

‘*Candidatus* Phytoplasma prunorum’

Scientific Name

‘*Candidatus* Phytoplasma prunorum’ Seemüller and Schneider, 2004

Synonyms:

Apricot chlorotic leaf roll, plum leptoncrosis, 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:** Achleplasmatales, **Family:** Achleplasmataceae

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

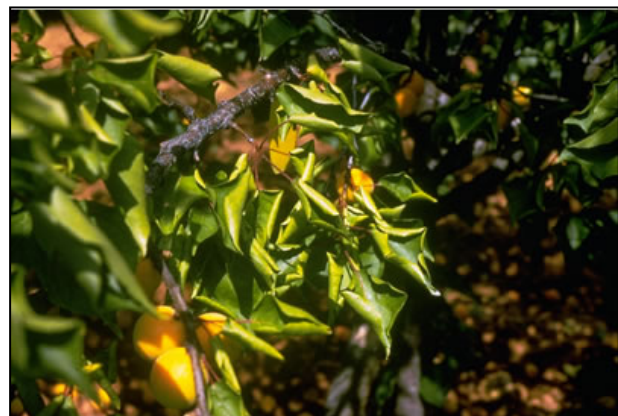


Figure 2. Leaf rolling symptom of ESFY in apricot. Photo courtesy of G. Morvan. EPPO.

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).

Jaraus 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.

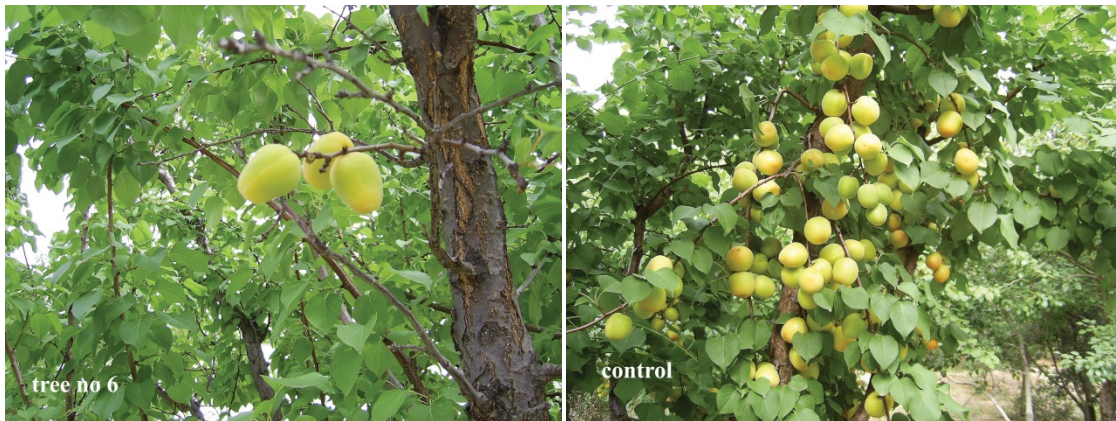


Figure 5: Fruit set of ‘*Ca. Phytoplasma prunorum*’ infected tree (left) compared with the control (non-infected) tree (right). Image from Gazel et al. (2009).

not clear, especially given that *C. pruni* is strictly oligophagous on *Prunus* species. Jarausch 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 (Marcone 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 Brompton, 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. coccinifolia* (coccinifolia) 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).

