

# EUROPEAN STONE FRUIT YELLOWS DISEASE AND ITS CAUSAL AGENT 'CANDIDATUS PHYTOPLASMA PRUNORUM'

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**Abstract:** The European stone fruit yellows (ESFY) caused by '*Candidatus Phytoplasma prunorum*' ('*Ca. P. prunorum*'), is a devastating, quarantine phytoplasma disease. This agent leads to serious damage in apricot and Japanese plum orchards, including decline of infected trees. The consequences are considerable economic losses in production. European plum and cherry are less susceptible to ESFY, although the '*Ca. P. prunorum*' was also detected in these trees in several countries. ESFY seems to be limited to Europe and the neighboring regions (Asia Minor) as its incidence was reported from most of the southern and central European countries, Turkey, and Azerbaijan. '*Ca. P. prunorum*' is transmitted by *Cacopsylla pruni* (Scopoli). The impact of '*Ca. P. prunorum*' on stone fruit trees, as well as the methods of detection and identification and control management are reviewed.

**Key words:** 16SrX, geographic distribution, hosts, symptoms, vectors, detection, identification, control

## INTRODUCTION

Phytoplasmas are wall-less, non-helical, Gram-positive prokaryotes which are associated with diseases of several hundreds of plant species in tropical, sub-tropical, and temperate regions of the world (Lee *et al.* 2000). They are obligate parasites of plant phloem tissue and transmitting insects (vectors). In 1992, the Subcommittee on the Taxonomy of Mollicutes decided on the use of the name Phytoplasma in place of the term mycoplasma-like organism (MLO) (ICSB/STM 1993). The name phytoplasma was adopted, and currently the *Candidatus* status (IRPCM 2004) name is used for non-culturable bacteria. Phytoplasmal diseases of pome and stone fruits are economically important in some areas of Europe and North America. Up until now, twenty-eight phytoplasma species have been formally described and 15 other phytoplasma strains are potentially new '*Candidatus Phytoplasma*' species with pairwise sequence similarity scores ranging from 97.6 to 99% (Zhao *et al.* 2010). Several phytoplasmas may infect *Prunus* sp. plants. The most common among them is '*Candidatus Phytoplasma prunorum*' ('*Ca. P. prunorum*') causing European stone fruit yellows (ESFY) disease. ESFY leads to economic damage in *Prunus* species throughout Europe and Asia Minor. The issues regarding ESFY and its causal agent '*Ca. P. prunorum*' are reviewed in this paper. Less common phytoplasmas infecting stone fruit trees in different regions of the world will be described in another paper.

### European stone fruit yellows phytoplasmas

Diseases of stone fruits associated with phytoplasma infection including apricot chlorotic leaf roll (Morvan

1977), plum leptonecrosis (Giunchedi *et al.* 1978), peach yellowing (Poggi Pollini *et al.* 1993), and declining of plum, peach and almond (Lederer and Seemüller 1992; Poggi Pollini *et al.* 1993, 1995), were found to have a common etiology. It was decided to give them a single name: European stone fruit yellows (ESFY) (Lorenz *et al.* 1994). Further studies have shown that the disease is associated with the '*Candidatus Phytoplasma prunorum*' infection (Seemüller and Schneider, 2004). The issues regarding ESFY disease and its causal agent were discussed in several papers (Carraro and Osler 2003; Kamińska 2008; Marcone *et al.* 2010; Sullivan 2010).

The 16S rDNA sequence data indicated that '*Ca. P. prunorum*' belongs to subgroup B of the apple proliferation (AP) phytoplasma group (16SrX) beside '*Candidatus Phytoplasma mali*' (16SrX-A) inducing apple proliferation (AP), and '*Candidatus Phytoplasma pyri*' – the causal agent of pear decline (PD) and peach yellow leaf roll (PYLR) diseases. Both are classified to subgroup C of the 16SrX group (Seemüller *et al.* 1998a; Lee *et al.* 1998). However, these agents are distinguished by their biological, serological and molecular properties and they are transmitted by different vectors. Pairwise, 16S rRNA gene sequence identity scores among these three agents are between 98.6 and 99% (Zhao *et al.* 2010), with differences in the 16S rDNA sequences of AP/PD, AP/ESFY and PD/ESFY phytoplasmas 1.0–1.1, 1.3–1.5 and 1.2–1.3%, respectively (Seemüller and Schneider 2004). Sequence dissimilarities in the *Imp* gene of 36% between '*Ca. P. prunorum*' and '*Ca. P. mali*', 29% between '*Ca. P. prunorum*' and '*Ca. P. pyri*' and 28% between '*Ca. P. mali*' and '*Ca. P. pyri*' were

