

# Chapter 6

## Fruit Crop Phytoplasmas



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**Abstract** The present chapter focuses on phytoplasmas and phytoplasma diseases affecting stone, pome, and small fruit worldwide. An outlook is also provided on other fruit tree species growing mainly in tropical and subtropical areas that are often infected by phytoplasma diseases usually associated with crop losses or loss of fruit quality and marketability.

**Keywords** Stone fruit · Pome fruit · Small fruit · Detection · Phytoplasma identification · Symptoms

### 6.1 Introduction

Phytoplasma detection in fruit crops and in insect vectors is now mainly based on molecular tools as for all phytoplasma diseases. For a number of these species grown in temperate areas, phytoplasma specific protocols that allow a sensitive and

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reliable detection were developed and validated. For phytoplasmas in the 16SrX group, specific primers (Lorenz et al. 1995; Lee et al. 1995a) that usually also require RFLP analysis of the amplified fragments can be employed. Specific primer pairs are also available that target non-ribosomal genes (Jarausch et al. 1998; Martini et al. 2007a). Several qPCR assays based either on SybrGreen® (Martini et al. 2007b; Yvon et al. 2009; Jarausch et al. 2010) or TaqMan® (Pignatta et al. 2008; Thébaud et al. 2009; Nikolić et al. 2010) technologies have been developed; group specific qPCR systems (Torres et al. 2005; Lee and Lin 2011) can also be used. Finally, a LAMP-based protocol has been recently developed (de Jonghe et al. 2017). Several of the above mentioned PCR and qPCR protocols have been validated through interlaboratory ring tests (The EUPHRESKO FruitPhytoInterlab group 2011; Pasquini et al. 2013). In the pome and stone fruits, several other non species-specific phytoplasmas were detected worldwide as summarized in Table 6.1.

## 6.2 Pome Fruit Phytoplasmas

The main phytoplasma historically associated with diseases in apple and pear are ‘*Candidatus* Phytoplasma mali’ and ‘*Ca. P. pyri*’, respectively (Seemüller and Schneider 2004). They both belong to 16SrX-A and 16SrX-C subgroups (Lee et al. 1998a, b; Pastore et al. 1996). Due to the ecological complexity of ‘*Ca. P. mali*’ biological cycle, including plant hosts and insect vectors, several studies focused on the identification of molecular markers allowing the distinction of strains related to specific biological features. Sequence analyses of a non-ribosomal genomic fragment (nitroreductase-like gene) and of the *rplV-rpsC* genes (coding the ribosomal proteins L22 and S3, respectively) distinguished three AP phytoplasma subtypes (AT-1, AT-2, and AP-15) and four rpX subgroups (rpX-A, -B, -C, and -D), respectively (Jarausch et al. 2000b; Martini et al. 2007b, 2008). Moreover, nucleotide sequence analyses of multiple genomic regions revealed genetic diversity among ‘*Ca. P. mali*’ populations (Paltrinieri et al. 2010; Casati et al. 2010, 2011; Franova et al. 2013; Cieślińska et al. 2015). The full genome of ‘*Ca. P. mali*’ has been sequenced and as for ‘*Ca. P. pyri*’ was determined to be linear and not circular, as reported for the other available phytoplasma genomes. Genome analysis revealed the presence of SAP11 (effector)-like protein and other phage-related elements putatively involved in the interaction with hosts (Kube et al. 2008). Full length genome sequencing also identified genes coding AAA+ ATPases and HflB/FstH proteases, suitable for distinguishing closely related strains showing a range of virulence during the infection of apple trees (Seemüller and Schneider 2007; Schneider and Seemüller 2009; Seemüller et al. 2013).

**Apple** ‘*Ca. P. mali*’ is associated with apple proliferation (AP) disease, one of the most important diseases of apple trees. Under intense cultivation the disease is the major constraint for the sustainability of this crop. ‘*Ca. P. mali*’ is a quarantine pathogen for EPPO in Europe and NAPP0 in North America, depleting vigor of the

**Table 6.1** Non species-specific phytoplasmas detected in stone and pome fruits

Host species	Ribosomal group/ subgroup	Geographic origin	Reference
Almond ( <i>Prunus dulcis</i> , syn. <i>Prunus amygdalus</i> )	16SrIX-C; 16SrII-B; 16SrXII-A; 16SrVI-A	Iran	Salehi et al. (2006); Zirak et al. (2009b)
	16SrIII-A	USA	Uyemoto and Kirkpatrick (2011)
	16SrX-B	Turkey	Sertkaya et al. (2005)
Apple ( <i>Malus</i> Mill spp.)	16SrI-B, 16SrI-C, 16SrXII-A	United Kingdom, Czech Republic	Bertaccini et al. (2001)
Apricot ( <i>Prunus armeniaca</i> L.)	16SrI-B	Italy	Lee et al. (1995a)
	16SrX-A	Slovenia	Mehle et al. (2007)
	16SrIII-A	USA	Uyemoto and Kirkpatrick (2011)
European plum ( <i>Prunus domestica</i> L.)	16SrI-B	Czech Republic; Lithuania	Navrátil et al. (2001a); Valiūnas et al. (2007)
	16SrX-A	Slovenia	Mehle et al. (2007)
Japanese plum ( <i>Prunus salicina</i> Lindl.)	16SrI-B; 16SrIII-B; 16SrV; 16SrX-A; 16SrXII-A	Italy	Lee et al. (1995a); Paltrinieri et al. (2004)
	16SrII; 16SrXII-A	Iran	Zirak et al. (2009a)
Nectarine [ <i>Prunus persica</i> (L.) Batsch]	16SrX-A; 16SrX-C	Poland	Cieślińska and Morgaś (2011)
	16SrXII-A; 16SrX-A	Italy	Paltrinieri et al. (2001)
Peach [ <i>Prunus persica</i> (L.) Batsch]	16SrIII-A	USA, Canada	Uyemoto and Kirkpatrick (2011)
	16SrI-A; 16SrV-B	India	Singh et al. (2014); Thakur et al. (1998); Khan et al. (2013)
	16SrX-C	Czech Republic	Navrátil et al. (2001a)
	16SrXII-B	Bolivia	Jones et al. (2005)
	16SrV-B; 16SrVII-A, 16SrXII-A	Italy	Paltrinieri et al. (2003, 2006)
	16SrI-B	Croatia	Krizanac et al. (2010)
	16SrXV-A	Azerbaijan	Balakishiyeva et al. (2011)
	16SrVII-A; 16SrI-W	Canada	Zunnoon-Khan et al. (2010); Arocha-Rosete et al. (2011a, b)
	16SrI-C; 16SrV-B	China	Zhang et al. (2013); Li et al. (2014b)
	16SrIII-B; 16SrX-C	Argentina	Fernández et al. (2013, 2017)
	16SrX-C	Spain	Sabaté et al. (2014)
	16SrIX-B	Iran	Zamharir (2014)
	16SrXII-A	Chile	Paltrinieri et al. (2006)
Peach and nectarine	16SrIX-B	Lebanon	Abou-Jawdah et al. (2009)
Pear ( <i>Pyrus</i> spp.)	16SrI-B; 16SrXII-A	Croatia	Krizanac et al. (2010)
	16SrX-A	Hungary	Del Serrone et al. (1998)
Sour cherry ( <i>Prunus cerasus</i> L.)	16SrI-R; 16SrI-Q	Lithuania	Jomantiene et al. (2011); Valiūnas et al. (2009a)
	16SrI-B	Poland	Cieślińska and Smolarek (2015)