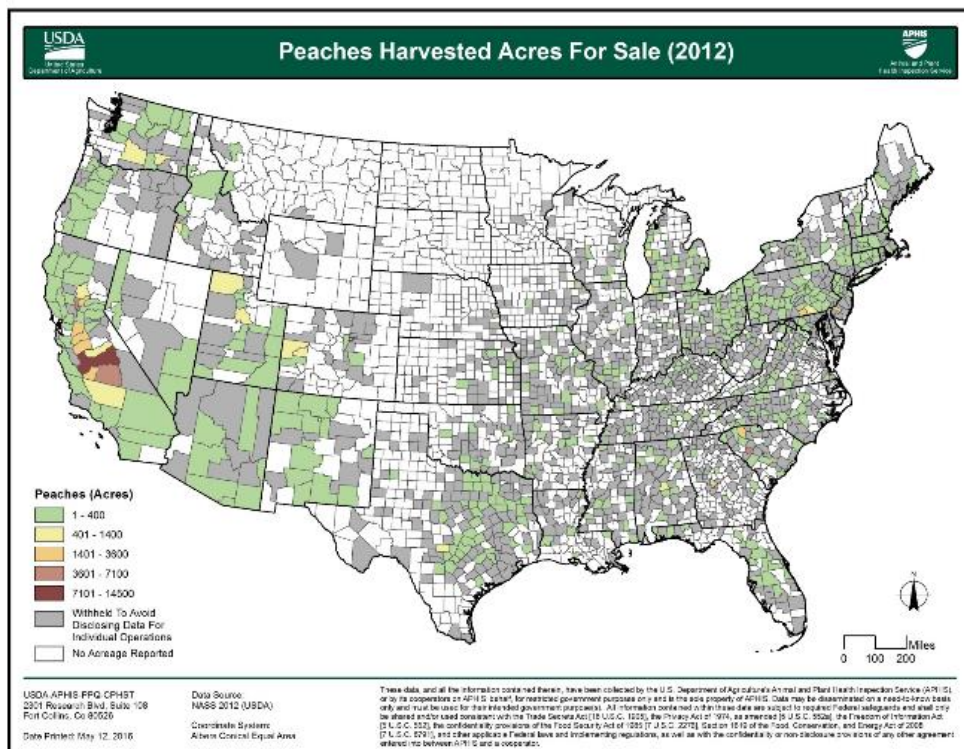
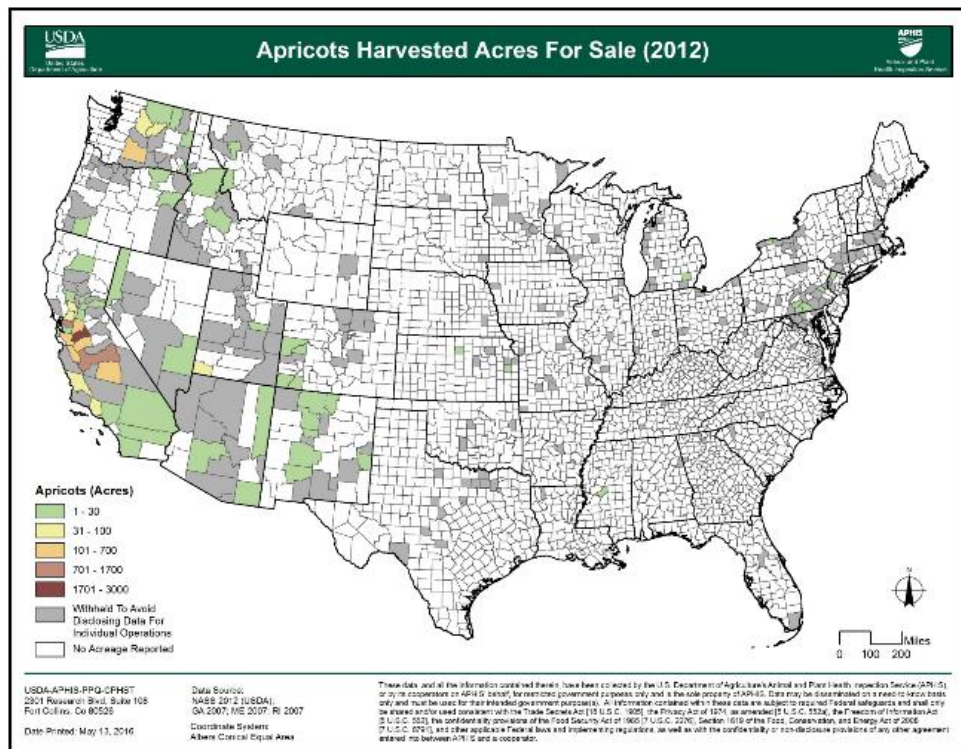


Figure 8. Commodity acreage maps for apricot and peach within the continental United States by county. Map courtesy of USDA-APHIS-PPQ-CPHST.

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 (Marcone 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 (Marcone 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.

References

Ahrens, U., and Seemüller, E. 1992. Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. *Phytopathology* 82(8): 828-832.

Aldaghi, M., Massert, S., Roussel, S., and Jijaki, M.H. 2007. Development of a new probe for specific and sensitive detection of *Candidatus* Phytoplasma mali in inoculated apple trees. *Annals of Applied Biology* 151: 251-258.

Aldaghi, M., Massert, S., Roussel, S., Dutrecq, O., and Jijaki, M.H. 2008. Adaptation of real-time PCR assay for specific detection of apple proliferation phytoplasma. *Acta Horticulturae* 781: 387-393.

