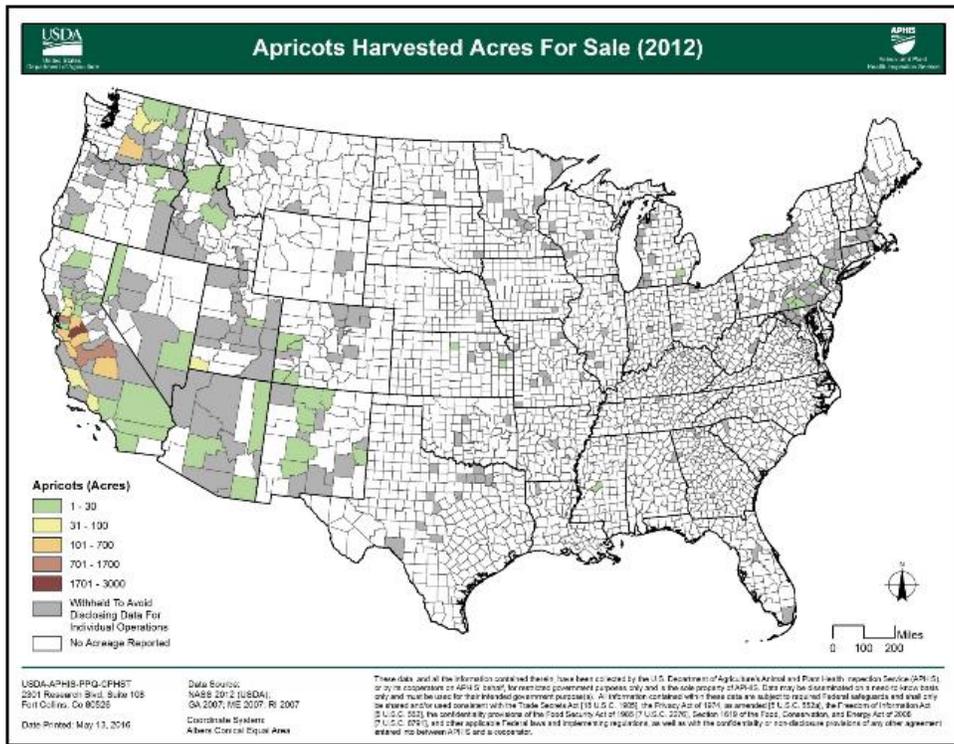


Figure 8. Commodity acreage maps for apricot and peach within the continental United States by county. Map courtesy of USDA-APHIS-PPQ-CPHST.

Pathway

According to Federal Order DA-2013-18, effective May 20, 2013, the import of *Prunus* spp. propagative material is currently prohibited from all countries except Canada and the Netherlands to restrict import of host material of *Anoplophora chinensis* (Chinese longhorned beetle) and *Anoplophora glabripennis* (Asian longhorned beetle) (USDA, 2013). Prior to this Federal Order, import of *Prunus* spp. propagative material was allowed from the following countries known to have 'Ca. P. prunorum': Belgium, France, Germany, and the United Kingdom (USDA, 2013).

Since 2004, there have been shipments of *Prunus* spp. plant material from the following host countries: Czech Republic (2), France (31), Germany (1), Hungary (1), Italy (1), Netherlands (13), Spain (2), and the United Kingdom (7). The largest of these shipments was from the Netherlands and contained 46,000 plant units (AQAS, 2014). There have also been a total of 73 interceptions of *Prunus* spp. plant material intended for propagation from 15 different host countries since 2004 (AQAS, 2014).

Potential Distribution within the United States

Areas at risk would be in apricot, Japanese plum, and peach growing areas/orchards in eastern and western United States. At this time, commodity acreage maps for apricot and peach are available (Fig. 8). A map of Japanese plum acreage is not available. The map describes the relative density of these susceptible hosts at a county level. This map shows that portions of California are at the greatest risk from this phytoplasma based on host availability. Most of the continental United States has a fairly low level of risk for 'Ca. Phytoplasma prunorum' establishment based on host availability.

Survey

Approved Method for Pest Surveillance*:

The CAPS-approved survey method is to collect symptomatic plant tissue by visual survey. Sensitive stone fruit species (apricot and Japanese plum) can indicate the presence of ESFY in a given area; thus particular attention should be paid to these hosts.