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found. Respective values for the *aceF* marker were 11, 12 and 10%, for *pnp* marker – 7, 6 and 5%, and for *secY* gene – 8, 7 and 10% (Danet *et al.* 2007). These three phytoplasmas share 94.3–94.6% of *rp* gene sequence similarity (Martini *et al.* 2007). As the agents cause economically devastating fruit tree diseases, they are listed by European and Mediterranean Plant Protection Organization (EPPO) as quarantine organisms.

### Natural hosts and geographic distribution

Wild and cultivated *Prunus* sp. plants are the natural hosts of ‘*Candidatus Phytoplasma prunorum*’ (Lederer and Seemüller 1992; Navrátil *et al.* 2001; Carraro *et al.* 2002; Laviña *et al.* 2004). Carraro *et al.* (2004) showed that the most common natural hosts of ‘*Ca. P. prunorum*’ were apricot (*Prunus armeniaca* L.), cherry plum (*P. cerasifera* Ehrh.), European plum (*P. domestica* L.), peach (*P. persica* L.), Japanese plum (*P. salicina* Lindley), and blackthorn (*P. spinosa* L.). Sweet cherry (*P. avium* L.) and mahaleb (*P. mahaleb* L.) were considered as non-common natural hosts of the agent. It was found that wild *Prunus* spp. (*P. spinosa*, *P. cerasifera*, *P. amygdalus*, *P. armeniaca*, *P. domestica*) were naturally infected by ‘*Ca. P. prunorum*’ (Jarausch *et al.* 2001; Carraro *et al.* 2002). Plants of the other species were also reported to be natural hosts of ‘*Ca. P. prunorum*’. The pathogen was detected in France in wild growing hackberry (*Celtis australis* L.), ash (*Fraxinus excelsior* L.) and dog rose (*Rosa canina* L.) (Jarausch *et al.* 2001) as well as in grapevine in Hungary (Varga *et al.* 2000) and Serbia (Duduk *et al.* 2004). Previous findings of European stone fruit yellows phytoplasma presence in red currant in the Czech Republic were not confirmed (Navrátil *et al.* 2007).

The incidence of ‘*Ca. P. prunorum*’ depends on the region and species of the tree. ESFY is mainly known in Europe and severe outbreaks were observed over the years especially in the Mediterranean basin (Morvan 1977; Giunchedi *et al.* 1982; Rumbos and Bosalidis 1985; Lederer and Seemüller 1992; Poggi Pollini *et al.* 1993, 1995; Marcone *et al.* 1996; Mornado *et al.* 1998; Desvignes *et al.* 1999; Paltrinieri *et al.* 2001, 2004, 2008; Laviña *et al.* 2004; Torres *et al.* 2004). ‘*Ca. P. prunorum*’ was also detected in the other regions where the stone fruits were grown – the Czech Republic (Navrátil *et al.* 2001), Austria (Laimer da Camara Machado *et al.* 2001), England (Davies and Adams 2000), Bulgaria (Topchiiska *et al.* 2000), Hungary (Süle 1999), Romania (Ionica 1985), Slovenia (Brzin *et al.* 2001), Switzerland (Ramel *et al.* 2001), Bosnia-Herzegovina (Delić *et al.* 2008), Poland (Cieślińska and Morgaś 2010, 2011), Azerbaijan and Turkey (Jarausch *et al.* 2000).