Ambrozic Turk, B., Mehle, N., Brzin, J., Skerlavaj, V., Seljak, G., and Ravnikar, M. 2008. High infection pressure of ESFY phytoplasma threatens the cultivation of stone fruit species. *Journal Central European Agriculture* 9(4): 795-802.

AQAS. 2014. Agricultural Quarantine Activity Systems. Queried April 24, 2014 from, www.aqas.aphis.usda.gov/.

Balakishiyeva, G., Danet, J.L., Qurbanov, M., Mamedov, A., Kheyr-Pour, A., and Foissac, X. 2010. First report of phytoplasma infections in several temperate fruit trees and vegetables crops in Azerbaijan. *Journal of Plant Pathology* 92: S4.107-S4.122.

Bertolini, E., Torres, E., Olmos, A., Martin, M.P., Bertaccini, A., and Cambra, M. 2007. Co-operational PCR coupled with dot blot hybridization for detection of 16SrX grouping of phytoplasmas. *Plant Pathology* 56: 677-682.

CABI. 2009. Crop protection compendium: global module. Commonwealth Agricultural Bureau International, Wallingford, UK. <http://www.cabi.org/compendia/cpc/>.

Carraro, L., and Osler, R. 2003. European stone fruit yellows: a destructive disease in the Mediterranean Basin. In: Myrta, A., Di Terlizzi, B., and Savino, V. (eds). *Virus and virus-like diseases of stone fruit with particular reference to the Mediterranean region*, CIHEAM. Options Méditerranéennes Serie B 45: 113-117.

Carraro, L., Osler, R., Refatti, E., and Favali, M.A. 1992. Natural diffusion and experimental transmission of plum "leptoncrosis". *Acta Hort.* 285-290.

Carraro, L., Loi, N., Ermacora, P., and Osler, R. 1998a. High tolerance of European plum varieties to plum leptoncrosis. *European Journal of Plant Pathology* 104: 141-145.

- Carraro, L., Osler, R., Loi, N., Ermacora, P., and Refatti, E.** 1998b. Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni*. *Journal of Plant Pathology* 80: 233-239.
- Carraro, L., Loi, N., and Ermacora, P.** 2001a. Transmission characteristics of the European stone fruit yellows phytoplasma and its vector *Cacopsylla pruni*. *European Journal of Plant Pathology* 107: 695-700.
- Carraro, L., Osler, R., Loi, N., Ermacora, P., and Refatti, E.** 2001b. Fruit tree phytoplasma disease diffused in nature by psyllids. *Acta Hort* 550: 345-350.
- Carraro, L., Ferrini, F., Ermacora, P., and Loi, N.** 2002. Role of wild *Prunus* species in the epidemiology of European stone fruit yellows. *Plant Pathology* 51: 513-517.
- Carraro, L., Ferrini, F., Labonne, G., Ermacora, P., and Loi, N.** 2004a. Seasonal infectivity of *Cacopsylla pruni*, vector of European stone fruit yellows phytoplasma. *Ann. Appl. Biol.* 144: 191-195.
- Carraro, L., Ferrini, F., Ermacora, P., and Loi, N.** 2004b. Transmission of European Stone Fruit Yellows phytoplasma to *Prunus* species by using vector and graft transmission. *In: Llacer G. (ed.). ISHS Acta Horticultura: XIX International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops – Fruit Tree Diseases* 657: 449-453.
- Davies, D.L., and Adams, A.N.** 2000. European stone fruit yellows phytoplasmas associated with a decline disease of apricot in southern England. *Plant Pathology* 49: 635-639.
- Davis R., Zhao Y., dally E.L., Lee I.-M., Jomantiene R., and Douglas S.M.** 2013. ‘*Candidatus* Phytoplasma pruni’, a novel taxon associated with X-disease of stone fruits, *Prunus* spp.: multilocus characterization based on 16S rRNA, *secY*, and ribosomal protein genes. *International Journal of Systematic and Evolutionary Microbiology* 63:766-776.
- Delic, D., Martini, M., Ermacora, P., Myrta, A. and Carraro, L.** 2007. Identification of fruit tree phytoplasmas and their vectors in Bosnia and Herzegovina. *EPPO Bulletin* 37: 444-448.
- Deng, S., and Hiruki, C.** 1991. Amplification of 16S rRNA from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* 14: 53-61.
- Desvignes, J.C., and Cornaggia, D.** 1982. Observation on apricot chlorotic leaf roll (ACLR): sensitiveness of different *Prunus* species detection, spread in plum orchards. *Acta Hort* 130: 249-256.
- Dosba, F., Lansac, M., Mazy, K., Garnier, M., and Eyquard, J.P.** 1991. Incidence of different diseases associated with mycoplasma-like organisms in different species of *Prunus*. *Acta Hort* 283: 311-320.
- Doyle, J.J., and Doyle, J.B.** 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- EPPO.** 2014. PQR - EPPO database on quarantine pests (available online). <http://www.eppo.int>
- Ferrini, F., Carraro, L., Ermacora, P., and Loi, N.** 2002. Vector and graft transmission of European stone fruit yellows phytoplasma to *Prunus* species. *In: Proceeding ‘Il Incontro Nazionale sulle Malattie da Fitoplasmii’* Roma, 3-4 Oct. 2002. Pp: 22-23.
- Fialova, R., Navratil, M., Valova, P., Lauterer, P., Kocourek, F., and Poncarova-Vorackova, Z.** 2004. Epidemiology of *European Stone Fruit Yellows* phytoplasma in the Czech. Republic. *Act Hor.* 658: 483-487.
- Fialova, R., Navratil, M., Lauterer, P., and Navrkalova, V.** 2007. ‘*Candidatus* Phytoplasma prunorum’: the phytoplasma infection of *Cacopsylla pruni* from apricot orchards and from overwintering habitats in Moravia (Czech. Republic). *Bulletin of Insectology* 60(2): 183-184.

