Offered Review

CURRENT STATUS OF PHYTOPLASMA DISEASES OF FOREST AND LANDSCAPE TREES AND SHRUBS

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SUMMARY

This paper summarizes the current knowledge of phytoplasma diseases of forest and landscape trees and shrubs, with special reference to molecular and taxonomic aspects of the associated phytoplasmas in the light of the plethora of data generated over the last years. Phytoplasma diseases of such kind of trees and shrubs are of considerable economic and ecological significance throughout the world, either because of the local impact or of their widespread distribution. However, information is still missing on many aspects of some of these diseases, including insect vectors, phytoplasma-vector relationships, phytoplasma-host plant interactions, strain virulence, strain interference, host tolerance, host range, and impact of the infections on the growth and yield of affected plants. In some instances, phytoplasma-infected forest and landscape trees and shrubs can serve as reservoirs of phytoplasmas affecting agricultural crops. Latent phytoplasma infections in some forest and landscape trees and shrubs are common.

Key words: '*Candidatus* Phytoplasma' species, elm yellows, alder yellows, ash yellows, symptomatology, taxonomic group.

INTRODUCTION

Phytoplasmas are a large group of plant-pathogenic wall-less, non-helical, unculturable bacteria associated with diseases, collectively referred to as yellows diseases, of more than a thousand plant species, including economically and ecologically important forest and landscape trees and shrubs (McCoy *et al*., 1989; Seemüller *et al*., 1998, 2002; Lee *et al*., 2000; Bertaccini, 2007; Bertaccini and Duduk, 2009; Griffiths, 2013). In diseased plants, phytoplasmas reside almost exclusively in the sieve tubes and are transmitted from plant to plant by phloem-feeding homopteran insects, mainly leafhoppers and planthoppers, less frequently by psyllids (Weintraub and Beanland, 2006). Most of the host plants are angiosperms in which a wide range of symptoms are induced.

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Taxonomically, phytoplasmas are placed in the class *Mollicutes*, closely related to acholeplasmas, and are currently classified within the provisional genus '*Candidatus* Phytoplasma' based primarily on 16S rDNA sequence analysis (IRPCM, 2004; Martini *et al*., 2014). Based on phylogenetic analysis of the 16S rDNA sequences, approximately 20 major phylogenetic groups or subclades have been identified within the phytoplasma clade. This figure is broadly in accordance with the number of phytoplasma groups established by restriction fragment length polymorphism (RFLP) analysis of PCR-amplified rDNA (Lee *et al*., 1998, 2000; Seemüller *et al*., 1998, 2002). Each phytoplasma subclade (or corresponding 16Sr group) is considered to represent at least one distinct species under the provisional taxonomic status '*Candidatus*' (IRPCM, 2004).

Within most of the phytoplasma groups, several distinct subgroups (16Sr subgroups) have been delineated, based on the RFLP analysis of 16S rDNA sequences (Lee *et al*., 2007). Recently, the number of 16Sr groups has grown to 32 and that of subgroups to more than 100 upon the use of a computer-simulated RFLP analysis (Wei *et al*., 2007; Davis *et al*., 2012, 2013; Zhao *et al*., 2013; Nejat *et al*., 2013).

To date, 37 '*Candidatus* Phytoplasma' species have been formally described (IRPCM, 2004; Harrison *et al*., 2014; Martini *et al*., 2014). In addition, multi-locus sequence typing (MLST) employing genes with varying degrees of genetic variability has provided new insights into the genetic diversity of some phytoplasmas that are relatively homogeneous at the 16S rDNA sequence level. MLST proved to be a most useful tool for the identification of genetically close but pathologically and/or ecologically distinct strains. Identification of these strains is essential and much relevant for epidemiological studies and for a more precise definition of '*Ca*. Phytoplasma' species (Lee *et al*., 2010, 2012; Davis *et al*., 2013).

Unlike the phytoplasma diseases of fruit trees, those of forest and landscape trees and shrubs have not been extensively reviewed. For this reason and taking into account the plethora of data generated over the last years, an attempt is made to fill this gap by summarizing the current knowledge on these diseases, with a focus on the molecular and taxonomic aspects of the associated phytoplasmas. However, lethal yellowing and lethal yellowing-type diseases of palms, for which detailed reviews are available (Harrison and Jones, 2004; Harrison and Oropeza, 2008)

are not dealt with, the same as: (i) early descriptions that were not confirmed either by molecular and/or microscopical methods; (ii) reports of detection of low phytoplasma numbers in trees and shrubs that could not be clearly associated with a disease. Names, causal agent(s), host range and geographic distribution of the diseases addressed are reported in Table 1.

Elm yellows

Elm yellows (EY), formerly called elm phloem necrosis, is a serious disease of *Ulmus* (elm) species and hybrids, known to occur in North America and Europe. EY was first described in Ohio in 1938 (Swingle, 1938). However, there is evidence that it was present in this US state as well as in Kentucky, Indiana and Illinois long before, perhaps as early as the late 1800s (Garman, 1893, 1899). The disease has been observed in Italy at least since 1918 (Pantanelli, 1918; Massalongo, 1924; Goidànich, 1951; Gualaccini, 1963; Pisi *et al*., 1981; Conti *et al*., 1987; Lee *et al*., 1993, 1995; Marcone *et al*., 1994a, 1997a) and recorded in other European countries, including former Czechoslovakia, France, Germany and Serbia (Boiñanský, 1969; Mäurer *et al*., 1993; Seemüller *et al*., 1998; Jarausch *et al*., 2001; Arnaud *et al*., 2007; Jović *et al*., 2008, 2011a; Navrátil *et al*., 2009).

EY is caused by '*Candidatus* Phytoplasma ulmi' (Lee *et al*., 2004a), a member of the EY phytoplasma group or 16SrV group, subgroup 16SrV-A. Other members of

Table 1. Phytoplasma diseases of forest and landscape trees and shrubs.