### Symptomatology and economical impact of ESFY

Infected trees may show numerous symptoms such as chlorotic leaf roll, leaf deformation, reduced terminal growth, necrosis on the cross section of trunk and branches, die-back and decline (Morvan 1977; Giunchedi *et al.* 1982; Lederer and Seemüller 1992; Marcone *et al.* 1996; Desvignes *et al.* 1999; Poggi Pollini *et al.* 2001; Torres *et al.* 2004). Apricot, peach and Japanese plum are among the fruit trees species which are most sensitive to ESFY (Kison and Seemüller 2001). ESFY causes considerable economic losses due to the high mortality of apricot and Japanese

plum trees (Morvan 1977; Giunchedi *et al.* 1982). The infected trees of apricot and Japanese plum show yellows and leaf roll followed by leaf reddening, necrosis, and decline. The symptoms on peach are milder and consist of premature reddening of the leaves, leaf roll and vein enlargement (Marcone *et al.* 1996; Poggi Pollini *et al.* 2001). European plum shows high tolerance to ESFY (Desvignes and Cornaggia 1982; Carraro *et al.* 1998a; Jarausch *et al.* 2000; Kison and Seemüller 2001), however, the trees of some cultivars may show mild symptoms (Jarausch *et al.* 2000; Paltrinieri *et al.* 2004). *Prunus cerasifera*, *P. mahaleb*, *P. padus* L. (bird cherry), *P. spinosa*, and *P. tomentosa* Thunb. (nanking cherry) are highly tolerant to ESFY and rarely indicate any symptoms (Kison and Seemüller 2001), however Carraro *et al.* (2002) reported that *P. cerasifera*, *P. spinosa*, and *P. tomentosa* were fairly susceptible. Ermacora *et al.* (2010) indicated that in the trial conditions *P. salicina*, *P. armeniaca*, *P. persica* and *P. tomentosa* were most susceptible to ESFY and the authors confirmed the high tolerance of *P. domestica*, *P. cerasifera*, and *P. spinosa* to this disease. It is known that infection with ‘*Ca. P. prunorum*’ does not lead to devastation of sweet and sour cherry orchards, as these trees are latent infected or show only mild symptoms (Giunchedi *et al.* 1982; Jarausch *et al.* 1999; Kison and Seemüller 2001).

Jarausch *et al.* (1998), using primers designed specifically for ESFY phytoplasma isolates, detected this agent in 82% of examined samples collected from orchards. These were orchards representing the major stone fruit growing regions of France. A relatively high incidence of this phytoplasma was also noted in apricot (about 80% of infected trees) in some regions of Germany (Jarausch *et al.* 2004), and in Japanese plum (25–80% of infected trees) grown in Spain (Laviña *et al.* 2004; Torres *et al.* 2004). Depending on rootstock, cultivar and age of the trees, the incidence of ESFY in apricot orchards in Austria range from 5 to 40% (Desvignes *et al.* 1999; Laimer da Camara Machado *et al.* 2001; Torres *et al.* 2004) and over 74% in Switzerland (Genini and Ramel 2004). In some orchards in Italy, nearly 93% of apricot and 40% of plum were infected with the ‘*Ca. P. prunorum*’ (Pastore *et al.* 1999; Poggi Pollini *et al.* 1995). Carraro *et al.* (1992) showed that 50–70% of plum trees in northeastern Italy became infected within 3–4 years of planting. Despite the fact that ‘*Ca. P. prunorum*’ was mainly detected in tested *Prunus* sp. trees, it does not seem to be widespread in Poland. Depending on the tree species, the phytoplasma was detected in 3–9% of the tested samples from Poland. (Cieślińska and Morgaś 2010).

### Transmission

‘*Ca. P. prunorum*’ is transmitted by plum psyllid [*Cacopsylla pruni* (Scopoli)] (Carraro *et al.* 1998b; Jarausch *et al.* 2001; Fialová *et al.* 2004; Ferretti *et al.* 2010) in a persistent manner (Carraro *et al.* 2001). The psyllid vector completes one generation per year. Adult forms of *C. pruni* overwinter on conifers and at the end of winter move to stone fruit trees where the new generation feeds from May till the beginning of July. The minimum acquisition period of plum psyllid is 2–4 days; the minimum latent period is 2–3 weeks; and the minimum inoculation period is 1–2 days. The infectivity retention of the vector lasts through

the winter and the following spring (Carraro *et al.* 2001). It took 4–5 months for *Prunus* plants to show typical ESFY symptoms after phytoplasma transmission by *C. pruni* (Carraro *et al.* 1998b). Naturally infected individuals of *C. pruni* were found in Italy (Carraro *et al.* 1998b), France (Yvon *et al.* 2004), Spain (Laviña *et al.* 2004), the Czech Republic (Fialová *et al.* 2004), Germany (Jarausch *et al.* 2007), and Bosnia-Herzegovina (Delić *et al.* 2008). In high pressure infection the annual rate of newly infected plants can reach 20% (Carraro *et al.* 1992).