- Gazel, M., Caglayan, K., Serce, C.U., and Son, L.** 2009. Evaluations of apricot trees infected by *Candidatus Phytoplasma prunorum* for horticultural characteristics. *Romanian Biotechnological Letters* 14(1): 4123-4129.
- Gibb, K.S., Constable, F.E., Moran, J.R., and Padovan, A.C.** 1999. Phytoplasmas in Australian grapevines - detection, differentiation and associated diseases. *Vitis* 38(3): 107-114.
- Giunchedi, L., Poggli Pollini, D., and Credi, R.** 1982. Susceptibility of stone fruit trees to the Japanese plum tree decline causal agent. *Acta Hort.* 130: 285-290.
- Green, M.J., Thompson, D.A., and MacKenzie, D.J.** 1999. Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. *Plant. Dis.* 83: 482-485.
- Gundersen, D.E., and Lee, I.-M.** 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopath. Medit.* 35: 144-151.
- Harrison, N.A., Gundersen-Rindal, D., and Davis, R.E.** 2011. Genus I. "*Candidatus Phytoplasma*" gen. nov. IRPCM.
- Harrison, N.A., Davis, R.E., Oropeza, C., Helmick, E.E., Narvaez, M., Eden-Green, S., Dollet, M., and Dickinson, M.** 2014. '*Candidatus Phytoplasma palmicola*', a novel taxon associated with a lethal yellowing-type disease (LYD) of coconut (*Cocos nucifera* L.) in Mozambique. *International Journal of Systematic and Evolutionary Microbiology* 64: *In Press*. doi:10.1099/ijs.0.060053-0. <http://ijs.sgmjournals.org/content/early/2014/02/28/ijs.0.060053-0.full.pdf>.
- Heinrich, M., Botti, S., Caprara, L., Arthofer, W., Strommer, S., Hanzer, V., Katinger, H., Bertaccini, A., Machada, M.L.D.C.** 2001. Improved detection methods for fruit tree phytoplasmas. *Plant Molecular Biology Reporter* 19: 169-179.
- IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (IRPCM).** 2004. '*Candidatus Phytoplasma*', a taxon for wall-less, non-helical prokaryotes that colonize plant phloem and insects. *International Journal of Systemic and Evolutionary Microbiology* 54: 1243-1255.
- Jarausch, W., Eyquard, J.P., Lansac, M., Mohns, M., and Dosba, F.** 2000a. Susceptibility and tolerance of new French *Prunus domestica* cultivars to European stone fruit yellows phytoplasma. *J. Phytopathology* 148: 489-493.
- Jarausch, W., Eyquard, J.P., Mazy, K., Lansac, M., and Dosba, F.** 1999a. High levels of resistance of sweet cherry (*Prunus avium* L.) towards European stone fruit yellows phytoplasmas. *Advances in Horticultural Science* 13: 160-173.
- Jarausch, W., Fuchs, A., and Jarausch, B.** 2010. Establishment of a quantitative real-time PCR assay for the specific quantification of *Ca. Phytoplasma prunorum* in plants and insects. *Julius-Kühn-Archiv*, 427, retrieved from, http://www.jki.bund.de/fileadmin/dam_uploads/veroeff/JKI_Archiv/JKI_Archiv_427.pdf.
- Jarausch, W., Jarausch-Wehrheim, B., Danet, J.L., Broquaire, J.M., Dosba, F., Saillard, C., and Garnier, M.** 2001. Detection and identification of European stone fruit yellows and other phytoplasmas in wild plants in the surroundings of apricot chlorotic leaf roll-affected orchards in southern France. *European Journal of Plant Pathology* 107: 209-217.
- Jarausch, W., Lansac, M., and Dosba, F.** 1999b. Seasonal colonization pattern of European stone fruit yellows phytoplasma in different *Prunus* species detected by specific PCR. *J. Phytopathology* 147: 47-54.