(continued)

**Table 6.1** (continued)

Host species	Ribosomal group/ subgroup	Geographic origin	Reference
Sweet and Chinese cherry	16SrV-B	China	Lee et al. (1995b); Zhu et al. (1997); Jung et al. (2003)
Sweet and sour cherry	16SrI-B; 16SrIII-T	Lithuania	Valiūnas et al. (2007, 2009b)
	16SrIII-J	Chile	González et al. (2011)
Sweet cherry ( <i>Prunus avium</i> L.)	16SrIII-A	USA	Uyemoto and Kirkpatrick (2011)
	16SrX-C; 16SrXII-A; 16SrI-B; 16SrV-B; 16SrIII-B	Italy	Paltrinieri et al. (2001, 2008)
	16SrX-A	Czech Republic	Navrátil et al. (2001a)
	16SrX-A	Slovenia	Mehle et al. (2007)
	16SrX-C	Poland	Cieślińska and Smolarek (2015)
	16SrXII-A	Bulgaria	Avramov et al. (2011)
	16SrI-B	Turkey	Çaglayan et al. (2013)
	16SrI-S	China	Gao et al. (2011)

trees whose fruit cannot be commercialized because of their poor organoleptic qualities. AP was described for the first time in Italy in 1950 and occurs in many countries in the European areas: Albania, Austria, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Moldova, the Netherlands, Norway, Poland, Romania, Southern Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, and Ukraine. In Asia AP has only been reported to occur in Turkey. Epidemics of the disease were reported in several Italian northern regions where *Malus domestica* cultivars are the main hosts of ‘*Ca. P. mali*’ and most cultivars are susceptible. Apple cultivars known to be affected by AP phytoplasma include Belle de Boskoop, Gravestain, Golden Delicious, Winter Banana, Florina, Prima, Priscilla, Idared, McIntosh, Starking, Starkrimson, Roja de Benejama, Antonokova, Cortland, Spartan, Yellow Transparent, and Wealthy. In northern Italy severe epidemics have been reported in cultivars Golden Delicious, Florina, Canadian Renette, and Granny Smith grafted on different rootstocks. The recorded host range includes apple, periwinkle, bindweed, hazelnut, Bermuda grass, oriental lily, magnolia, cherry, apricot, plum, Japanese plum, pear, and rose (Kaminska and Sliwa 2004; Mehle et al. 2007). AP is associated in the most severe cases with productivity losses from 50% to 80%, depending on the variety, rootstock, environmental conditions, and agronomic management adopted (Zawadzka 1976; Osler and Loi 1986; Kunze 1989; Musetti et al. 2008). The main symptom is proliferation of the buds occurring mostly in the upper part of the plant due to development of axillary buds. The first symptoms appear the year after infection when the presence of the phytoplasma stimulates the growth of the secondary shoots, usually more sensitive to powdery mildew and then the witches’ brooms. The disease then causes lack of vigor; in spring the flowers may show phyllody. The leaves are smaller, roll downward, and become brittle; also, they are finely and irregularly serrated, sometimes showing reddening or yellowing and often early fall. Petioles are shorter, but, at the



**Fig. 6.1** Apple proliferation disease. Upper row from left to right. Symptoms of proliferation on the upper part of the plant with premature development of axillary buds on the trunk, and proliferation on secondary shoot with powdery mildew. Lower row left, leaves with abnormal stipules at right healthy leaf; right, fruit of smaller size and deformed elongated petioles

basis of the leaf, abnormally long stipules appear, and the fruits are usually smaller and the flavor is poor, since both sugar and acidity are very low (Fig. 6.1). ‘*Ca. P. mali*’ is transmitted from infected plants to healthy ones by psyllids. In Italy, *Cacopsylla melanoneura* (Förster) is the main vector of ‘*Ca. P. mali*’ in the north-western regions (Tedeschi and Alma 2007), while *C. picta* (Förster) (main vector of ‘*Ca. P. mali*’ in Germany) (Jarausch et al. 2007; Mayer et al. 2009) in the northeastern regions (Carraro et al. 2001). Additional putative vectors and natural plant hosts have been reported (Tedeschi and Alma 2006; Tedeschi et al. 2009). The long-distance spread of ‘*Ca. P. mali*’ is however mainly due to the movement of infected saplings, cuttings, and rootstock materials. The disease is graft-transmissible, with different rates of efficiency; in addition, since the colonization of aerial parts of apple trees shows seasonal variations, transmission rates vary up to 30%. Spring was reported to be the season when scion wood was least likely to be infected (up to 0.08%) and the vegetatively propagated rootstocks are especially hazardous as they are generally symptomless (Pedrazzoli et al. 2008). Available tools are very poor for reliable and effective disease containment for AP. In the focus areas, eradication of infected plants and pesticides application against the insect vectors are mandatory.



**Fig. 6.2** On the left pear decline infected pear tree (with red leaves) near to healthy pear; on the right a diseased tree producing just one fruit

**Pear** Pear decline (PD) associated with the presence of ‘*Ca. P. pyri*’ is a serious disease that, after its first reports in North America, occurs in many of the countries where pear is cultivated. It had long been known as “moria” in northern Italy (Refatti 1964) where the phytoplasma was detected as PD and studied after 1970 (Giunchedi et al. 1982; Giunchedi and Poggi Pollini 1984; Bertaccini et al. 1994; Pastore et al. 1996; Camele et al. 1998; Guerrini et al. 2000) as well as in many other countries in the European and North American areas (Blatný and Váňa 1974b; Westwood and Cameron 1978; Behnke et al. 1980; Davies et al. 1985; Eleftheriou 1985; Del Serrone et al. 1998; Ember et al. 2004; Duduk et al. 2005a, b; Süle et al. 2007; Krizanac et al. 2008; Çaglayan et al. 2008; Hunter et al. 2010) and recently also in Iran, Turkey, and in Chile (Salehi et al. 2008; Sertkaya et al. 2008; Facundo et al. 2017). In the 1960s in California (USA), about two million plants died, and those surviving after the PD epidemic showed reduced yield of marketable fruits (Hibino and Schneider 1970). There are two symptom types, quick and slow decline. In the quick decline, the plants die quickly in summer time without showing previous symptoms. The slow decline reduces vigor and induces leaf reddening and rolling (Fig. 6.2).

The disease can spread through propagation with infected buds and/or rootstocks or by insects of the genus *Cacopsylla*: *C. pyri*, *C. pyricola*, and *C. pyritsuga* are the reported vectors (Davies et al. 1992; Carraro et al. 1998c; Sanchez and Ortín-Angulo 2011). Phytoplasmas are consistently detected in the PD diseased trees:

they are usually unevenly distributed (Garcia Chapa et al. 2003) and in low concentration. The clear correlation of phytoplasma presence with symptom expression is still unresolved for PD, and it has been shown that phytoplasmas could be present also in asymptomatic trees (Lee et al. 1995a). In an experimental trial, 105 pear plants showing symptoms possibly linked to phytoplasma presence were tested by molecular assays, and no correlation was found between phytoplasma presence and symptomatology. However pear cultivars grafted on Quince A rootstock were more susceptible to phytoplasma infection (58–85%), while cvs William and Decana del Comizio appear to be less susceptible, especially when grafted on “franco comune” rootstock (29–43% of infection). Plants of cv William grafted on Farold 40 and Farold 87 showed a similar degree of phytoplasma infection (57%), but all the phytoplasma-positive trees were asymptomatic (Pastore et al. 1998). It was shown that grafting PD infected materials in winter time allow PD transmission (Errea et al. 2002). However, a recent 3-year-long experiment on PD infected pear plants under insect-proof nets showed a reduced presence of symptoms over the years; moreover in the last year, all the plants were PD negative at both crown and root levels. These results suggest no seasonal translocation of ‘*Ca. P. pyri*’ in the absence of the insect vector and confirmed phytoplasma elimination in the new phloem (Paltrinieri et al. 2017).