Disease	Phytoplasma(s) associated[a]	Plant host	Geographic distribution	Reference
Elm yellows	16SrV-A ('*Ca*. P. ulmi')	*Ulmus* spp.	North America, Europe	Lee *et al*., 2004a; Arnaud *et al*., 2007; Jović *et al*., 2011a Jović
	16SrV-A	*Zelkova serrata*	Italy	Romanazzi and Murolo, 2008; Murolo and Romanazzi, 2008
	16SrVI-C	*Ulmus americana*	USA (Illinois)	Jacobs *et al*., 2003
	16SrI, 16SrXII	*Ulmus* spp.	Italy	Lee *et al*., 1995
	16SrV-B, 16SrI-B, 16SrI-D	*Ulmus parvifolia*	China	Zhu *et al*., 2008; Gao *et al*., 2011
Alder yellows	16SrV-C	*Alnus* spp.	Europe	Lee *et al*., 2004a; Arnaud *et al*., 2007; Cvrković *et al*., 2008
	N.D.	*Alnus rubra*	USA (Washington State)	Lederer and Seemüller, 1991; Mäurer *et al*., 1993
Poplar witches' broom	16SrI-B, 16SrI-A, 16SrI-P	*Populus* spp.	Europe	Berges *et al*., 1997; Cousin *et al*., 1999; Šeruga *et al*., 2003; Mitrović *et al*., 2011
	16SrI	*Populus nigra*	Colombia	Perilla-Henao *et al*., 2012
Spartium witches' broom	16SrX-D ('*Ca*. P. spartii'), 16SrV-C	*Spartium junceum*	Italy	Marcone *et al*., 1996a, 2004; Arnaud *et al*., 2007; Spallino *et al*., 2013
	16SrX-D	*Spartium junceum*	Spain	Torres *et al*., 2002
Sarothamnus scoparius witches' broom	'*Ca*. P. spartii'-related	*Sarothamnus scoparius*	Italy	Marcone *et al*., 1997c, 2004
Buckthorn witches' broom	16SrXX-A ('*Ca*. P. rhamni')	*Rhamnus catharticus*	Europe	Mäurer and Seemüller, 1996; Marcone *et al*., 2004
Eucalyptus little leaf	16SrV, 16SrI-B, 16SrI-C	*Eucalyptus* spp.	Italy	Marcone *et al*., 1996b, 1997a; Camele *et al*., 1999
	N.D.	*Eucalyptus* spp.	Asia, Sudan	McCoy *et al*., 1989
Pine shoot proliferation	16SrXXI-A ('*Ca*. P. pini')	*Pinus* spp.	Europe	Schneider *et al*., 2005; Śliwa *et al*., 2008; Kamińska *et al*., 2011
Unnamed	16SrXXI-A	*Abies procera*, *Tsuga canadensis*, *Picea pungens*	Poland, Czech Republic	Kamińska *et al*., 2011; Kamińska and Berniak, 2011
Unnamed	16SrIII, 16SrI	*Cupressus* sp., *Picea abies*, *P. glauca*, *P. pungens*	Italy, Poland	Paltrinieri *et al*., 1998; Kamińska and Berniak, 2011
Unnamed	16SrXXI-A	*Taxodium distichum* var. *imbricarium*	China	Huang *et al*., 2011
Larch dwarfed needle proliferation	16SrI-B	*Larix* sp.	Ukraine	Jomantiene *et al*., 2011
Juniper witches' broom	16SrIX-F	*Juniperus occidentalis*	USA (Oregon)	Davis *et al*., 2010
Unnamed	16SrVI	*Araucaria heterophylla*	India	Gupta *et al*., 2010
Sandal spike	16SrI-B	*Santalum album*	India	Khan *et al*., 2008; Lee *et al*., 2004b

Table 1 continued.

Disease	Phytoplasma(s) associated[a]	Plant host	Geographic distribution	Reference
Ash yellows	16SrVII-A (‘*Ca*. P. fraxini’)	*Fraxinus americana*, *F. pennsylvanica*, *F. velutina* and other *Fraxinus* spp.	North America	Sinclair *et al*., 1996, 2000b; Griffiths *et al*., 1999
	16SrI, 16SrVII	*Fraxinus excelsior*, *F. uhdei*	Poland, Colombia	Filgueira *et al*., 2004; Kamińska and Berniak, 2009; Perilla-Henao *et al*., 2012
Lilac witches’ broom	16SrVII-A	*Syringa* spp.	North America	Sinclair *et al*., 1996, 2000b; Griffiths *et al*., 1999
Paulownia witches’ broom	16SrI-D	*Paulownia* spp.	Asia	Hiruki, 1999; Lee *et al*., 2004b
Mulberry dwarf	16SrI-B	*Morus alba*, *M. bombycis*, *M. multicaulis*	Japan, Korea	Sato *et al*., 1996; Ji *et al*., 2009; Gai *et al*., 2014a, 2014b
Black locust witches’ broom	16SrIII	*Robinia pseudoacacia*	USA	Davis and Dally, 2000; Chapman *et al*., 2001
	16SrV-B (‘*Ca*. P. ziziphi’)	*Robinia pseudoacacia*	China	Ren *et al*., 2014
Chestnut witches’ broom	16SrXIX-A (‘*Ca*. P. castaneae’)	*Castanea crenata*	Japan, Korea	Jung *et al*., 2002
Chinese chestnut yellow crinkle	16SrXIX-A	*Castanea mollissima*	China	Lin *et al*., 2011
Willow witches’ broom	N.D.	*Salix rigida*, *S. caprea*	USA	Seliskar and Wilson, 1981
	16SrVI, 16SrI-B	*Salix bebbiana*, *S. discolor*, *S. exigua*, *S. petiolaris*, *S. tetradenia* and other *Salix* sp.	Canada, China, Lithuania	Hiruki and Wang, 1999; Wang and Hiruki, 2005; Lee *et al*., 2004b; Mou *et al*., 2014
	16SrI-C, 16SrVI-A, 16SrXII	*Salix babylonica*	China, Spain	Wei *et al*., 2009; Zhang *et al*., 2012; Alfaro-Fernández *et al*., 2011
	N.D.	*Salix babylonica*	Italy	Ragozzino *et al*., 1977
Walnut witches’ broom	16SrIII-G	*Juglans nigra*	USA	Chen *et al*., 1992a, 1992b; Davis *et al*., 2013
Salt cedar witches’ broom	16SrXXX (‘*Ca*. P. tamaricis’)	*Tamarix chinensis*	China	Zhao *et al*., 2009
Japanese maple witches’ broom	16SrI-D	*Acer palmatum*	China	Li *et al*., 2012
Unnamed	16SrI-B	*Acer negundo*	Poland	Kamińska and Śliwa, 2006
Japanese raisin witches’ broom	16SrV-B	*Hovenia dulcis*	Asia	Kamala-Kannan *et al*., 2011
Chinese tallow tree yellows	16SrIII-Y	*Sapium sebiferum*	China	Gao *et al*., 2015
Goldenrain tree little leaf	16SrI-B	*Koelreuteria paniculata*	Korea	Kamala-Kannan *et al*., 2010
China tree decline	16SrIII-B, 16SrXIII-C	*Melia azedarach*	Argentina, Paraguay	Galdeano *et al*., 2004, 2013; Arneodo *et al*., 2005, 2007
	16SrIII-B	*Melia azedarach*	Brazil	Duarte *et al*., 2009
Chinaberry yellows	16SrIII-J, 16SrXIII-C	*Melia azedarach*	Bolivia	Harrison *et al*., 2003
	16SrI-B	*Melia azedarach*	Vietnam	Harrison *et al*., 2006
Melia witches’ broom	16SrI	*Melia azedarach*	China	Qui *et al*., 1998
	16SrI-B	*Melia azedarach*	Korea	Han *et al*., 2014
Sophora japonica witches’ broom	16SrV-B (‘*Ca*. P. ziziphi’)	*Sophora japonica*	China	Yu *et al*., 2012; Ren *et al*., 2014
Unnamed	16SrXII-A, ‘*Ca*. P. japonicum’-related	*Sophora japonica*	China	Duduk *et al*., 2010
Linden laciniated leaf	16SrI-B	*Tilia platyphyllos* var. *laciniata*	Lithuania	Jomantiene *et al*., 2013
Linden laciniated leaf witches’ broom	16SrI-(L/L)V	*Tilia platyphyllos* var. *laciniata*	Lithuania	Jomantiene *et al*., 2013
Allocasuarina yellows	‘*Ca*. P. allocasuarinae’	*Allocasuarina muelleriana*	Australia	Gibb *et al*., 2003; Marcone *et al*., 2004
Balanites witches’ broom	‘*Ca*. P. Balanitae’	*Balanites triflora*	Myanmar	Win *et al*., 2013
Magnolia stunt	16SrI-B, 16SrX	*Magnolia* spp.	Poland	Kamińska *et al*., 2001; Kamińska and Śliwa, 2003
Apple proliferation	16SrX-A (‘*Ca*. P. mali’)	Various ornamental *Malus* spp.	Germany	Kartte and Seemüller, 1988; Seemüller *et al*., 2011b
European stone fruit yellows	16SrX-F (‘*Ca*. P. prunorum’)	*Prunus serrulata*, *P. cerasifera*, *P. subhirtella*	Europe	Lederer and Seemüller, 1992; Marcone *et al*., 2010, 2011
Flowering cherry decline	16SrV-B	*Prunus serrulata*	China	Wang *et al*., 2014
X-disease	16SrIII-A (‘*Ca*. P. pruni’)	*Prunus virginiana*, *P. pensylvanica*	North America	Hiruki and Wang, 1999; Davis *et al*., 2013