- Jarausch, W., Lansac, M., Saillard, C., Broquaire, J.M., and Dosba, F.** 1998. PCR assay for specific detection of European stone fruit yellows phytoplasma and its use for epidemiological studies in France. *European Journal of Plant Pathology* 104: 17-27.
- Jarausch, B., Mühlenz, I., Beck, A., Lampe, I., Harzer, U., and Jarausch, W.** 2008. Epidemiology of European Stone Fruit Yellows in Germany. *Acta Hort.* 781: 417-422.
- Jarausch, W., Saillard, C., Broquaire, J.M., Garnier, M., and Dosba, F.** 2000b. PCR-RFLP and sequence analysis of a non-ribosomal fragment for genetic characterization of European stone fruit yellows phytoplasmas infecting various *Prunus* species. *Molecular and Cellular Probes* 14: 171-179.
- Khalifa, M. B. and Fakhfakh, H.** 2011. First report of *Candidatus* Phytoplasma prunorum infecting almonds in Tunisia. *Phytoparasitica* DOI: 10.1007/s12600-011-0166-4.
<http://www.springerlink.com/content/tw46568665024550/fulltext.pdf>.
- Kirkpatrick, B.C., Stenger, D.C., Morris, T.J., and Purcell, A.** 1987. Cloning and detection of DNA from a nonculturable plant pathogenic mycoplasma-like organism. *Science* 238: 197-200.
- Kison, H., and Seemüller, E.** 2001. Differences in strain virulence of European Stone Fruit Yellows phytoplasma and susceptibility of stone fruit trees on various rootstocks to the pathogen. *J. Phytopathology* 149: 533-541.
- Kison, H., Kirkpatrick, C., and Seemüller, E.** 1997. Genetic comparison of the peach yellow leaf roll agent with European fruit tree phytoplasmas of the apple proliferation group. *Plant Pathology* 46: 538-544.
- Lauterer P.** 1999. Results of the investigations on Hemiptera in Moravia, made by the Moravian Museum (Psylloidea 2). *Acta Musei Moraviae, Scientiae biologicae* 84:71-151.
- Lee, I.M., Bertaccini, A., Vibio, M., and Gundersen, D.E.** 1995. Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. *Phytopathology* 85: 728-735.
- Lee, I.M., Gundersen-Rindal, D.E., Davis, R.E., and Bartoszyk, I.M.** 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* 48(4): 1153-1169.
- Lee, I.M., Davis, R.E., Gundersen-Rindal, D.E.** 2000. Phytoplasma: Phytopathogenic Mollicutes. *Ann. Rev. Microbiol.* 54: 221-255.
- Lorenz, K.H., Dosba F., Poggi Pollini, C., Llacer, G., and Seemüller, E.** 1994. Phytoplasma diseases of *Prunus* species in Europe are caused by genetically similar organisms. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 101: 567-575.
- Machado, M.L.D.C., Paltrinieri, S., Hanzer, V., Arthofer, W., Strommer, S., Martini, M., Pondrelli, M., and Bertaccini, A.** 2001. Presence of European stone fruit yellows (ESFY or 16SrX-B) phytoplasmas in apricots in Austria. *Plant Pathology* 50: 130-135.
- Maixner, M., Ahrens, U., and Seemüller, E.** 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. *European Journal of Plant Pathology* 101: 241-250.
- Malisano, G., Firraro, G., and Locci, R.** 1996. 16S rDNA-derived oligonucleotide probes for the differential diagnosis of plum leptonecrosis and apple proliferation phytoplasmas. *EPPO Bulletin* 26: 421-428.