### 6.3 Stone Fruit Phytoplasmas

**Almond** Almond witches’ broom (AlmWB) disease is associated with the presence of ‘*Candidatus Phytoplasma phoenicium*’, 16SrIX-B (Verdin et al. 2003). The first epidemic of a lethal devastating almond [*Prunus dulcis* (Mill.) D.A. Webb] disease occurred in the south of Lebanon in the early 1990s; it was later reported in north Lebanon and in Iran starting in 1995 (Salehi and Izadpanah 1995; Abou-Jawdah et al. 2002; Molino Lova et al. 2011). During the last two decades, the outbreak of AlmWB has led to a rapid decline of almond trees in northern regions and in the Bekaa Valley in Lebanon (Choueiri et al. 2001; Abou-Jawdah et al. 2002) and in Fars province and in other southern provinces in Iran (Salehi et al. 2006). In Lebanon, the disease, rapidly spread and killed almost 100,000 trees over a period of 10 years from coastal areas to high mountainous areas. In 2009, ‘*Ca. P. phoenicium*’ was also identified in association with a severe disease of peach (*P. persica*) and nectarine (*P. persica* var. *nucipersica*) in southern Lebanon (Abou-Jawdah et al. 2009), and more than 40,000 newly diseased almond trees were observed in 2010 throughout the country, in 16 out of 24 Lebanese districts (Molino Lova et al. 2011). In the case of peach and nectarine trees, the first symptom observed is early flowering, followed by development of buds in infected branches. In addition, phyllody at the flowering period and serrate, slim, light green leaves and witches’ brooms developing from the trunk and the crown of the trees several months after are observed (Fig. 6.3). Even if the presence of witches’ broom is more common in almond trees



**Fig. 6.3** Symptoms due to ‘*Ca. P. phoenicium*’ in peach and nectarine trees: early flowering and development of all the buds of the infected branches (a); phyllody at the flowering (b); abnormal fruits (c); serrate, slim, light green leaves (d); witches’ broom (e–f)

than in peach/nectarine, in the latter phyllody, never recorded on almond is the differential symptomatology (Molino Lova et al. 2011). In Iran, ‘*Ca. P. phoenicium*’ was not identified in peach and nectarine but in other plant species, such as GF-677 (*P. amygdalus* × *P. persica*) and wild almond (*P. scoparia*) (Salehi et al. 2015). A total loss of production happens 1–2 years after the initial appearance of the symptoms (Abou-Jawdah et al. 2002). AlmWB was found to infect properly managed orchards, abandoned orchards, and isolated wild trees. The most characteristic symptoms in almond trees are (i) shoot proliferation on the main trunk with the appearance of a witches’ broom, (ii) perpendicular development of many axillary buds with small and yellowish leaves, and (iii) general tree decline with final die-back (Fig. 6.4). In Lebanon field surveys conducted in AlmWB almond and peach orchards and surroundings detected ‘*Ca. P. phoenicium*’ in the leafhopper *Asymmetrasca decedens* (prevalent in almond) and in the cixiids *Cixius* sp., *Tachycixius* spp., and *Eumecurus* spp. (prevalent in *Smilax aspera* L. and *Anthemis* sp.) and in crops and wild plants where the insects were collected. Transmission trials demonstrated that *A. decedens*, *T. viperinus*, and *T. cf. cypricus* are able to transmit ‘*Ca. P. phoenicium*’. AlmWB epidemiological cycle in Lebanon involves *A. decedens*, possibly responsible for the transmission from almond to almond, and cixiids of the genus *Tachycixius*, possibly responsible for the transmission from weeds to almond (Abou-Jawdah et al. 2014; Tedeschi et al. 2015). Multiple gene typing analyses of ‘*Ca. P. phoenicium*’ strains infecting almond, peach, and nectar-





**Fig. 6.4** Main symptoms of AlmWB on almond trees: shoot proliferation on the main trunk (a); perpendicular development of axillary buds on the branches, with small and yellowish leaves (b); general decline of the tree with final dieback (c)

ine in Lebanon revealed a substantial genetic homogeneity within the analyzed phytoplasma populations based on housekeeping gene sequence analyses and allowed the identification of distinct AlmWB-associated phytoplasma strains from diverse host plants based on *inmp* (integral membrane protein) gene sequence analysis. This evidence, along with reports of multiple insect vectors suggests that AlmWB could be associated with phytoplasma strains derived from the adaptation of an original strain to diverse hosts (Quaglino et al. 2015). ‘*Ca. P. phoenicium*’ strains, classified in 16SrIX-F and 16SrIX-G subgroups (Molino Lova et al. 2011), are considered as genetic variants of subgroup 16SrIX-B due to common biological traits (Casati et al. 2016). Healthy plant material and vector control are the main measures applied for AlmWB containment. Other phytoplasma diseases reported in almond worldwide are AlmWB-like diseases, inducing broomings in Iran (Verdin et al. 2003) in association with phytoplasmas classified in subgroup 16SrIX-C (Salehi et al. 2006).

**Apricot** The phytoplasma associated with the disease named apricot chlorotic leaf roll belongs to the 16SrX-B subgroup, also known as European stone fruit yellows (ESFY) or ‘*Ca. P. prunorum*’ (Seemüller and Schneider 2004), and is considered the most destructive pathogen of apricot (Lederer and Seemüller 1992; Navrátil et al. 1998, 2001a; Jarausch et al. 2001; Torres et al. 2004; Sertkaya et al. 2005; Ambrožič Turk et al. 2008; Ciešlińska and Morgaś 2011; Ludvíková et al. 2011; Mehle et al. 2011; Tarcali 2013; Žežlina et al. 2016). ESFY is associated with high mortality of apricot trees (Morvan 1977); a study carried out in France has shown that the average annual mortality of infected plants is 5% (Gentit et al. 1998). The prevalence of infected plants in each orchard depends on the variety, rootstock, age of orchard, environmental conditions, infecting strain, and insect vector populations. In some orchards in Germany, the prevalence of infected plants has been up to 80% (Jarausch et al. 2004). Prevalence values range from 5% to 40% (Desvignes et al. 1999; Kison

and Seemüller 2001; Laimer da Camara Machado et al. 2001; Torres et al. 2004); in Italy and Switzerland, they reach 93% and 4%, respectively (Pastore et al. 1999; Genini and Ramel 2004). During the spring, infected plants initially produce leaves instead of flowers, while from the summer end until the middle of autumn, the main symptoms consist of yellows and upward rolling of the leaves, followed by leaf reddening, branch and phloem necrosis, and decline.