[a] 16Sr group and subgroup designation according to Davis *et al*. (2012, 2013), Nejat *et al*. (2013) and Harrison *et al*. (2014), based on computer-simulated RFLP analysis.

N.D., not determined.

this group are phytoplasmas causing mainly diseases of woody plants such as flavescence dorée of grapevine, alder yellows, Palatinate grapevine yellows, spartium witches' broom, rubus stunt, eucalyptus little leaf, cherry lethal yellows, flowering cherry decline, peach yellows (in India), and jujube witches' broom (Jung *et al*., 2003; Lee *et al*., 2004a; Arnaud *et al*., 2007; Gao *et al*., 2011; Malembic-Maher *et al*., 2011; Wang *et al*., 2014). '*Ca.* P. ulmi' differs from other members of the EY group including '*Ca.* P. rubi' (the rubus stunt agent) and '*Ca.* P. ziziphi' (the jujube witches' broom agent) by less than 2.5% in 16S rDNA sequence, the cut-off threshold for assigning the rank of species to phytoplasmas under the status '*Candidatus*' (IRPCM, 2004). However, supporting data for separating the above taxa at the putative species level were obtained by examining other molecular markers and considering biological properties, such as host range and insect vector specificity (Jung *et al*., 2003; Lee *et al*., 2004a; Malembic-Maher *et al*., 2011). Nucleotide sequence comparisons revealed that four strains of '*Ca.* P. ulmi' from North America and Italy shared 99.9% sequence identity in the 16S rRNA gene, 99.7% in the ribosomal protein genes *rplV* and *rpsC*, and 99.5% in the traslocase protein gene *secY* (Lee *et al*., 2004a). However, typing of additional European strains based on RFLP and sequence analyses of 16S rRNA, *rplV*, *rpsC*, *secY* and *map* genes, showed genetic diversity within this pathogen (Arnaud *et al*., 2007; Jović *et al*., 2008, 2011a). For example, four different subtypes, designated EY-S1, EY-S2, EY-S3 and EY-S4, were identified among '*Ca.* P. ulmi' strains infecting elm trees in Serbia (Jović *et al*., 2011a), which differ from the '*Ca.* P. ulmi' reference strain EY1 by single base substitutions in the signature sequences defined by Lee *et al*. (2004a) as characteristic of this taxon.

A phytoplasma of the clover proliferation group (=16SrVI group), subgroup 16SrVI-C, was identified in nine American elm trees with symptoms similar to those of EY disease in a suburb west of Chicago (Illinois, USA), whereas double or multiple infections with the EY agent and a phytoplasma of the aster yellows group (16SrI) and/or the stolbur group (16SrXII) were detected in diseased elm and elm hybrids in northern and central Italy (Lee *et al*., 1995; Jacobs *et al*., 2003; Hiruki and Wang, 2004). However, in doubly or multiply infected trees, the EY agent was predominant and readily detectable by direct PCR, while the other phytoplasmas could be detected only by nested PCR. Subgroup 16SrV-B, 16SrI-B and 16SrI-D phytoplasmas were associated with EY disease in China (Zhu *et al*., 2008; Gao *et al*., 2011).

Symptoms of EY vary among the elm species. In those native to North America such as *U. americana* (American or white elm), *U. rubra* (red or slippery elm), *U. alata* (winged elm), *U. serotina* (September elm) and *U. crassifolia* (cedar elm) symptoms include leaf epinasty, leaf curl, chlorosis, premature casting of the leaves, a yellow to brown discoloration of the phloem in the roots and stem, and tree death, that usually occurs within 1 or 2 years from the appearance of foliar symptoms (Fig. 1A). Red elm, which usually dies in the second year of symptom expression, often shows witches' brooms symptoms that occur over the entire crown and progressively increase in severity giving the tree a starved appearance. Discolored phloem tissue of American, winged, September and cedar elms have a characteristic odor of wintergreen oil (methyl salicylate), whereas a pleasant scent like that of maple syrup is released by red elm. In *U. minor* (= *U. carpinifolia*, European field elm), the most characteristic symptoms are outstanding witches' brooms at the tips of twigs and branches and at the root level (Fig. 1B, C, E). For this reason, the disease of European field elm is often called elm witches' broom. Other symptoms include leaf epinasty, yellowing, stunting, small leaves and premature leaf shedding. Brooming and stunting are also the typical symptoms of *U. glabra* (Scots elm) and *U. parvifolia* (Chinese elm) (Fig. 1D and G) (Seemüller, 1992; Braun and Sinclair, 1979; Lee *et al*., 1993; Mittempergher *et al*., 2000). Leaf yellowing or reddening, reduced terminal growth, witches' broom formation, dieback and decline have also been observed in several other European and Asian elm species including *U. pumila* (Siberian elm) (Fig. 1F), *U. chenmoui* (Chenmou elm), *U. villosa* (cherry bark elm) (Fig. 1H), *U. laevis* (European white elm), *U. wallichiana* (Himalayan elm), *U. wilsoniana* (Wilson elm), *U. japonica* (Japanese elm) and *U.* x *hollandica* (Dutch elm) (Conti *et al*., 1987; Mittempergher *et al*., 1990, 2000; Lee *et al*., 1995; Mittempergher, 2000; Sfalanga *et al*., 2002). However, phloem discoloration is not know to occur in any of the European or Asian species.

In *U. rubra* x *U. pumila* hybrids, differences in symptom expression have been observed (Braun and Sinclair, 1979). Trees most closely resembling *U. pumila* show localized brooming and chlorosis only in the branches. The most severely affected trees are those with the strongest expression of *U. rubra* characters. Such trees, in addition to foliar symptoms show also phloem discoloration in the roots and stem. Thus, in these hybrids, the diverse parental genetic contribution may account for the variation in symptom expression.