Marcone, C., Jarausch, B., Jarausch, W. 2010. 'Candidatus Phytoplasma prunorum', the causal agent of European stone fruit yellows: an overview. *Journal of Plant Pathology* 92 (1): 19-34.

Marcone, C., and Seemüller, E. 2001. A chromosome map of the European stone fruit yellows phytoplasma. *Microbiology* 147: 1213-1221.

Marcone, C., Raggozzino, A., and Seemüller, E. 1996. European stone fruit yellows phytoplasma as the cause of peach vein enlargement and other yellows and decline diseases of stone fruits in southern Italy. *Phytopathology* 144: 559-564.

Martini, M., Loi, N., Ermacora, P., Carraro, L., and Pastore, M. 2007. A real-time PCR method for detection and quantification of 'Candidatus Phytoplasma prunorum' in its natural host. *Bulletin of Insectology* 69(2): 251-252.

Maskova, V., Krska, B., and Necas, T. 2009. Comparison of methods of isolating DNA for ESFY phytoplasma detection. *Acta Hort* 825:213-220.

McCoy, R.E., Caudwell, A., Chang, C.J., Chen, T.A., Chykowski, L.N., Cousin, M.T., Dale, J.L., de Leeuw, G.T.N., Golino, D.A., Hackett, K.J., Kirkpatrick, B.C., Marwitz, R., Petzold, H., Sinha, R.C., Sugiura, M., Whitcomb, R.F., Yang, I.L., Zhu, B.M., and Seemüller E. 1989. Plant diseases associated with mycoplasma-like organisms. In: *The Mycoplasmas*, Vol. 5. Whitcomb, R.F., and Tully, J.G. (ed.). Academic Press, New York.

Mehle, N., Brzin, J., Boben J., Hren, M., Frank, J., Pertovik, N., Gruden, K., Dreo, T., Zezlina, I., Seljak, G., and Ravnika, M. 2007. Pregled rezultatov dolocanja fitoplazem na koscicarjih v letih 2000-2006 v Sloveniji (The result of phytoplasma testing in stone fruits (*Prunus* spp.) in Slovenia (2000-2006)). *Radenci*, 6-7, Marec. 2007. 139-143.

Morvan, G. 1977. Apricot chlorotic leaf roll. *EPPO Bulletin* 7: 37-55.

Morvan, G., and Castelain C. 1972. Induction d'une résistance de l'abricotier à l'enroulement chlorotique par le greffage sur *Prunus spinosa* L. *Annales de Phytopathologie* 4:418 (Abstr.).

Myrta, A., Ermacora, P., Stamo, B., and Osler, R. 2003. Identification of European Stone Fruit Yellows from apricot and Japanese plum in Albania. In: Myrta, A., Di Terlizzi, B., and Savino, V. (eds). *Virus and virus-like diseases of stone fruit with particular reference to the Mediterranean region*, CIHEAM. Options Méditerranéennes Serie B 45: 119-121.

Navratil, M. Valova, P., Fialova, R., Petrova, K., Franova, J., Nebesarova, J., Poncarova-Vorackova, Z., and Karesova, R. 2001. Survey for stone fruit phytoplasmas in the Czech Republic, *Acta Hort*. 550: 377-382.

Necas, T., and Krska, B. 2005. Detection of phytoplasma ESFY in apricot trees using phloem and petioles. *Plant Protect. Sci.* 41: 132-140.

Necas, T., and Krska, B. 2006. Selection of woody indicators and optimum plant material and sampling time for phytoplasma ESFY detection. In: Romojaro, Dicenta, F., and Martinez-Gomez, P. *Proc. XIIIth IS on Apricot Breeding and Culture*. *Acta Hort* 717: 101-104.

Nemeth, M. 1986. Apricot chlorotic leaf roll. In: Kiada, A.T. (ed.) *Virus, Mycoplasma, and Rickettsia Diseases of Fruit Trees*. Budapest, Hungary. pp: 626-633.

Nikolic, P., Mehle, N., Gruden, K., Ravnika, R. Dermastia, M. 2010. A panel of real-time PCR assays for specific detection of three phytoplasmas from the apple proliferation group. *Molecular and Cellular Probes* 24(5): 303-309.