### Detection and identification

In the past, before molecular techniques were developed, the diagnosis of phytoplasma diseases was difficult and based mainly on observation of symptoms. Although grafting the fragments of tested trees onto *Prunus persica* GF305 indicator is very useful (Desvignes *et al.* 1999), it is time consuming and difficult to apply on a large scale. The first symptoms usually appear 4–5 months or one year after grafting in the greenhouse and in the field, respectively (Desvignes *et al.* 1999; Németh *et al.* 2001).

Ultra-thin sections of the phloem tissue from suspected phytoplasma infected stone fruit trees, were positively examined for phytoplasmas by fluorescence microscopy (Lederer and Seemüller 1992; Desvignes *et al.* 1999; Carraro *et al.* 1998b; Thakur *et al.* 1998) and electromicroscopy (Verdin *et al.* 2003). A Southern blot hybridization assay was applied for differentiation of phytoplasmas belonging to apple proliferation aster yellows, and X-disease groups (Lee *et al.* 1992; Kison *et al.* 1997; Kison and Seemüller 2001).

Phytoplasma diagnostic is routinely based on the 16S rRNA gene and 16S-23S rRNA spacer region using Polymerase Chain Reaction/Restriction Fragment Length Polymorphism (PCR/PFLP) analysis. Genes encoding 16S ribosomal DNAs are highly conserved and the wide range of primers were designed for amplification of their fragments. Routinely, two-step PCR protocols (nested PCR) with two different primer pairs are used for amplification of phytoplasmal DNA fragment. This is because concentration of these agents in infected plants is usually very low. The first amplification is usually conducted with phytoplasma-universal primers P1/P7 (Deng and Hiruki 1991; Schneider *et al.* 1995) followed by the PCR with nested-universal primers R16F2n/R16R2 (Lee *et al.* 1993; Gundersen and Lee 1996). 'Ca. P. prunorum', in contrast to 'Ca. P. mali' and 'Ca. P. pyri', may be detected in naturally infected trees from spring until late autumn (Carraro *et al.* 1998a; Seemüller *et al.* 1998b; Jarausch *et al.* 1999; Davies and Adams 2000).

Some of the primers used in nested PCR are highly specific for 'Ca. P. prunorum' (Jarausch *et al.* 1998; Seemüller and Schneider 2004). The others are 'universal' for phytoplasmas and require an RFLP analysis which allows for the accurate identification of different species and strains of phytoplasmas (Lee *et al.* 1995, 1998; Seemüller *et al.* 1998a). The apple proliferation group-specific primers: R16(X)F1/R1 (Lee *et al.* 1995) or f01/r01 (Lorenz *et al.* 1995) are routinely used in the nested PCR. For the second PCR, primers fAT/rPRUS, specific for ESFY phytoplasma, are also recommended (Smart *et al.* 1996). Com-

puter simulated RFLP analysis of the 16S rRNA gene is a useful tool for identification of phytoplasmas and rapid delineation of phytoplasma subgroups (Wei *et al.* 2007). The *AluI* and *RsaI* restriction patterns of the 16S rDNA are characteristic and diagnostic for the 16SrX group (Schneider *et al.* 1993). 'Ca. P. prunorum' can be distinguished by 16S rDNA digestion with *RsaI*, *BsaAI*, *SspI* and *SfcI* from 'Ca. P. mali', and from 'Ca. P. pyri' by using *RsaI* and *SfcI* (Kison *et al.* 1997; Seemüller and Schneider 2004; Ferraro *et al.* 2005). If f01/r01 primers were used, the amplification products may be digested by *BsaAI*, *SspI* and *SfcI* endonucleases (Lorenz *et al.* 1995; Carraro *et al.* 1998b) for differentiation of these three phytoplasmas.