Cross-inoculation experiments have revealed that inocula from EY-affected American and red elms induced in European field elm the typical symptoms occurring in naturally infected European field elm and *vice versa*. Furthermore, when grafted on Chinese, Siberian, or European white elms, inocula from symptomatic European field elm or red elm caused symptoms similar to those displayed by naturally infected Chinese, Siberian and European white elm trees (Gualaccini, 1963; Sinclair *et al*., 1976, Sinclair, 1981; Braun and Sinclair, 1979). Histopathological studies have shown that in diseased American elm trees, the first detectable anatomical alteration is an abnormal callose deposition on the sieve plates and lateral sieve areas followed by collapse and necrosis of sieve tubes and companion

cells. As a reaction to the loss of conducting tissue, hyperactivity of the cambium follows, resulting in the formation of additional, excessive phloem, which is usually referred to as replacement phloem. Sieve tubes of replacement phloem are smaller than normal and necrotize soon. Hyperplasia and hypertrophy of phloem parenchyma cells are common. Therefore, phloem transport is severely impaired. Starch accumulates in the stem, especially in the leaves, while the roots starve to death. The severity of anatomical alterations of phloem tissue correlates with the severity of phloem discoloration. Furthermore, although a very low number of '*Ca.* P. ulmi' cells occur in diseased *U. americana* and *U. rubra* trees, their sieve tubes are so sensitive to the EY agent infection that they collapse before the pathogen reaches a high titer (McLean, 1944; Wilson *et al*., 1972; Braun and Sinclair, 1976, 1978, 1979). In contrast, '*Ca.* P. ulmi'-colonized phloem tissues of *U. minor* and *U. parvifolia* trees are usually only slightly damaged and do not differ significantly from the phloem of healthy trees. In fact, the sieve tubes of these elm species are rather tolerant, thus allow multiplication of the pathogen to a relatively high titer. Phloem necrosis rarely occurs in the other European or Asian elm genotypes so far examined (Braun and Sinclair, 1979; Pisi *et al*., 1981; Marcone *et al*., 1994a, 1997a). '*Ca.* P. ulmi' infections induce also stomatal closure and an elevated diffusive resistance of the leaves of *U. americana* and *U. rubra* trees. Stomatal closure is in turn associated with an abnormally high water potential of diseased trees throughout the day as well as in darkness (Matteoni and Sinclair, 1983).

EY is widespread in the eastern half of the USA where several spectacular epidemics have killed hundreds to tens of thousands American and red elm trees. During these epidemics, elms of European and Asian origin remained apparently unaffected, except for single disease records in *U. parvifolia* and *U. rubra* x *U. pumila* hybrids (Braun and Sinclair, 1979; Lee *et al*., 1993; Mäurer *et al*., 1993). The reasons why European and Asian elms seem to escape damage from EY in North America may be ascribed to food preference of vectors (Sinclair, 2000). In Italy, significant EY outbreaks have occurred in three experimental fields established in the 1980s in northern and central Italy to test the adaptability of a number of elm species and 33 hybrid clones to local environmental conditions (Conti *et al*., 1987; Mittempergher *et al*., 1990, 2000; Lee *et al*., 1995; Mittempergher, 2000). Like in North America, *U. americana* seedlings were severely affected by EY, dying within 5 years from planting. Other most affected genotypes were *U. villosa*, *U. parvifolia*, *U. parvifolia* x *U. wallichiana* (= clone P628), (*U. glabra* 'Exoniensis' x *U. wallichiana*) x *U.* x *hollandica* 'Bea Schwarz' selfed (= clone 'Lobel') (Fig. 1I), (*U. glabra* x *U. wallichiana*) x *U. minor* open-pollinated (= clone 808), (*U. wallichiana* x (*U.* x *hollandica* 'Vegeta' x *U. minor*) (= clone 793), [(*U. wallichiana* x *U. minor)*] x *U. laciniata*) x *U. laciniata* 'Nikkoensis' open-pollinated (= clone 1094), and (*U. wallichiana* x *U. minor*) x *U. laciniata* open-pollinated (= clone 1098) (Fig. 1J) (Mittempergher, 2000). In the experimental field located at San Rossore near Pisa (central Italy), the percentage of affected trees was 30% (320 trees planted) five years after disease outbreak, reaching nearly 80% within 14 years. Such a high infection rate reflected the occurrence of an efficient vector.

EY epidemics of European field elm have been observed in the Po valley and Friuli- Venezia Giulia (northern Italy) (Conti *et al*., 1987; Mittempergher *et al*., 1990; Mittempergher, 2000; Carraro e*t al*., 2004) and are currently known to occur in the Agri valley (Basilicata, southern Italy) (C. Marcone, unpublished information). In Friuli-Venezia Giulia a widespread occurrence of EY on Siberian elm has also been recorded (Carraro e*t al*., 2004). Although in nature '*Ca.* P. ulmi' preferentially infects plants in the genus *Ulmus*, it was also identified in central Italy in plants of *Zelkova serrata* (Japanese zelkova) showing symptoms of yellowing, foliar reddening, witches' brooms, reduced terminal growth and stunting (Romanazzi and Murolo, 2008; Murolo and Romanazzi, 2008).

The white-banded elm leafhopper, *Scaphoideus luteolus*, is the only confirmed vector of '*Ca.* P. ulmi' in North America, although other vectors are likely to be involved in its natural spread. This likelihood is supported by the fact that numerous homopteran insects belonging to genera that comprise known phytoplasma vectors have been found on elm and probably feed on it to some extent, and that *S. luteolus* is rare or absent in some areas where severe EY outbreaks occur (for reviews see Sinclair, 1981, 2000; Lanier *et al*., 1988). By real-time PCR '*Ca.* P. ulmi' was also detected in several leafhoppers belonging to the genera *Allygus*, *Colladonus*, *Empoasca*, *Erythronneura*, *Graphocephala*, *Homalodisca*, *Orientus*, *Scaphoideus*, and *Typhlocyba*, which were captured in the Pennsylvania State University, University Park campus that hosts one of the largest populations of mature elm trees in the USA (Herath *et al*., 2010). However, it remains to be demonstrated if the above leafhopper species can transmit the pathogen. *S. luteolus* is not known to occur in Europe. Carraro *et al*. (2004) have shown that *Macropsis mendax* is a natural vector of the EY agent in Friuli- Venezia Giulia (Northern Italy). This leafhopper is strictly monophagous, completes one generation per year and overwinters as eggs on elm. It is unknown whether *M. mendax* is involved in the spread of the EY agent in other parts of Europe, and there is no information on its transmission efficiency.

The EY agent may also spread among closely spaced trees of the same species trough natural root grafts. This mode of transmission has been considered an important cause of shade tree losses in urban epidemics in North America (Seliskar and Wilson, 1981; Sinclair, 1981). The pathogen has also been transmitted from diseased elm trees to *Catharanthus roseus* (periwinkle) via dodder (*Cuscuta epithymum*) bridges and is efficiently transmitted among periwinkle plants by several dodder species

including *C. ceanothi*, and grafting (Braun and Sinclair, 1979; Mäurer *et al*., 1993). '*Ca*. P. ulmi' strains transmitted to periwinkle, *i.e.* EY1 from North America and ULW from France, induce identical symptoms in this host.