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 are typically conducted for ESFY phytoplasma (Jarausch et al., 2008). Delic et al. (2007) carried out surveys during autumn (October) and spring (April). Several hectares of orchards were visually inspected, and stone fruit trees were checked for symptoms of phytoplasma infection. The symptoms considered were leaf roll, yellowing, and phloem necrosis. In Thébaud et al. (2006), visual surveys were mostly based on the early break of leaf buds, a characteristic winter symptom. Fialova et al. (2004) also conducted visual surveys in apricot and peach orchards, experimental plantings, and private gardens for symptoms of ESFY phytoplasma. Twigs from symptomatic stone fruit trees exhibiting yellows, leaf rolling, or decline were cut for phytoplasma tests during vegetative growth stages. Myrta et al. (2003) collected leaf samples from symptomatic samples and assayed the mid-ribs and petioles by PCR (polymerase chain reaction).

Insect vectors were shaken from *Prunus domestica* and *P. salicina* trees onto an underlying net and grouped using an aspirator (Carraro et al., 2004a). The population of the vector in the orchards was high, however, in this site. Fialova et al. (2007) used sweep-netting to capture psyllids during the vegetative season (April through mid-July). Fialova et al. (2004) used a limb-jarring technique; while Jarausch et al. (2008) used a beat-tray method.

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:

Culture: The phytoplasma that causes European stone fruit yellows is an obligate intercellular parasite and cannot be cultured on microbiological growth media.

Biological Indexing: Greenhouse indexing, which consists of graft-transmission onto a woody indicator, often using peach GF 305 as a test plant, is a time-intensive method (Desvignes and Cornaggia, 1982). Waterworth and Mock (1999) and Polak et al. (2007) found that this method is not as reliable as a nested PCR for detection of phytoplasmas. The best woody indicators for fast diagnostic detection of the ESFY phytoplasma in the Czech Republic were Tomcot, Leskora, LE-2927 and Bergeron (strongest visual

symptoms); weak appearance of symptoms was found in genotypes Velkopavlovicka, Veecot, and M-LE-1 (Necas and Krska, 2006).

Fluorescence Microscopy: For large scale diagnosis, the DAPI (4', 6'-diamidino-2-phenylindole, 2HCl) staining method (Seemüller, 1976) can be used, although the percentage of false negative can reach high levels. False negatives generally occur when phytoplasma colonization of plants is poor or uneven. This test detects fluorescence of phytoplasmas in the sieve tubes of the leaf veins.

Biological indexing and DAPI staining are time-consuming and do not often allow specific identification of phytoplasmas (Poggi Pollini et al., 2001).

Molecular: Seemüller and Scheider (2004) offer a summary of the molecular studies conducted on the apple proliferation, European stone fruit yellows, and pear decline phytoplasmas. The authors conclude that the phytoplasmas are coherent and discrete taxa and can be distinguished as distinct species with the proposed names 'Ca. *Phytoplasma mali*' (apple proliferation), 'Ca. *Phytoplasma prunorum*' (European stone fruit yellows), and 'Ca. *Phytoplasma pyri*' (pear decline). A chromosome map of the ESFY phytoplasma is available (Marccone and Seemüller, 2001).

Necas and Krska (2005, 2006) found that DNA isolated from phloem gave a more reliable reaction than that isolated from leaf-stalks. The best time to collect phloem samples was June and September in the Czech Republic; while August was the worst month. Jarausch et al. (1999b) found that colonization of trees by the ESFY phytoplasma was systemic from July until leaf fall, and that the ESFY phytoplasma could be detected in off-season grown leaves during winter until March. Almost no phytoplasma could be detected in normally grown leaves in April and May (Jarausch et al., 1999b).

Kirkpatrick et al. (1987), Ahrens and Seemüller (1992), and Maixner et al. (1995) developed a procedure to enrich extracts with phytoplasma DNA. Most authors working with the ESFY phytoplasma used these procedures or some modification (e.g., Malisono et al., 1996) of these procedures (Marccone et al., 1996; Kison et al., 1997; Carraro et al., 1998a,b; Jarausch et al., 1998; Davies and Adams, 2000; Kison and Seemüller, 2001; Fialova et al., 2004; Sertkaya et al., 2005; Bertolini et al., 2007; Delic et al., 2007). The method of Doyle and Doyle (1990) was employed for isolating DNA from the insect vector (Carraro et al., 1998b; Carraro et al., 2001a,b; Carraro et al., 2004a; Delic et al., 2007). Green et al. (1999) developed an 'easy and efficient' DNA extraction method from woody plants for detection of phytoplasmas by PCR. Maskova (2009) evaluated four methods for DNA extraction of the ESFY phytoplasma, including the Ahrens and Seemüller (1992) method, and found that none of the methods provided the consistent quality and quantity of DNA necessary for ESFY phytoplasma detection. The authors, however, did not offer an alternative strategy.