Two groups of isolates with a well defined geographical distribution were identified in 'Ca. P. prunorum', on the basis of restriction analysis of the *tuf* gene using *NlaIII* endonuclease (Ferretti *et al.* 2010). Differences of virulence of ESFY phytoplasma strains were conducted using sequence analyses of *tuf*, *rpsC*, *tlyC*, *imp* and *fol* genes (Marccone *et al.* 2010) and *aceF* (Martini *et al.* 2010). Multilocus sequence typing (MLST) of *Imp*, *pnp*, *aceF* and *secY* markers revealed the genetic diversity of 'Ca. P. mali', 'Ca. P. pyri' and 'Ca. P. prunorum', and confirmed the existence of inter-species recombination (Danet *et al.* 2007, 2011).

More recently, real-time PCR technique was developed that allows for quantifying the titre of phytoplasmas within the plant. A higher sensitivity and shorter time of processing new assays in a comparative study with the conventional PCR was proved. Real-time PCR was successfully used for specific detection and quantification of 'Ca. P. prunorum' in naturally infected stone fruit trees and insects (Jarausch *et al.* 2004; Galetto *et al.* 2005; Torres *et al.* 2005; Martini *et al.* 2007; Yvon *et al.* 2009; Delić *et al.* 2010; Jarausch *et al.* 2010; Nikolić *et al.* 2010; Torres *et al.* 2010). Pignatta *et al.* (2008) used a multiplex real-time PCR procedure (TaqMan) with two primers/probe combinations for detection of 'Ca. P. prunorum' in apricot, several wild *Prunus* spp., and *Cacopsylla pruni*. The pathogen was detected in 93% of the apricot trees showing ESFY symptoms and in ~33% of the insect groups and in several wild species collected from different locations.

### Control

The control of fruit tree phytoplasma diseases is based on prevention. It is very important to use healthy plant material and to avoid planting the stone fruit orchards in areas where 'Ca. P. prunorum' and its potential vector occur. It was evidenced that the wild *Prunus* species (*P. spinosa*, *P. cerasifera*, *P. amygdalus*, *P. armeniaca*, *P. domestica*) with ESFY symptoms, *Celtis australis*, as well as symptomless *Fraxinus excelsior* and *Rosa canina* surrounding apricot chlorotic leaf roll-affected orchards in southern France, were the reservoir of ESFY phytoplasma (Jarausch *et al.* 2001). Carraro *et al.* (2002) showed that *P. spinosa* and *P. cerasifera* play an important role in the epidemic cycle of ESFY. The possible role of alternate plant hosts such as bindweed (*Convolvulus arvensis* L.) and bermudagrass [*Cynodon dactylon* (L.) Pers.] in the spread of ESFY phytoplasma was suggested by Sanchez-Capuchino *et al.* (1982).

Management of phytoplasmal diseases used to focus on the spraying of insecticides to control the vector population. However, the study conducted by Poggi Pollini *et al.* (2007) on the application of diverse pesticides to control the vector *Cacopsylla pruni* did not confirm the efficacy in limiting ESFY in the Trentino region (Italy).

Tissue culture can be used to eliminate the phytoplasmas from plants in order to produce clones of healthy plant material. Thermo-therapy and meristem culture *in vitro* were applied for elimination of ESFY phytoplasmas from apricot (Laimer 2003; Bertaccini *et al.* 2004).

Phytoplasmas infecting *Prunus* species are also controlled by the breeding and planting of disease resistance rootstocks and cultivars of stone fruits. The study showed low susceptibility of plum trees grafted on GF 8/1 or several clones of *P. insititia* to ESFY (Morvan 1977; Desvignes and Cornaggia 1982; Dosba *et al.* 1990). Torres *et al.* (2010) indicated the differences in susceptibility of *Prunus* species trees to ESFY: the apricot, Japanese plum and the peach trees were more susceptible than the myrobalan and European plum genotypes. Significant diversity in susceptibility of new cultivars of European and Japanese plums grafted on some rootstock was identified (Landi *et al.* 2010). Ermacora *et al.* (2010), using real-time PCR analyses, showed that in the apricot trees infected by hypo-virulent strains, the colonization of the '*Ca. P. prunorum*' was lower than in the plants infected by the hyper-virulent strains of the pathogen. Apricot trees propagated from mother plants infected with a hypo-virulent strain of this phytoplasma did not show any symptoms. The trees from asymptomatic mother plants infected with hyper-virulent strains showed severe symptoms.

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