Several elm genotypes of Eurasian and American origin, which are resistant to the Dutch elm disease have been examined for EY resistance or tolerance. Following graft inoculation with a North American '*Ca*. P. ulmi' strain and observing disease development over a 3-year period, inoculated trees of the Eurasian cvs Pioneer (*U. glabra* x *U. minor*), Frontier (*U. minor* x *U. parvifolia*), Pathfinder (*U. parvifolia*), Prospector (*U. wilsoniana*), and the complex hybrids 'Homestead' and 'Patriot', differed considerably in their response to '*Ca*. P. ulmi' infections (Sinclair *et al*., 2000a). The responses ranged from no apparent reaction to phloem necrosis and death. Diseased trees of cvs Frontier, Pathfinder and Patriot showed symptoms of foliar yellowing and reddening, witches' brooms, reduced terminal growth and stunting. Lethal decline and phloem discoloration or necrosis, *i.e.* symptoms occurring in *U. americana* and *U. rubra*, were not observed. However, a longer observation period is required to determine whether diseased trees decline slowly.

Considerably more affected were trees of cv. Pioneer, most of which (13 of 16) became infected, as shown by PCR and DAPI (4'-6-diamidino-2-phenylindole) fluorescence assays, and died (12 trees) within the observation period. Only two out of twenty inoculated cv. Prospector trees were infected, one of which died. None of the 14 inoculated cv. Homestead plants tested phytoplasma-positive. These plants showed localized phloem necrosis below inoculum bark patches which could have resulted from a defense reaction that killed conductive phloem, thus preventing the systemic movement of the pathogen. The low infection rate recorded for most genotypes examined could hardly be ascribed to graft failures, because more that 75% of the bark patches used as inoculum fused with the recipient stems, nor was the absence of phytoplasma infections in the inoculum likely (Sinclair *et al*., 2000a).

Trees of *U. americana* cv. American Liberty grafted with bark patches from a naturally infected American elm sapling, proved to be highly susceptible to EY infections as shown by their high mortality rate, a finding confirmed by natural infections. In fact, the death of EY-affected cv. American Liberty trees was observed in at least one municipal planting and one arboretum in New York state (USA) (Sinclair *et al*., 1994; Sinclair, 2000). Since EY is lethal to North American species while some Eurasian species may be tolerant or resistant, it appears that the disease rather than North American, seems to be of European origin (Sinclair, 2000). Tolerance or resistance would be expected in regions where '*Ca*. P. ulmi' and its natural hosts have co-evolved, while the lack of tolerance or resistance could indicate a recent introduction of the pathogen.

Alder yellows

Alder yellows (ALY) is a decline disease that affects several *Alnus* (alder) species including *A. glutinosa*, *A. cordata*, *A. incana*, *A. hirsuta*, *A. rugosa*, *A. subcordata*, *A. tenuifolia* and *A. rubra*. The disease has been reported from several European countries such as Germany, Italy, Austria, France, Switzerland, Hungary, Serbia, Slovenia and Lithuania (Seemüller and Lederer, 1988; Lederer and Seemüller, 1991; Marcone *et al*., 1994b, 1997a; Seemüller *et al*., 1998; Valiunas *et al*., 2001; Arnaud *et al*., 2007; Cvrković *et al*., 2008; Malembic-Maher *et al*., 2007; Mehle *et al*., 2011). It has also been observed in the Washington state (USA) on *A. rubra* (red alder) (Lederer and Seemüller, 1991; Mäurer *et al*., 1993). However, molecular identification and classification of the phytoplasma(s) infecting *A. rubra* have not yet been accomplished.

Diseased *A. glutinosa* (European alder) trees are particularly widespread in Europe. In all affected *Alnus* species the symptoms are similar. These include yellowing, sparse foliage, premature autumn coloration, small leaves, reduced terminal growth, dieback and decline (Fig. 1K, L, M). Sometimes, these symptoms occur in the crown while sprouts at the base of trunks show normal growth. Latent infections are also common (Lederer and Seemüller, 1991; Maixner and Reinert, 1999). Of the 500 European alder trees examined by Lederer and Seemüller (1991) using DAPI fluorescence, ALY infections could be detected in all trees older than *ca.* 5 years, irrespective of symptom expression. About 80% of infected trees were symptomless, whereas only 20% showed ALY symptoms. Usually, healthy-looking trees were more heavily colonized by phytoplasmas than symptomatic trees. The phytoplasma population was always higher in petioles and young twigs than in aged branches, trunks and roots. Colonization was characterized by an uneven distribution of phytoplasmas in adjacent sieve tubes, with some tubes packed with pathogen's cells while others contained a considerably lower number of them.

A comparable situation was observed in *A. incana* trees, all samples of which, with the exception of few relatively young trees, proved to be phytoplasma-positive and showed very high numbers of the colonizing agent, regardless of symptom expression (Lederer and Seemüller, 1991). Under natural infection conditions, a high detection rate of more than 85% of ALY phytoplasma infections on *A. glutinosa* was also recorded by PCR (Malembic-Maher *et al*., 2009). In *A. glutinosa* trees experimentally inoculated with the ALY agent and showing yellowing symptoms, phytoplasma concentrations were relatively uniform and in the upper range of values found for woody plants examined by competitive PCR (Berges *et al*., 2000). Values in the experimentally inoculated *A. glutinosa* trees ranged from 5.1×10^6 to 7.1×10^7 phytoplasma cells per gram of tissue.

The ALY phytoplasma is taxonomically assigned to subgroup 16SrV-C within the EY group (Lee *et al*., 2004a). It

shares 99.7-100% 16S rDNA sequence identity with flavescence dorée (FD), Palatinate grapevine yellows (PGY), spartium witches' broom (SpaWB) agents and phytoplasmas infecting *Clematis vitalba* in Italy and the Balkans, and hemp dogbane (*Apocynum cannabinum*) in New York state, respectively (Lee *et al*., 2004a; Filippin *et al*., 2009; Malembic-Maher *et al*., 2011). On the basis of 16S rDNA and 16S-23S rDNA spacer region sequences, ALY phytoplasma is a homogeneous pathogen throughout Europe. However, typing based on RFLP and sequence analyses of ribosomal protein genes (*rplV*, *rpsC*, *rplF* and *rplR*) and non-ribosomal loci, including *secY*, *map*, *tuf*, *uvrB* and *degV* genes, revealed a considerable molecular variability. Some strains proved to be very closely related or identical to either FD phytoplasma strains or PGY phytoplasma strains (Lee *et al*., 2004a; Arnaud *et al*., 2007; Malembic-Maher *et al*., 2007, 2009, 2011; Mehle *et al*., 2011; Ember *et al*., 2011).