**Peach and Nectarine** The ESFY phytoplasma in Mediterranean is associated with yellows and decline diseases (Lorenz et al. 1994; Delic et al. 2005; Sertkaya et al. 2005; Ambrožič Turk et al. 2008; Cieślińska and Morgaś 2011; Ludvíková et al. 2011; Mehle et al. 2011; Tarcali 2013; Etropolska et al. 2015; Žežlina et al. 2016; Etropolska and Lefort 2017). Peach species, along with those of apricot and plum, are the most susceptible to ESFY (Kison and Seemüller 2001; Ermacora et al. 2010). Infected plants are less productive and vigorous and may die within a few years. In peach orchards in Spain, the prevalence of infected plants varied from 5% to 25% (Battle et al. 2012). In Northern-Central Italy, the prevalence of the disease varied between 1% and 4%, with the highest infection rates concerning the cultivars Venus and Super Crimson Gold, grafted on GF 677 (Poggi Pollini et al. 2001). In peach and nectarine during winter and early spring, the leaf buds of infected plants break early. From the end of the summer and during the autumn, in some cultivars early fall and moderate rolling of leaves occurs. In others, these symptoms appear later in the season and are accompanied by reddening, upward rolling of the leaves, and vein enlargement (Poggi Pollini et al. 2001).

**Plum** ESFY phytoplasma is responsible for several decline diseases described over the years in *Prunus* spp. in Europe (Ahrens et al. 1993; Lorenz et al. 1994). On plum, it is associated with decline diseases referred to also as plum leptonecrosis in Italy on *P. salicina* (Goidanich 1933; Giunchedi et al. 1978) and as “Molières decline” in France (Bernhard et al. 1977). In Europe, ‘*Ca. P. prunorum*’ is the major and most economically important phytoplasma affecting plum. On Japanese plum it induces economically significant damages (Dosba et al. 1991), and it represents one of the major limiting factors in the production of this crop in countries bordering the Mediterranean Sea (Spain, France, Italy, Balkans), where the cultivation of Japanese plum and other susceptible *Prunus* species is widespread. A high percentage of naturally infected trees often associated with a high mortality rate of plants make orchards unproductive 8–10 years after planting. Infection percentages up to 100% have been recorded on susceptible Japanese plums cvs Ozark Premier and Shiro after 7 years of growth (Carraro et al. 1998a). Cultivar also affects the mortality rate of the infected plants that is generally higher on susceptible varieties than on plants grafted on susceptible rootstocks. A different response to ‘*Ca. P. prunorum*’ infection is reported for some Japanese plum varieties under natural pressure of ESFY disease and cvs. Bragiolla, Brarossa, Fortune, and Ruby Crunch are reported as not affected after 5 years of observations (Landi et al. 2010). The impact of the ESFY disease on the European plum is variable: severe symptoms resembling those



**Fig. 6.5** Off-season growth in plum due to an early break of leaf bud in late winter–early spring: ESFY infected tree is already at the leaf stage, while the healthy is still covered by flowers (**b**); upward rolled leaves with a brownish-red coloration (**a**)

occurring on Japanese plum were first observed in Italy on “Susina di Dro” (Poggi Pollini et al. 1995). A 5-year monitoring period carried out in orchards located in a natural and severely ESFY-affected area showed that only 6 out of 39 cultivars/selections observed were found infected and 4 of these showed only slight symptoms (Landi et al. 2010). A different response to ‘*Ca. P. prunorum*’ infection, ranging from little/not affected to moderately/highly susceptible, were also observed in experimental trials on French *P. domestica* cultivars (Jarausch et al. 2000a). Moreover, European plum can be latently infected and act as reservoirs of the pathogen. As for other *Prunus* species, symptoms induced on plum are different depending on the vegetative growth stage and season. The most typical symptom is off-season growth due to an early break of leaf bud before flowering that can be observed in late winter–early spring (Jarausch et al. 2008) (Fig. 6.5a). In summer, typical symptoms consist of smaller and narrowed leaves, upward and longitudinally rolled, and with a brownish-red coloration that became thick and brittle (Fig. 6.5b). Phloem necrosis can also appear, especially after winter frost. A poor fruit production is observed; fruits are smaller, ripen later than the healthy trees and may fall prematurely. Finally, an earlier defoliation generally occurs in summer or early fall. Initially, the disease can involve one branch, but in 2–3 years, the whole plant can be affected and die. An abnormal production of suckers from the rootstock usually occurs that can survive even after the aerial part of the tree has died. *P. salicina* is reported as highly susceptible to ‘*Ca. P. prunorum*’ (Ferrini et al. 2002; Carraro et al. 2004), and the majority of Japanese plum varieties exhibit severe symptoms when infected. *P. domestica* is generally more tolerant (Carraro et al. 1998a) and can also be latently infected. Under experimental conditions considerable differences were observed among *Prunus* rootstocks in response to ‘*Ca. P. prunorum*’ infection (Kison and Seemüller 2001). With regard to rootstocks commonly

used in Europe for plum varieties, myrobolan (*P. cerasifera*) and Ishtara [(*P. cerasifera* × *P. persica*) × *P. salicina*] were found to be moderately susceptible, whereas GF677 (*P. dulcis* × *P. persica*) and *P.* ‘Marianna’ GF 8/1 were only slightly affected.

The psyllid *Cacopsylla pruni* (Scopoli) is the only known vector of ‘*Ca. P. prunorum*’ (Carraro et al. 1998b, 2001) to *Prunus* species, although the two known genetic groups of *C. pruni* (Sauvion et al. 2007) appear to constitute divergent biological species (Peccoud et al. 2013). A strong host preference is reported, and Japanese plum trees are considered to be the more suitable hosts (Thébaud et al. 2006). ‘*Ca. P. prunorum*’ can be efficiently transmitted also by grafting throughout the year as it persists in the stem during winter (Seemüller et al. 1998). Infected rootstocks and/or scions can contribute to disease establishment and spread. Monitoring of ESFY in a plum growing area in Italy showed that myrobolan rootstocks infected by ‘*Ca. P. prunorum*’ resulted in 100% infection of newly grafted scions within one winter season (Paltrinieri et al. 2004).

**Sweet and Sour Cherry** A ESFY-related disease, named “Molière decline”, was for the first time observed in France in sweet cherry (Bernhard et al. 1977), but a clear relationship between ‘*Ca. P. prunorum*’ presence and cherry “Molière decline” was not established. The symptoms observed were yellowing of leaves, decline of shoots, and death of the plant. The first indications about the destructive nature of ‘*Ca. P. prunorum*’ are from Kison and Seemüller (2001). Later there was a report on five cherry rootstocks (Gisela 1, Gisela 3, Gisela 5, F 12/1, Weihroot 158) graft inoculated with a ESFY strain from flowering cherry (*P. serrulata*) showing decline symptoms (Lederer and Seemüller 1992).

Flowering cherry was used as scion on the rootstocks, and all the plants showed symptoms on leaves were less vigorous, and finally the entire plant or the scion died. ‘*Ca. P. prunorum*’ was detected then in sweet and sour cherry in Poland and Hungary and only in sour cherry in the Czech Republic where out of ten sour cherry plants infected by ‘*Ca. P. prunorum*’ only one was asymptomatic (Ludvíková et al. 2011). In the same country, a ESFY-infected sour cherry plant showed stunting, leaf rolling, and yellowing (Navrátil et al. 2001a). In Poland, three out of six sweet cherry plants infected by ESFY were asymptomatic, while the others showed chlorotic leaf roll, short internodes, and wilting, but in the same country, only one out of three sour cherry plants infected with ‘*Ca. P. prunorum*’ exhibited short internodes and decline (Cieślińska and Morgaś 2011) and leaf roll and yellowing of the leaves in sweet cherry varieties Kordia II.14 and Trzebnica (Cieślińska and Smolarek 2015). In Hungary the situation is more dramatic, with a high death rate of sweet and sour cherry plants (Tarcali 2013).