PCR amplification is now widely used for the sensitive and reliable diagnosis of phytoplasmas in fruit trees. Due to the close genetic relatedness of the apple proliferation group of phytoplasmas, specific identification often requires the digestion of

the amplicons with various endonucleases and subsequent RFLP analysis (Ahrens and Seemüller, 1992; Deng and Hiruki, 1991; Gundersen and Lee, 1996; Lee et al., 1995; Schneider et al., 1995; Smart et al., 1996; Kison et al., 1997; Gibb et al., 1999; Jarausch et al., 2000b; Heinrich et al., 2001).

Gundersen and Lee (1996) showed that nested PCR using two universal primer pairs for phytoplasmas increased the detection sensitivity 100-fold and readily detected phytoplasmas from all the woody hosts and insect vectors tested. Torres et al. (2004) used nested PCR with 16SrX group-specific primers and were able to detect the ESFY phytoplasma in 50% of asymptomatic trees that showed symptoms the following year. Ambrozic Turk et al. (2008) used PCR and nested PCR to detect the ESFY phytoplasma in 100% of Japanese plums, 70% of apricots, 13% of peaches/nectarines, 0% of cherries, and 51% of European plum (asymptomatic) trees sampled in Slovenia. Poggi Pollini et al. (1997, 2001) used immunoenzymatic detection of PCR products to detect phytoplasmas, including ESFY. Bertolini et al. (2007) developed a co-operational PCR coupled with dot blot hybridization for detection of 'Ca. Phytoplasma mali', 'Ca. Phytoplasma prunorum', and 'Ca. Phytoplasma pyri'. The sensitivity of this method was at least one hundred times greater than conventional PCR and similar to that achieved by nested PCR and real-time PCR.

A primer pair (ECA1/ ECA 2), designed from conserved chromosomal sequences, showed no cross reaction in PCR amplification with other phytoplasmas of the apple proliferation group and proved to be highly specific for ESFY phytoplasma (Jarausch et al., 1998). Rubio-Cabetas and Sancho (2009) evaluated nested PCR with group-specific primers followed by RFLP and direct PCR with specific primers for 'Ca. Phytoplasma prunorum' from Jarausch et al. (1998). Rubio-Cabetas and Sancho (2009) recommend the nested PCR followed by RFLP analysis for routine diagnosis rather than the direct PCR.

Real-time PCR: Torres et al. (2005) developed a real-time PCR that detects 'Ca. Phytoplasma mali', 'Ca. Phytoplasma prunorum', and 'Ca. Phytoplasma pyri' (three phytoplasmas in apple proliferation group of quarantine importance). Martini et al. (2007) and Yvon et al. (2009) developed a specific PCR and real-time PCR assay for 'Ca. Phytoplasma prunorum' in plants and insect vectors. Pignatta et al. (2008) developed a specific multiplex real-time PCR procedure that allows the simultaneous detection of ESFY phytoplasma and host DNA, in order to avoid false negatives due to PCR inhibition. Jarausch et al., (2010) developed a real-time PCR using ESFY phytoplasma specific primer pairs of ECA1/ECA2. Nicolic et al. (2010) used the specific AP, ESFY, and PD sets of primers/probes, UniRNA to confirm the presence of tested phytoplasmas and/or to detect possible other undetermined phytoplasma species, and 18S rRNA amplicon as an endogenous control for a quality of DNA extraction.

Easily Confused Species

The ESFY phytoplasma is phylogenetically closely related to the apple proliferation (AP) and pear decline (PD) phytoplasmas. The peach yellow leaf roll (PYLR) phytoplasma from California was found by Kison et al. (1997) to also be closely related to AP, PD,

and ESFY. The PYLR agent could clearly be distinguished from the AP and ESFY phytoplasmas by Southern blot hybridization with DNA fragments from the AP phytoplasma and by RFLP analysis of ribosomal DNA employing *SspI*, *BsaAI*, and *RsaI* restriction endonucleases. The PYLR phytoplasma, however, was indistinguishable from the PD phytoplasma by PCR-amplified ribosomal DNA (Kison et al., 1997).

Aldaghi et al. (2007) developed a real-time PCR protocol for 'Ca. Phytoplasma mali'. This probe could distinguish a single mismatch between 'Ca. Phytoplasma mali' and 'Ca. Phytoplasma prunorum', but late fluorescent curves were obtained from European stone fruit yellows isolates. Aldaghi et al., (2008) developed a new probe and adapted the original procedure to eliminate the late fluorescent curves.

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Update History:

April, 2014: Added Pathway section and updated Pest Importance section.

July, 2014: -Incorporated reviewer comments from Batlle, Jarausch, Serce, and Thébaud. Added orchard photographs to Figures 6 and 7.

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

July, 2016: Updated figure 8 maps.