The ALY phytoplasma is transmitted by *Oncopsis alni* (Maixner and Reinert, 1999), an univoltine leafhopper extremely abundant on alder, with a strictly oligophagous feeding behaviour. The distribution of *O. alni* is clearly correlated with that of ALY disease in Europe (Maixner and Reinert, 1999). ALY phytoplasma infections were also detected in specimens of the psyllid *Psylla alni* collected from ALY-diseased alder trees. However, is spite of the high numbers of infected individuals that were recorded, no successful transmission of the ALY phytoplasma to healthy alder seedlings was achieved with transmission trials using ALY-infected *P. alni* psyllids (Maixner and Reinert, 1999). Molecular and epidemiological evidence indicates that ALY phytoplasma strains infecting alder trees in the Palatinate region of Germany also infect grapevines growing in neighboring vineyards, inducing the disease described as Palatinate grapevine yellows (PGY). *O. alni*, which feeds occasionally on grapevine, can transmit ALY strains from alder to grapevine (Maixner *et al*., 1995, 2000; Reinert and Maixner, 1997; Angelini *et al*., 2001). Therefore, diseased alder trees residing in close proximity of vineyards may serve as pathogen reservoirs. Also, due to the close phylogenetic relationship of ALY phytoplasma with the FD agent, diseased alder trees may also function as pathogen reservoirs in areas where FD disease occurs. In these cases, some ALY phytoplasma strains may be transmitted to grapevine by occasional grapevine-feeding vectors, such as *O. alni*, leading to FD disease, which is then spread from grapevine to grapevine by *Scaphoideus titanus*, the ampelophagous vector of FD phytoplasma strains. *S. titanus* is not known to occur in the Palatinate (Arnaud *et al*., 2007; Malembic-Maher *et al*., 2007, 2009; Mehle *et al*., 2011). ALY phytoplasma has also been transmitted from naturally infected *A. glutinosa* trees to periwinkle via dodder (*C. odorata*) bridges. The symptoms in dodder-inoculated periwinkle plants consist of yellowing, reduced vigor and small flowers (Marcone *et al*., 1997b).

As mentioned above, a high percentage of alder trees infected by the ALY agent does not develop obvious symptoms in nature. The reasons were elucidated by Berges and Seemüller (2002) through graft-inoculation of healthy alder seedlings with scionwood from differently affected and symptomless alder trees and observation of disease development over a 5-year period. It resulted that the severity of symptoms shown by inoculated plants largely corresponded to the severity of inoculum sources. Thus, it was concluded that ALY phytoplasma is pathogenic to alder and may induce severe symptoms, but avirulent and low virulent strains occurring within this taxon account for the latent infections that are widespread in Europe. Also, it is likely that avirulent strains mediate cross protection and prevent infection by severe strains since infected alder trees remain symptomless over time in spite of the presence of an abundant and efficient insect vector such as *O. alni*. Differences in strain virulence are also known for other phytoplasmas, including the agents of apple proliferation, European stone fruit yellows, ash yellows and aster yellows, whereas antagonistic interactions between distinct strains of the same taxon have been described for a number of phytoplasma-plant host combinations (for reviews see Marcone, 2010; Seemüller *et al*., 2010, 2011a).

Poplar witches' broom

Poplar witches' broom (PoWB) was first observed in 1973 in Bulgaria on *Populus nigra* 'Italica' (Lombardy or black polar) and *P. canadensis* (Canadian poplar) (Atanasoff, 1973). Later, the disease was first reported from the Netherlands on *P. alba* (white poplar), *P. canescens* (grey poplar) and Lombardy poplar (Van der Meer, 1980, 1981), then from France on Lombardy and white poplar, Germany on *P. tremula* (aspen), Lombardy and white poplar, Hungary on white poplar, and Croatia and Serbia on Lombardy poplar (Sharma and Cousin, 1986; Seemüller and Lederer, 1988; Mäurer *et al*., 1994; Cousin, 1996; Berges *et al*., 1997; Cousin *et al*., 1999; Šeruga *et al*., 2003; Mitrović *et al*., 2011). The disease is characterized mainly by witches' broom (Fig. 1N and P) although in some instances, especially on *P. nigra* 'Italica' trees, only nonspecific symptoms such as yellowing and undersized leaves, sparse foliage, stunting, dieback and decline may be present (Fig. 1O). On aspen, the disease is frequently associated with foliar reddening, yellowing and decline symptoms, whereas witches' brooms may occasionaly develop on vigorous shoots (Fig. 1N).

PoWB is associated with the aster yellows agent '*Ca.* P. asteris', subgroup 16SrI-B (Berges *et al*., 1997; Marcone *et al*., 2000; Lee *et al*., 2004b). However, subgroup 16SrI-A and 16SrI-P phytoplasma strains have also been identified in PoWB-affected *P. nigra* 'Italica' trees in Croatia and Serbia (Šeruga *et al*., 2003; Mitrović *et al*., 2011). Work based on RFLP analyses of 16S rDNA and 16S-23S rDNA spacer region sequences, revealed the presence of three different RFLP profiles among strains infecting *P. nigra* 'Italica',

P. alba and *P. tremula* in Germany, France and Hungary, following digestion with *Alu*I restriction enzyme. One of the profiles was shown by French strains from *P. nigra* 'Italica'. The second type of profile, which differed from that of the French strains by the lack of a restriction site in the 16S-23S rDNA spacer region, was shown by German strains from *P. nigra* 'Italica'. The third profile, which was most probably the result of sequence heterogeneity in the two 16S RNA genes, was presented by strains infecting *P. alba* and *P. tremula* (Berges *et al*., 1997). Differences between French and German strains infecting *P. nigra* 'Italica' trees were also obtained by heteroduplex mobility assays using 16S rDNA and 16S-23S rDNA spacer region sequences (Cousin *et al*., 1998).

A wide range of phytoplasma concentrations were detected in both naturally infected and experimentally inoculated poplar trees. Value ranged from 3.4×10^4 to 8.3×10^8 cells per gram of tissue in *P. nigra* 'Italica', whereas intermediate concentrations were estimated in *P. alba* and *P. tremula* (Berges *et al*., 2000). Nevertheless, in some naturally infected trees of *P. nigra* 'Italica' and *P. tremula*, the pathogen concentration was so low that nested PCR was required to detect infections (Berges *et al*., 1997, 2000). A 16SrI phytoplasma was also found in a diseased *P. nigra* tree in Colombia (Perilla-Henao *et al*., 2012). Plants micropropagated from diseased *P. alba* trees in France (Cousin *et al*., 1990) proved to be an useful diagnostic tool and allowed to maintain the PoWB agent in its natural host for more than three years (Cousin *et al*., 1990).

The strictly oligophagous leafhoppers *Rhytidodus decimusquartus* and *Tremulicerus vitreus*, which are particularly abundant on *Populus* species, were identified as vectors of the PoWB agent in France (Cousin, 1996; Cousin *et al*., 1999).

Spartium and *Sarothamnus scoparius* witches' broom

Spartium witches' broom (SpaWB) is a lethal disease of *Spartium junceum* (Spanish broom), a fabaceous shrub up to 3 m tall, common in Mediterranean areas. SpaWB occurs in Italy and Spain (Marcone *et al*., 1996a, 1997a, 1998; Torres *et al*., 2002; Spallino *et al*., 2013) where its most characteristic symptoms are prominent witches' brooms, shortened internodes, off-season growth and death of the plants (Fig. 1Q, R, S, T). Other symptoms include stunting, twisting and fasciation of twigs, and purple discoloration and rugosity of the bark, which may also show cracks. Affected plants die within one or a few years from the appearance of the first symptoms.