Widespread epidemics of X-disease in cherry were reported in the USA associated with the presence of ‘*Ca. P. pruni*’ which belongs to the ribosomal subgroup 16SrIII-A (Davis et al. 2013). The disease was first reported in California in sweet cherry plants and then in Connecticut in peach trees; X-disease was found only in peach in Canada besides the USA (Uyemoto and Kirkpatrick 2011). In addition to sweet and sour cherry, the phytoplasma infects peach, nectarine, almond, Japanese



**Fig. 6.6** Cherry trees infected by phytoplasmas in Chile: from left to right, plant on the left showing decline infected by strain 16SrIII-J; plant with shortened internodes; dead plant; phloem necrosis in the trunk of infected plant

and European plum, apricot, sour cherry (*Prunus emarginata*), and chokecherry (*P. virginiana*). The latter species represents an important reservoir of the phytoplasma, along with some spontaneous herbaceous species such as *Erigeron canadensis*, *Solidago rugosa*, *Medicago hispida*, and *Asclepias syriaca* (Douglas 1986; Uyemoto and Kirkpatrick 2011). The severity of X-disease depends on climatic conditions, being more destructive in California than in Washington State. On the other hand, sweet cherry scions grafted on *P. mahaleb* show a rapid decline, because of the hypersensitivity reaction of this rootstock that is reported as resistant to the pathogen. In rootstocks susceptible to the disease, such as Mazzard, Colt, and Stockton Morello, the scion declines slowly, and the fruits have a reduced size and are conical and scarcely colored, with the peduncle thick and short. The canopy is less developed, with smaller and reddish leaves, whose margins folded upwards (Kirkpatrick et al. 1995; Uyemoto and Kirkpatrick 2011). The phytoplasma can be transmitted by several species of leafhopper, as *Colladonus clitellarius*, *C. montanus*, *C. geminatus*, *Euscelidius variegatus*, *Fieberiella florii*, *Graphocephala confluens*, *Gyponana lamina*, *Keonella confluens*, *Norvellina seminuda*, *Osbornellus borealis*, *Paraphlepsius irroratus*, *Scaphytopius delonghi*, and *S. acutus* (Rice and Jones 1972; McClure 1980; Larsen and Whalen 1988; Kirkpatrick et al. 1990). The disease has not been reported in the last decade after extensive uprooting of the symptomatic orchards. In Chile 16SrIII-J phytoplasmas were identified in plants showing dieback, shortened internodes, and decline (Fig. 6.6) (González et al. 2011).

#### 6.4 Small Fruit Phytoplasmas

***Vaccinium* species** Blueberry stunt (BBS) was first described in New Jersey, USA as a virus disease (Wilcox 1942). In the mid-1900s, the disease became widespread in major blueberry production regions in the USA. It has been reported in eastern Canada and in several states of eastern and southeastern USA (Gocio and Dale

1982; Ramsdell and Stretch 1987; Bagadia et al. 2013; Arocha-Rosete et al. 2015). All cultivars of highbush blueberry and several other species of *Vaccinium* (*V. angustifolium*, *V. vacillans*, *V. atrococcum*, *V. stramineum*, *V. myrtilloides*) are susceptible to stunt disease (Ramsdell and Stretch 1987). Infected bushes of the most susceptible cultivars are usually less than half the size of healthy plants. Fruit production is reduced causing economical losses. Symptoms on highbush blueberry include overall dwarfing, witches' broom, and shortened internodes. Leaves of infected plants are small, spoon-shaped, and cupped slightly downward and turn red in summer or early autumn (Fig. 6.6). The sharp-nosed leafhopper [*Scaphytopius magdalensis* (Provancher)], and perhaps related species feeding on *Ericaceae* plants, transmits the BBS phytoplasma; it was also experimentally transmitted by *S. acutatus* and *S. frontalis* (Tomlinson et al. 1950; Maramorosch 1955; Tozzi et al. 1993), by dodder to periwinkle (Tomlinson et al. 1950), and by grafting of infected scions onto healthy blueberry plants (Ramsdell and Stretch 1987). Diseased samples collected from USA harbored phytoplasmas classified in the aster yellows phytoplasma subgroup 16SrI-E (Lee et al. 1998b); further study on BBS strains from Michigan and Quebec confirmed the presence of subgroup 16SrI-E phytoplasmas (Arocha-Rosete et al. 2015). Moreover BBS-diseased plants exhibiting typical stunt syndrome in New Jersey were infected with both 16SrI-E and 16SrIX-E subgroup phytoplasmas; the latter were identified in 4.4% of the bushes (Bagadia et al. 2013). Blueberry witches' broom is a disease reported in wild European blueberry (bilberry) plants (*Vaccinium myrtillus*) and described in The Netherlands, Germany, the then Czechoslovakia, Yugoslavia, Scotland, France, Lithuania, and Austria (Bos 1960; Blattný and Vána 1974; de Leeuw 1975; Valiūnas et al. 2004; Borroto-Fernández et al. 2007).

Besides *V. myrtillus*, the disease affects *V. vitis-idaea* (lingonberry), *V. uliginosum* (bog bilberry), and *V. oxycoccus* (small cranberry); in the 1960s it had been a serious problem in *V. myrtillus* production in the former Czechoslovakia with 15% fruit losses (Blattný and Blattný 1970). Infected European blueberry plants exhibit symptoms of shoot proliferation and bushy growth (Fig. 6.7). The size of branches and leaves is reduced. The shoots of plants may have an erect position, while the normal, healthy plants have plagiotropic shoots. Sometimes the leaves may prematurely turn red, but they usually drop later in the autumn than leaves of healthy plants which may result in higher sensitivity to frost damage. Tomenius and Ahman (1983) reported little leaf disease of *V. vitis-idaea* and wild *V. myrtillus* in Sweden. The leafhopper *Idiodonus cruentatus* Panz. was reported as a vector of the witches' broom agent in *V. myrtillus* (Blattný 1963); however the disease also occurred in *I. cruentatus*-free areas indicating that other leafhoppers can be involved in disease spread. On the basis of RFLP, nucleotide sequence and phylogenetic analyses of 16S rDNA, the phytoplasma strain detected in Lithuania was classified in the 16SrIII-F group (Valiūnas et al. 2004) as that detected in Germany (Paltrinieri et al. 1999). Phytoplasmas belonging to ribosomal group 16SrVI were also identified in *V. myrtillus* exhibiting symptoms of shoot proliferation in Austrian forests (Borroto-Fernández et al. 2007).



**Fig. 6.7** Left: marginal yellowing and cupping of leaves and branch stunting of blueberry infected with 16SrI-E phytoplasma in Canada (Courtesy of Y. Arocha-Rosete). Right: shoot proliferation and bushy growth of blueberry infected by phytoplasma in Austria (Courtesy of M. Laimer)

**Blueberry Reddening Disease** Yellowing and reddening on the upper leaves, shoot proliferation, and uneven ripening of the fruits were observed on the blueberry plants cvs. Bluecrop, Duke, and Spartan grown in central Serbia (Starović et al. 2013). More than 20% of plants in the single field were infected, and the incidence of the disease increased up to 50% in the following year. “Stolbur” phytoplasmas (16SrXII-A subgroup) were detected; however its association with blueberry reddening disease was not confirmed.