Pastore, M., Paltrinieri, S., Raffone, E., Simone, A.M., Priore, R., and Bertacini, A. 2002. Preliminary results of a study performed in Campania and Latium to evaluate fruit tree phytoplasma transmission ability of *Empoasca decedens* Paoli. *Petria* 12(3): 421-422.

Pastore, M., Raffone, E., Santonastaso, M., Priore, R., Paltrinieri, S., Bertaccini, A., and Simeone, A.M. 2004. Phytoplasma detection in *Empoasca decedens* and *Empoasca* spp. and their possible role as vectors of European stone fruit yellows (16SrX-B) phytoplasma. *Acta Hort* 657: 507-511.

Peccoud, J., Labonne, G., and Sauvion, N. 2013. Molecular Test to Assign Individuals within the *Cacopsylla pruni* Complex. *PLoS ONE* 8(8): e72454. doi:10.1371/journal.pone.0072454

Pignatta, D., Poggi Pollini, C., Giunchedi, L., Ratti, C., Reggiani, N., Gobber, M., Miorelli, P., Forno, F., Mattedi, L., and Ropelato, E. 2008. A real-time PCR assay for the detection of European Stone Fruit Yellows phytoplasma (ESFYP) in plant propagation material. *Acta Hort*. 781: 499-503.

Poggi Pollini, C., Bissani, R., Giunchedi, L., and Vindimian, E. 1995. Occurrence of phytoplasma infection in European plums (*Prunus domestica*). *J. Phytopathology* 143(11-12): 701-703.

Poggi Pollini, C., Bissani, R., Mordenti, G.L., Giunchedi, L., Cravedi, P., and Nicoli Aldini, R. 1996. Reperimento di fitoplasmi in cicadellidi mediante la tecnica della nest-PCR. Abstract Convegno SIPaV, Udine: C80.

Poggi Pollini, C., Giunchedi, L., and Bissani, R. 1997. Immunoenzymatic detection of PCR products for the identification of phytoplasmas in plants. *J. Phytopathology* 145: 371-374.

Poggi Pollini, C., Bissani, R., and Glunchedi, L. 2001. Occurrence of European stone fruit yellows phytoplasma (ESFYP) infection in peach orchards in Northern-Central Italy. *Journal of Phytopathology* 149(11-12): 725-730.

Polak, J., Salava, J., Bryxiova, M., and Svoboda, J. 2007. Problems in detection of European stone fruit yellows phytoplasma in apricot trees in the Czech. Republic. *Bulletin of Insectology* 60(2): 261-262.

Quaglino F., Zhao Y., Casati P., Bulgari D., Bianco P.A., Wei W., and Davis R.E. 2013. '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur and bois noir related diseases of plants. *International Journal of Systematic and Evolutionary Microbiology* doi:1099/ijs.0.044750-0

Ramel, M.E., and Gugleri, P. 2004. Epidemiological survey of European stone fruit yellows phytoplasma in two orchards in western Switzerland. *Acta Hort* 657: 459-463.

Rubio-Cabetas, M.J., and Sancho, S. 2009. Detection and identification of '*Candidatus* Phytoplasma prunorum' in *Prunus* germplasm. *Spanish Journal of Agriculture Research* 7(2): 439-446.

Sabaté J., Laviña A., and Batlle, A. 2007. Survey of *Cacopsylla pruni* in different fruit tree producing areas of Spain. *Bulletin of Insectology* 60 (2): 193-194.

Sabaté, J., Laviña, A., and Batlle, A. 2014. Incidence and spread of '*Candidatus* Phytoplasma prunorum' and its vector *Cacopsylla pruni* in wild and cultivated *Prunus* in Spain. *Plant pathology* (In press).

Schneider, B., Seemüller, E., Smart, C.D., Kirkpatrick, B. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S., and Tully, J.D. (eds.) *Molecular and Diagnostic Procedures in Mycoplasmatology*. Pp. 369-380. Academic Press, San Diego, CA.

Seemüller, E. 1976. Investigation to demonstrate mycoplasma-like organisms in diseased plants by fluorescence microscopy. *Acta Hort* 67: 109-112.