Whereas in Spain only '*Ca.* P. spartii' was reported to be associated with SpaWB (Torres *et al*., 2002), in Italy two genetically different phytoplasmas which induce the same symptoms were found: (i) '*Ca.* P. spartii', a member of the apple proliferation (AP) phytoplasma group (16SrX group), subgroup 16SrX-D and (ii) a phytoplasma belonging to EY group, subgroup 16SrV-C (Marcone *et al*., 1996a, 2004; Lee *et al*., 2004a, Arnaud *et al*., 2007). Most of the diseased plants are doubly infected with the two phytoplasmas, one of which is predominant and readily detectable by direct single-step PCR assays, while the other has a very low titer and can be detected only by the more sensitive nested PCR (Marcone *et al*., 1996a, 1997a, 1998, 2004).

A '*Ca.* P. spartii'-related strain has been identified in plants of *Sarothamnus scoparius* [syn. *Cytisus scoparius* (broom)] affected by witches' broom (SSWB) in southern Italy (Marcone *et al*., 1997c; Marcone, 2002). Most of the symptoms shown by diseased plants are similar to those of SpaWB (Fig. 1U). '*Ca.* P. spartii' can be distinguished from the SSWB agent by RFLP analysis of 16S rDNA and 16S-23S rDNA spacer region sequences using *Hha*I restriction endonuclease (Marcone *et al*., 1997c, 2004).

Recent work has shown that the yield of volatile fraction, *i.e.* essential oils, extracted from flowers of SpaWB-affected Spanish broom plants is lower than that of healthy plants (Mancini *et al*., 2010a). Also, substantial amounts of sesquiterpenes and a marked decrease in the amount of *n*-alkanes and aliphatic compounds occur in the volatile fraction from flowers of diseased plants. Sesquiterpenes could not be detected in the volatile fraction of healthy plants (Mancini *et al*., 2010a). Great differences were also identified in the content of alkaloid compounds between healthy and diseased Spanish brooms (Mancini *et al*., 2010b), the latter containing seven different alkaloids that were not present in healthy plants. These compounds included *N*-methylcytisine, its isomer, *N*-formylcytisine and a hydroxy-substituted derivative of sparteine. Four alkaloids including hydroxy-derivatives of cytisine and anagyrine were shared by both healthy and diseased plants. All identified alkaloids were quinolizidine alkaloids (Mancini *et al*., 2010b). Collective data indicated that changes in the composition of secondary metabolites of SpaWB-affected Spanish broom plants can be related to the role of phytoplasma infections in triggering plant defense responses (Mancini *et al*., 2010a, 2010b).

Buckthorn witches' broom

Rhamnus catharticus (buckthorn) is a woody shrub or small tree growing in various parts of Europe, western Asia, north Africa and North America. Buckthorn witches' broom (BWB), a phytoplasma disease reported from south-western Germany, causes brush-like witches' brooms arising mostly from the main stem or branches (Mäurer and Seemüller, 1996). These witches' brooms develop from young, premature shoots that are pushed in winter. Diseased plants bear distorted leaves, their vigor decreases steadily and show extensive discolouration of the phloem which is not visible in symptomless plants (Fig. 1V and W). BWB is associated with the presence of a