**Cranberry False Blossom** The disease reported in several northern USA states (Chen 1970; Stretch 1987; Xu and Chen 1996) was described only in American cranberry (*V. macrocarpon*) and European cranberry (*V. oxycoccus*). During the 1920s and 1930s, increasing occurrence of CBF and its vector *Limotettix vaccinii* (Dobroscky 1929) was a serious threat to cranberry production; however the control of the vector and the use of resistant cultivars have almost eliminated the disease. However, it reappeared in the late 1990s: infected plants exhibit abnormal floral structure including straight pedicels of the flowers, enlarged calyx, and shortened, discolored petals streaked with deep pink, red, or green, usually sterile flowers with abnormal pistils and stamens, and sometimes forming phyllody. Moreover, symptoms of upright growth above the level of normal vines and witches’ broom of shoots, leaves closely fitting to the stem, premature reddening of the leaves, and enlarged terminal flower buds can be also observed (Stretch 1987). *L. vaccinii* and *S. magdalenensis* are potential CBF vectors (Lee et al. 2014). The CBF phytoplasma was identified as belonging to 16SrIII-B subgroup (Xu and Chen 1996), but recent research assigns it to subgroup 16SrIII-Y (Lee et al. 2014).



**Fig. 6.8** From left: numerous weak and erect shoots in rubus stunt-affected blackberry (healthy plant at left); phyllody of flowers of loganberry infected with 16SrIII phytoplasmas; premature reddening of the leaves in rubus stunt-affected blackberry cv Loch Tay

**Rubus species** Several distinct phytoplasmas were associated with rubus stunt disease widely affecting *Rubus* species. The most common is ‘*Ca. P. rubi*’ (Malembic-Maher et al. 2011) classified in subgroup 16SrV-E and reported in many European countries (Marani et al. 1977; Mäurer and Seemüller 1994; Bertaccini et al. 1995; Marcone et al. 1997; Davies 2000; Ermacora et al. 2003; Sertkaya et al. 2004; Vindimian et al. 2004; Valiūnas et al. 2007; Cieślińska 2001, 2011; Ramkat et al. 2014). Recently, a blackberry phytoplasma strain was reported in Portugal and has been characterized and assigned to a new subgroup of the same group (16SrV-I) (Franova et al. 2016). ‘*Ca. P. asteris*’-related phytoplasmas were identified in wild raspberry and blackberry growing in Austrian forests (Borroto-Fernández et al. 2007), in blackberry in Pakistan (Fahmeed et al. 2009), in Poland (Cieślińska 2011), and in the United Kingdom (Reeder et al. 2010). ‘*Ca. P. solani*’, 16SrXII-A was reported only in Europe (Borroto-Fernández et al. 2007; Kuzmanović et al. 2011; Bobev et al. 2013). Phytoplasmas in *Rubus* are usually associated with stunting, small leaves, abnormal internodes (short or elongated), enlarged sepals, phyllody, virescence, proliferation of axillary buds, shoots and flowers, leaf reddening in early autumn, and fruit malformations (Fig. 6.8). The phytoplasma is transmitted by the leafhopper *Macropsis fuscula* (de Fluiter and van der Meer 1953) mostly to species of the genus *Rubus* but also to the dog rose *Rosa canina* (Mäurer and Seemüller 1994; Davies 2000; Jarausach et al. 2001). Phytoplasmas belonging to the X-disease group (16SrIII) were described in the United Kingdom (Davies 2000), Oregon, USA (Davis et al. 2001), and in Poland (Cieślińska 2011). PCR/RFLP analysis of the 16S rDNA fragment revealed that two distinct phytoplasmas belonging to 16SrIII and 16SrV groups were associated with rubus stunt in the United Kingdom. Results of a similar analysis of a phytoplasma found in *Rubus occidentalis* (black raspberry) in Oregon were the basis for its classification in the newly established subgroup 16SrIII-Q (Converse et al. 1982; Davis et al. 2001). “Stolbur” phytoplasma was detected in *R. idaeus* in Austria and in stunted *R. fruticosus* (cv. Evergreen Thornless) from central southern Bulgaria (Borroto-Fernández et al. 2007; Bobev et al. 2013) using PCR/RFLP or sequence analysis. Similar analyses showed that *R. fruticosus* from an Austrian forest was infected by phytoplasmas





**Fig. 6.9** From left: abnormal flower buds (upper) of full blossom disease-affected white currant “Blanka” in comparison with healthy flower buds (lower); flower malformation in a FBD white currant cv Blanka (courtesy by T. Malinowski). Sterile malformed flowers of black currant showing symptoms of full blossom disease

belonging to ribosomal group 16SrI-B (Borroto-Fernández et al. 2007). The PCR/RFLP and comparative genomic analysis of the 16S rRNA and *secY* genes revealed that phytoplasmas found in Poland were phylogenetically closely related to ‘*Ca. P. rubi*’, ‘*Ca. P. asteris*’ and X-disease phytoplasma strains (Cieślińska et al. 2014). Rapid and simultaneous detection of different groups of phytoplasmas infecting *Rubus* species was achieved by multiplex qPCR assay using TaqMan probes (Linck et al. 2017). This assay broadens the availability of tools useful for screening of nursery plant material and provides a new technique for the study of phytoplasmas infecting *Rubus* species.

***Ribes* species** Full blossom disease (FBD) was described on red currant “Houghton Castle” in Bojnice in former Czechoslovakia. Pleomorphic bodies were observed by transmission electron microscopy in the phloem tissue (Rakús et al. 1974; Rakús 1978). Later it has also been recorded in white currant cvs. Blanka, Meridián, and Primus (Špak et al. 2001). Since 1998, multiple cultivars of red and white currants with FBD symptoms have been observed in the Czech Republic in germplasm collections, propagation material, and commercial plantations (Špak et al. 2006). FBD was also recorded in Poland in red currant cv. Jonkheer van Tets and white currant cv. Blanka mostly infected with Blackcurrant reversion virus (BRV) but not tested for phytoplasma presence. The presence of phytoplasmas of aster yellows group (16SrI) in black currant cv. Karlštejnský dlouhohrozen with symptoms of the severe form of blackcurrant reversion disease was firstly detected in the Czech Republic (Špak et al. 2004). The incidence of the disease in the Czech Republic varied from 1% to 70% of infected bushes in different cultivars and locations. Similarly, the intensity of symptoms in the field observed between 1999 and 2004 varied from a few malformed flowers up to a massive occurrence of such flowers on whole branches. Yield losses of up to 70% were recorded in severely infected bushes of red currant cv. Vitan, and fruit quality was poor. The presence of both phytoplasma and BRV was confirmed in FBD infected bushes (Špak et al. 2006). The typical symptoms are malformations of flowers (Fig. 6.9) including the absence of stamens, presence of more than one style, enlarged petals and sepals, sterile flowers, and malformed fruit (van der Meer 1987). Diseased bushes are reduced in size and produce sparse crops of small berries (Rakús and Maliarčíková 1975). To determine the



**Fig. 6.10** From left: shoot proliferation and small berries from plant infected by phytoplasma (normal fruits from a healthy plant, at the top of the picture) in murtilla witches' broom disease. Strawberry fruit with leaves due to the phytoplasma presence (right)

role of phytoplasmas and BRV in FBD etiology and their potential to induce symptoms in *Ribes* sp., graft and dodder transmission experiments were conducted. A 16SrI-C phytoplasma was transmitted from FBD-symptomatic red currant by dodder to periwinkle, however at very low rate, and back transmission from periwinkle to red currant seedlings was by dodder as well as by grafting of cuttings from infected shoots to healthy red currant rootstocks (Špak et al. 2009; Příbylová et al. 2011). The problem with detection in symptomatic currants was associated with low concentration and erratic distribution of both BRV and phytoplasma (Špak et al. 2008). Phytoplasmas detected in red and white currant with full blossom symptoms in the Czech Republic were classified as subgroups 16SrI-B, 16SrX-A, 16SrX-B, and 16SrX-C (Navrátil et al. 2001b, 2004, 2007).