- Seemüller, E., and Schneider, B.** 2004. '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri', and '*Candidatus* Phytoplasma prunorum', the causal agents of apple proliferation, pear decline, and European stone fruit yellows, respectively. *International Journal of Systemic and Evolutionary Microbiology* 54: 1217-1226.
- Seemüller, E., Stolz, H., and Kison, H.** 1998. Persistence of the European stone fruit yellows phytoplasma in aerial parts of *Prunus* taxa during the dormant season. *J. Phytopathology* 146: 407-410.
- Seemüller, E., Garnier, M., and Schneider, B.** 2002. Mycoplasmas of plants and insects. in *Molecular Biology and Pathogenicity of Mycoplasmas*, S. Razin and R. Herrmann eds. Kluwer Academic/Plenum Publishers, Dordrecht, Netherlands Pgs. 91-116.
- Sertkaya, G., Martini, M., Ermacora, P., Musetti, R., Osler, R.** 2005. Detection and characterization of phytoplasmas in diseased stone fruits and pear by PCR-RFLP analysis in Turkey. *Phytoparasitica* 33(4): 380-390.
- Smart, C.D., Schneider, B., Blomquist, C.L., Guerra, L.J., Harrison, N.A., Ahrens, U., Lorenz, K.-H., Seemüller, E., and Kirkpatrick, B.C.** 1996. Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Applied and Environmental Microbiology* 62(8): 2988-2993.
- Thébaud, G., Sauvion, N., Chadoeuf, J., Dufils, A., and Labonne, G.** 2006. Identifying risk factors for European stone fruit yellows from a survey. *Phytopathology* 96: 890-899.
- Thébaud, G., Yvon, M., Alary, R., Sauvion, N., and Labonne, G.** 2009. Efficient transmission of '*Candidatus* Phytoplasma prunorum' is delayed by eight months due to a long latency in its host-alternating vector. *Phytopathology* 99: 265-273.
- Topchiiska, M., Marccone, C., and Seemüller, E.** 2000. Detection of pear decline and European stone fruit yellows in Bulgaria. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Diseases and Protection)* 107(6): 658-663.
- Torres, E., Martin, M.P., Paltrinieri, S., Vila, A., Masalles, R., and Bertaccini, A.** 2004. Spreading of ESFY phytoplasmas in stone fruit in Catalonia (Spain). *J. Phytopathology* 152: 432-437.
- Torres, E., Bertolini, E., Cambra, M., Monton, C., and Martin, M.P.** 2005. Real-time PCR for simultaneous and quantitative detection of quarantine phytoplasmas from apple proliferation (16SrX) group. *Molecular and Cellular Probes* 19: 334-340.
- USDA.** 2013. Plants for Planting Manual. Updated July 20, 2013. Retrieved from, http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/plants_for_planting.pdf.
- USDA-PCIT.** 2014. Phytosanitary Certificate Issuance & Tracking System. *Candidatus* Phytoplasma prunorum. Queried April 24, 2014 from, <https://pcit.aphis.usda.gov/PExD/faces/PExDReport.jsp>.
- Varga, K., Kolber, M., Martini, M.** 2000. Phytoplasma identification in Hungarian grapevines by two nested-PCR systems. Extended Abstracts of XIIIth Meeting of the International Council for the Study of Viruses and Virus-like Diseases of the Grapevine (ICVG). Adelaide, Australia, 12-17 March. Pp: 113-115.
- Verbeek, I. M. (Project leader).** 2008. **Project: Vectors of phytoplasma diseases in Dutch orchards.** Dutch Research Database (NOD). (2008, October 16). <http://www.onderzoekinformatie.nl/en/oi/nod/onderzoek/OND1331163/>.
- Waterworth, H.E., and Mock, R.** 1999. An assessment of nested PCR to detect phytoplasmas in imported dormant buds and intermodal tissues of quarantined fruit tree germplasm. *Plant Dis.* 83: 1047-1050.

Weintraub, P.G., and Beanland, L. 2006. Insect vectors of phytoplasmas. Annual Review of Entomology 51: 91-111.

Yvon, M. Thébaud, G., Alary, R., and Labonne, G. 2009. Specific detection and quantification of the phytopathogenic agent '*Candidatus Phytoplasma prunorum*'. Molecular and Cellular Probes 23: 227-234.

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Reviewers:

Dr. Assumpcio Batlle, IRTA, Catalunya, Spain.

Dr. Robert Davis, USDA Agricultural Research Service, Beltsville, MD.

Dr. Wolfgang Jarausch, AlPlanta - Institute for Plant Research, Germany

Dr. Cigdem Ulubas Serce, Nigde University, Turkey

Dr. Gaël Thébaud, INRA, France

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.