***Fragaria* species** Phytoplasma diseases of strawberry are mainly represented by virescence and phyllody symptomatology in both flowers and fruit (Fig. 6.10) that has been known for a long time (Posnette 1953) and determined as infectious disease transmissible by insect vectors or by grafting. At the end of the 1960s in New Zealand, symptoms on a lethal yellows disease were associated with phytoplasma presence (Stubbs 1968). Phytoplasmas were detected by transmission electron microscopy and molecular techniques in several countries worldwide. In Italy, Canada, and Czech Republic, 16SrI-C phytoplasmas were identified with losses reaching 30% in some severe cases (Franova Honetslegrova et al. 1996; Pastore et al. 2002; Contaldo et al. 2012). Phytoplasmas were detected in plants showing witches' broom symptoms (multiplier disease) and stunting in Florida and in other parts of the USA (Jomantiene et al. 1996, 1998). Phytoplasma strains distinguishable at the molecular level were also identified in strawberry with similar symptoms: 16SrI-J and 16SrVI groups in the USA (Harrison et al. 1997), 16SrI-B in Southern Italy (Bertaccini et al. 1997), and 16SrXII-B in New Zealand (Andersen et al. 1998). The strawberry lethal yellows (SLY) disease in Australia is associated with '*Ca. P. australiense*' and tomato big bud (Straten et al. 2005). '*Ca. P. australiense*' is also associated with strawberry green petal (SGP) disease.

In strawberry with a yellows disease in Lithuania, a new taxon named ‘*Ca. P. fragariae*’ was identified and assigned to ribosomal group 16SrXII-E (Valiūnas et al. 2006). Recently phytoplasmas of group 16SrIII were reported from Bolivia in strawberry plants displaying rosette formation and small fruits (Arocha et al. 2010). Subgroup 16SrI-F was detected in Cuba in nurseries with plants showing symptoms of leaf yellowing and reddening and fruit deformation and stunting (Ferriol-Marchena et al. 2013). In Spain “stolbur” and aster yellows were detected in plants with yellow leaves, stunting, reduced leaf size, and virescence (Oviedo Delgado and Ibeas Corcelles 2017), while in Mexico a similar symptomatology was associated with the presence of ‘*Ca. P. hispanicum*’ (16SrXIII-A subgroup) (Pérez-López and Dumonceaux 2016; Pérez-López et al. 2017). In strawberry red leaf in Argentina, 16SrXIII-F subgroup phytoplasmas were identified (Fernández et al. 2015).

**Myrtaceae Family** Murtilla (*Ugni molinae* Turcz) witches’ broom disease is only reported from Chile. In spring and summer, the infected plants showed small and yellow leaves at the end of summer which turned red during the autumn season, and the twigs become necrotic and dry. The berries, if present, are smaller and poor in sugar and flavorings (Fig. 6.10). The first report of this disease, based on symptoms, has been made in the early 1980s (Novoa 1982); however the first laboratory evidence for phytoplasma presence was obtained recently (Andrade et al. 2009). ‘*Ca. P. fraxini*’ and ‘*Ca. P. ulmi*’ (ribosomal subgroups 16SrVII-A and 16SrV-A, respectively) were detected in symptomatic plants (Arismendi et al. 2011).

## 6.5 Tropical/Subtropical Fruit

**Banana** *Musa* spp. is native to tropical Indomalaya and Australia and is likely to have been first domesticated in Papua New Guinea. They are grown in 135 countries, primarily for their fruit, and to a lesser extent to make fiber, banana wine, banana beer, and as ornamental plants. Phytoplasmas were detected from wilted cooking banana (plantain) plants in the Solomon Islands and Papua New Guinea and are most closely related to phytoplasmas in the 16SrXXII-A (‘*Ca. P. palmicola*’) and 16SrIV-A and 16SrIV-C subgroups. The plants showed yellowing and/or leaf death and unfilled fruit bunches, discontinuous necrotic vascular streaks, and pockets of rot and discoloration were also observed (Davis et al. 2012, 2015).

**Citrus** All citrus varieties are quite susceptible to phytoplasma infection although very often their presence is in association with ‘*Candidatus Liberibacter*’ species in a disease known as “huanglongbing” (HLB). Particularly in the Brazil epidemics, 16SrIX group phytoplasmas were detected, while in the Chinese epidemic, 16SrI-B group phytoplasmas were detected associated with HLB (Teixeira et al. 2008; Chen et al. 2009). Recently in Mexico, HLB was associated with the presence of 16SrI-B and 16SrI-S phytoplasmas in orange and lime (Arratia-Castro et al. 2014). In Puerto Rico 16SrIX group phytoplasmas are reported in *C. sinensis* and *C. limon* (Caicedo



**Fig. 6.11** Jujube young tree with severe JWB symptoms in China (left and center). Right, lime witches' broom ('*Ca. P. aurantifolia*') in Oman

et al. 2015). In China, grapefruit (*C. paradisi*) with HLB showed 16SrII-A phytoplasma presence in some of the infected plants (Lou et al. 2014). Historically the first described '*Candidatus Phytoplasma*' species (Zreick et al. 1995) is that detected in lime affected by the witches' broom disease in Oman (Fig. 6.11), Iran, and in the United Arab Emirates (Garnier et al. 1991; Bové et al. 2000; Al-Yahyai et al. 2015; Al-Abadi et al. 2016), but the disease in citrus was described in China, and phytoplasma presence was reported a long time ago (Chen et al. 1979). Acid lime is the other citrus species reported to be affected by these phytoplasmas in India (Ghosh et al. 1999), Iran (Salehi et al. 2000; Djavaheri and Rahimian 2004). This phytoplasma was also detected in limequat tree in Iran (Faghihi et al. 2017). Sweet orange (*C. sinensis*) and mandarin (*C. reticulata*) showing typical symptoms of witches' broom disease were detected in Egypt, and phytoplasma presence was demonstrated without molecular identification (EL Banna et al. 2015). Aster yellows were reported in citrus in Pakistan (Fahmeed et al. 2009).

**Jujube** For several decades jujube witches' broom (JWB), associated with '*Ca. P. ziziphi*' has caused a devastating disease of the jujube (*Ziziphus jujuba* Mill) industry in China (Tsai et al. 1988). Transmission electron microscopy showed that a phytoplasma was associated with the disease (Yi and La 1973). Diseased trees show the precocious development of proliferating secondary shoots which have an overabundance of abnormally small and sometimes chlorotic leaves (Fig. 6.11). Phyllody is another characteristic of diseased trees and symptomatic limbs do not bear fruit. Symptoms are at first limited to one or more branches, but then the disease spreads progressively throughout the entire crown. Trees of all ages are susceptible and die within a few years after symptoms first appear (La and Chang 1979). Fan et al. (2008) verified the presence of this phytoplasma in jujube trees belonging to diverse cultivars in both symptomatic and asymptomatic samples. The phytoplasma associated with JWB is '*Ca. P. ziziphi*' (Jung et al. 2003) belonging to subgroup 16SrV-B, and it was reported in China and India infecting also stone fruit (Zhu et al. 1997; Tian et al. 2000; Khan et al. 2008). The known insect vector of the phytoplasma is *Hishimonoides chinensis* Anufriev and *Hishimonus sallatus* Uhler (La and Woo 1980).

**Longan** (*Dimocarpus longan* Lour.) (Sapindaceae) is found commonly in most of Asia, primarily in China, Taiwan, Vietnam, and Thailand, and in the latter two countries, it is one of the major fruit plants promoted for domestic consumption and export. The witches' broom syndrome is a devastating epidemic in the majority of the longan-growing areas of Vietnam. The main symptoms include small and upward rolled leaves, shortened internodes, and witches' broom; small, stunted shoots with curved, rolled up margins, deformed leaves with blisters, and hairy patches on the underside; and abnormal development of flower structures, flower abortion, and failure to produce fruit or develop small fruit. Phytoplasmas detected belonged to 16SrI, 16SrII, 16SrV, and 16SrXII-A groups/subgroups (Nguyen et al. 2012; Hoat et al. 2016). Phytoplasmas are also reported in plants showing similar symptoms in Thailand (Chantrasri et al. 1999). During a recent study, a partial relationship between the symptoms and *Eriophyes dimocarpi* mites presence was verified (Hoat et al. 2017).

**Papaya** In *Carica papaya* (Caricaceae), phytoplasma diseases are reported from Australia, Cuba, and other parts of the Caribbean, Ethiopia, Hawaii, India, Israel, Oman, and South Africa. Mosaic, yellow crinkle, and dieback diseases of papaya are reported in Australia (Gibb et al. 1996, 1998; De La Rue et al. 1999). While in yellow crinkle and mosaic (PpYC and PpM) diseases, '*Ca. P. australasia*' (16SrII-D) phytoplasma was identified; in the dieback disease, '*Ca. P. australiense*' was detected (16SrXII-B) (White et al. 1998). PpYC disease consists of yellowing of leaves and bending down of the petioles. The crown leaves develop clear patches at the margins and between the veins, and these areas become brown and die; the older leaves die and fall leaving a bare stem with a few stunted leaves on top. The flowers may show abnormal distortion. In the PpM disease, stunted yellow leaves with clear margins and short stems with many side shoots are observed. The petioles and upper stems have "water-soaked" streaks, and the fruit may show light green areas. It could be problematic in some cases to distinguish among these two diseases. Papaya dieback (PpDB) disease shows inner crown leaves with bunched appearance, yellowing, followed by a slight bending of the stem tip. The affected leaves shrivel after a quick yellowing, the crown dies within 1–4 weeks, and the fruit falls off while still green or rot. PpDB dieback is an economically important disease limiting papaya production in southern and central Queensland, with outbreaks frequently causing losses of 10–100%. The phytoplasma associated with PpDB has been transmitted by dodder to tomato and other test plants. In the Caribbean a bunchy top disease is described beginning with yellow mottling of the upper leaves similar to the one present in the yellow crinkle disease, and then the leaves begin to die. Growth of leaves is slow, and the distance between them along the stem becomes shorter giving a "bunchy top" appearance. The oldest leaves fall down, leaving a few stunted leaves at the top of the stem. The plants may die, or new shoots appear from lower down the stem. In Cuba, '*Ca. P. caricae*' (16SrXVII group) (Arocha et al. 2005) and group 16SrI phytoplasmas (Acosta et al. 2011) were identified in diseased plants. Occurrence of aster yellows phytoplasmas is also reported in Sri Lanka in papaya dieback (Abeyasinghe et al. 2016) and in a papaya yellows disease in Taiwan (Bau et al.

2011). Phytoplasmas belonging to different 16Sr groups have been identified in the Asian continent including groups 16SrI and 16SrII in India associated with similar dieback symptoms (Rao et al. 2011) and axillary shoot proliferation (Verma et al. 2012); and group 16SrXII in Israel (Gera et al. 2005).

**Pomegranate** *Punica granatum* (Lythraceae) is grown in many subtropical countries especially in the Mediterranean region for its nutritional and medical properties. Phytoplasma presence in pomegranate is associated with yellows symptoms in Turkey where two phytoplasma strains belonging to subgroups 16SrI-B and 16SrXII-A were identified (Gazel et al. 2016) and in Iran where a group 16SrIII was associated with a slow decline, reduction in yield and fruit size and yellowing of the leaves, and foliar reddening with thickening of veins (Karimi et al. 2015), and a 16SrII-D subgroup was associated with little leaf symptoms (Salehi et al. 2016).

## 6.6 Nuts

**Chestnut** In Japan a chestnut yellows disease (Shimada and Kouda 1954) was shown by transmission electron microscopy to be associated with phytoplasma presence (Okuda et al. 1974). In Korea in 1993, chestnut trees with little leaf disease (CLL) showing small, yellow leaves were also associated with phytoplasma presence (Han et al. 1997). More recently Japanese chestnut trees (*Castanea crenata* Sieb. and Zucc.) showing a witches' broom disease, including abnormally small leaves and yellowing of young leaves, was found associated with the presence of '*Ca. P. castaneae*' (Jung et al. 2002). The disease named chestnut witches' broom (CnWB) differed from Korean CLL and the chestnut yellows of Japan in symptom severity and led to severe crop losses especially in some provinces.

**Hazelnut** Decline and yellows are diseases reported for a long time in several European hazelnut (*Corylus avellana* L., Betulaceae) growing areas and were associated with 16SrX group phytoplasma presence (Marcone et al. 1996). In stunted but also in asymptomatic plants a 16SrIII-B phytoplasma was identified in Oregon (USA) (Jomantiene et al. 2000); '*Ca. P. asteris*' was reported in a few stunted plants of European hazelnut in Poland (Cieślińska and Kowalik 2011). In the United Kingdom, '*Ca. P. fragariae*' (16SrXII-E) was detected in 10–15-year-old plants showing yellowing of leaves, leaf scorch, a lack of density of the tree canopy, and proliferation of small thin branches (Hodgetts et al. 2015). In Chile, hazelnut plants cv. Barcelona 12-year-old showing leaf and catkin malformation and yellowing in some branches were positive for the presence of phytoplasmas in 16SrI, 16SrIII, and 16SrV groups (Pérez Fuentealba et al. 2017). Nothing is reported about disease epidemiology and/or possible insect vectors.

**Peanut** Peanut witches' broom is the only disease reported in *Arachis hypogaea* L. (Fabaceae); the phytoplasmas detected in China and Taiwan resulted closely phylogenetically related and all belonging to 16SrII-A subgroup (Chen et al. 1994; Li et al. 2014a). The disease agent is transmitted by the insect vector *Orosius orientalis* (Yang 1975).

**Pecan** Pecan bunch is a widespread disease reported in commercially grown southern pecan, *Carya illinoensis* (Wangenh) K. Koch (Juglandaceae), in the USA. The tree is grown mainly in the America continent where it is the tree symbol for the state of Texas. The symptoms on diseased plants are bushy growths of slender willow shoots, resulting from an abnormal forcing of lateral buds. Symptoms may appear on only one branch or on many branches. Bunch disease is very conspicuous in the spring and early summer because the diseased shoots come out of dormancy earlier than non-infected shoots (Seliskar et al. 1974). The phytoplasmas detected in this species in Georgia belong to group 16SrIII-C (Lee et al. 1998b).

## 6.7 Conclusions

Management of phytoplasma diseases in fruit crops requires relevant efforts worldwide considering the huge number of diverse species that are affected or susceptible to these diseases. Early phytoplasma detection in mother plants together with the implementation of phytoplasma-free certified plants could greatly improve the sanitary status of these species and reduce the large diffusion of different phytoplasmas by propagation materials. It is very complicated to propose a scheme based on insect vector control since these are quite often not effective, and also their identity is quite often not known.

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