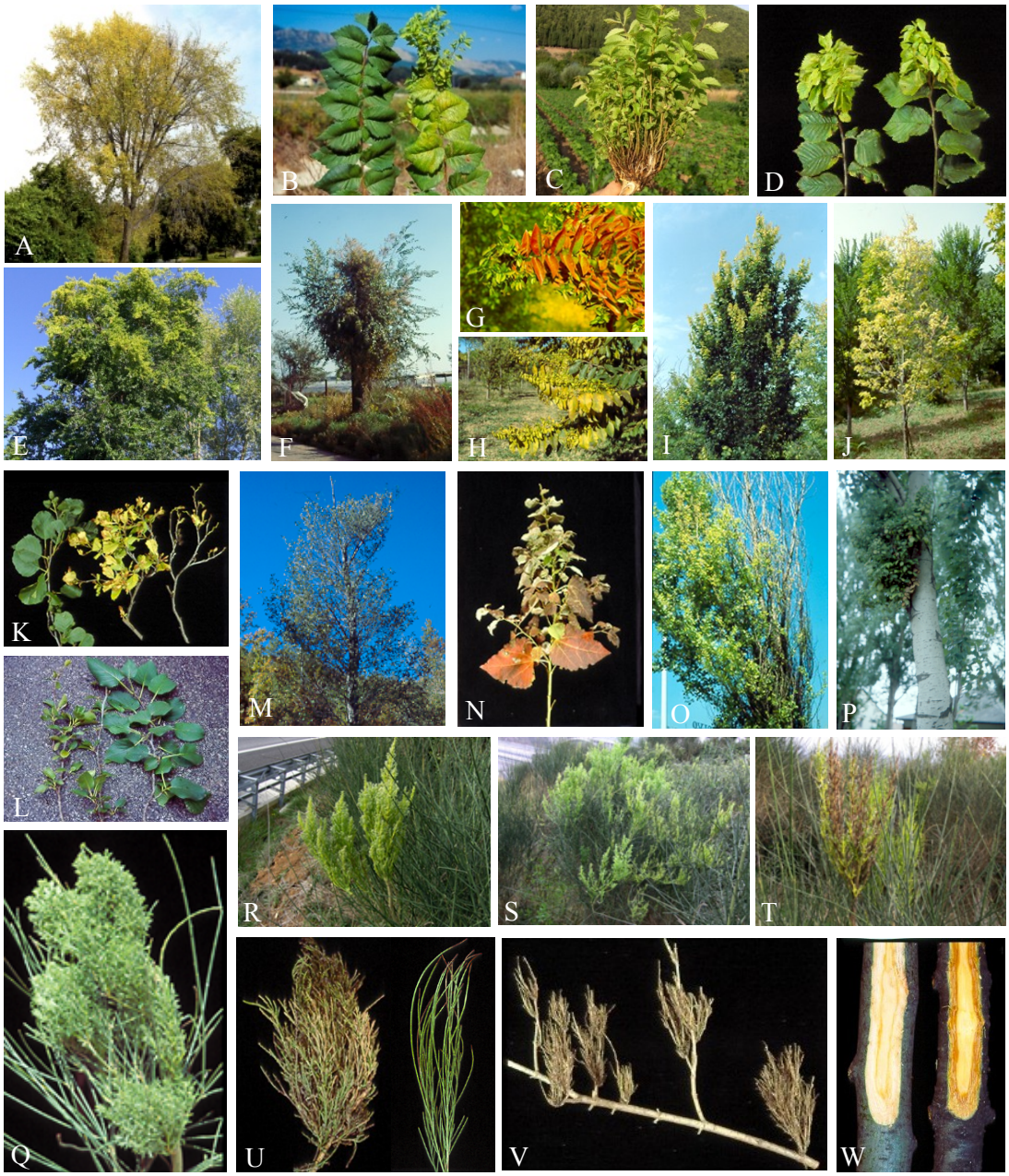

Fig. 1. A-J, elm yellows symptoms. A, large *Ulmus americana* (American elm) tree showing foliar yellowing and decline. B, small leaves and witches' broom at tip of *U. minor* (European field elm) shoot. Left, healthy shoot. C, witches' broom arising from a root of European field elm. D, witches' brooms on *U. glabra* (Scots elm). E, European field elm tree showing yellowing and witches' brooms in the upper portion of the crown. F, systemic brooming on *U. pumila* (Siberian elm). G, apical witches' brooms and foliar reddening on *U. parvifolia* (Chinese elm). H, stunted branches and severe yellowing on *U. villosa* (cherry bark elm). A healthy-looking branch is on the right. I, brooms at branch tips of (*U. glabra* 'Exoniensis' x *U. wallichiana*) x *U.* x *hollandica* 'Bea Schwarz' selfed (= clone 'Lobel') tree. J, sparse foliage and general chlorosis of (*U. wallichiana* x *U. minor*) x *U. laciniata* open-pollinated (= clone 1098) tree. K-M, alder yellows. K, symptoms of dieback, yellowing and leaf necrosis in *Alnus glutinosa* (European alder). Left, healthy branch. L, stunting and small leaves in *A. cordata* (right, healthy). M, European alder tree displaying abnormal branching and dieback. N-P, poplar witches' broom. N, witches' broom with decreasing leaf size towards the top on *Populus tremula* (aspen). O, dieback of declining *Populus nigra* 'Italica' (Lombardy polar) trees. P, witches' brooms on the trunk of *P. alba* (white poplar). Q-T, symptoms of spartium witches' broom on *Spartium junceum* (Spanish broom): pronounced witches' brooms (Q and R), off-season growth (S) and death of affected plant parts (T). U, witches' broom on *Sarothamnus scoparius* (broom) affected by the sarothamnus scoparius witches' broom. Right, healthy shoots. V and W, buckthorn witches' broom of *Rhamnus catharticus* (buckthorn): witches' brooms arising from a branch (V) and extensive browning of phloem and bark (W). In W, healthy control on the left. (A, courtesy W.A. Sinclair, Department of Plant Pathology, Cornell University, Ithaca, New York, USA; D, K, N, O, P, V and W, courtesy E. Seemüller, Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim, Germany; F, G, H, I and J, courtesy L. Mittempergher, Istituto per la Patologia degli Alberi Forestali, CNR, Firenze, Italy).

distinct phytoplasma, '*Ca.* P. rhamni', subgroup 16SrXX-A (Mäurer and Seemüller, 1996; Marcone *et al*., 2004; Davis *et al*., 2013), which shares 96% 16S rDNA sequence identity with fruit tree phytoplasmas of the AP group and 95% with '*Ca*. P. spartii' (Marcone *et al*., 2004). Greater differences occur in the sequences of the 16S-23S rDNA spacer region, where '*Ca*. P. rhamni' differs from the AP group fruit tree phytoplasmas in 14-17% of nucleotide positions and from '*Ca*. P. spartii' in 16% of the positions (Marcone *et al*., 2004). A few diseased buckthorn shrubs showing typical BWB symptoms from northern Italy proved to be infected by '*Ca*. P. rhamni' (Poggi Pollini *et al*., 2005). However, '*Ca*. P. rhamni' infections have also been detected in symptomless buckthorns in several European countries including Switzerland, Germany, Austria and Serbia (Jović *et al*., 2011b). In addition, '*Ca*. P. rhamni' was found in three psyllid species, *i.e. Trichochermes walkeri*, *Cacopsylla rhamnicola* and *Trioza rhamni* (Gassmann and Tosevski, 2014), which are common on *R. catharticus* in Europe, and may be regarded as potential vectors of '*Ca*. P. rhamni'.

Eucalyptus little leaf

Several *Eucalyptus* (eucalypt) species are affected by eucalyptus little leaf (ELL), a disease first described in India in 1971, where it was thought to be caused by a virus (Sastry *et al*., 1971). Later, ELL was observed by other Indian researchers, who discovered phytoplasma infections in affected trees by electron and light microscopy (Nayar, 1973; Nayar and Ananthapadmanabha, 1977; Sharma *et al*., 1983; Ghosh *et al*., 1984). The disease has also been reported from Italy, China and Sudan (Ragozzino and Cristinzio, 1980; Zhang *et al*., 1982; Dafalla *et al*., 1986; McCoy *et al*., 1989). The symptoms described from the various geographic areas and different *Eucalyptus* spp. are similar. They include abnormally minute leaves, yellowing, shortened internodes and premature growth from axillary buds, both giving the shoots a bushy, broom-like appearance (Fig. 2A and B). Diseased trees or their symptomatic parts do not set fruits, are stunted and dieback. Severely affected trees decline. A phytoplasma closely related to the EY agent was detected in ELL-affected trees in Italy (Marcone *et al*., 1996b). However, in the affected trees the phytoplasma concentration was so low that infections could be identified only through nested PCR assays. The ELL phytoplasma proved to be indistinguishable from those infecting *Rubus*, Spanish broom, and alder, using several restriction endonucleases (Marcone *et al*., 1997a, 1998, 1999). Also, phytoplasmas of 16SrI group, subgroups 16SrI-B and 16SrI-C, were identified in diseased eucalyptus trees in Italy (Camele *et al*., 1999). However, the identity of phytoplasmas infecting eucalyptus in India, China and Sudan has never been determined by molecular methods.

Pine shoot proliferation and other conifer diseases

After a few unconfirmed reports on the presence of phytoplasma infections in gymnosperms including representatives the families Pinaceae, Taxodiaceae and Cupressaceae using electron microscope observations (Koyama, 1970; Gopo *et al*., 1989; McCoy *et al*., 1989), phytoplasmas were detected and molecularly identified in several conifers over the last few years. Schneider *et al*. (2005) reported on the occurrence of a novel taxon, '*Ca*. P. pini', in *Pinus sylvestris* (Scots pine) and *P. halepensis* (Aleppo pine) trees grown in Germany and Spain, respectively. Symptoms shown by affected trees included yellowing, stunted growth, dwarfed needles and proliferation of shoots. In *P. sylvestris*, conspicuous shoot proliferation combined with dwarfed needles gives some affected branches a dense, ball-like appearance, whereas other branches are symptomless. These ball-like structures were not observed in *P. sylvestris*. '*Ca*. P. pini' infections were detected in symptomatic and symptomless plant parts of *P. sylvestris* and *P. halepensis* trees and some neighboring non-symptomatic trees proved to be infected by '*Ca*. P. pini'.

Following the report of Schneider *et al*. (2005), '*Ca*. P. pini' and related strains were detected in *P. sylvestris*, *P. nigra* (Austrian pine), *P. banksiana* (Jack pine), *P. tabuliformis* (Chinese pine), *P. mugo* (mountain pine), *Abies procera* (noble fir), *Tsuga canadensis* (Canadian hemlock), and *Picea pungens* (Colorado blu spruce) trees grown in Poland and the Czech Republic, in *Pinus* spp. and *P. mugo* in Croatia and in *P. sylvestris* in Lithuania. Most of the affected trees showed symptoms of shoot proliferation, stunted growth and dwarfed needles while ball-like structures similar to those observed in Germany on *P. sylvestris* were observed on the same species in Poland and the Czech Republic (Śliwa *et al*., 2008; Valiunas *et al*., 2010; Kamińska and Berniak, 2011; Kamińska *et al*., 2011; Ježić *et al*., 2012). Furthermore, '*Ca*. P. pini' was identified in China (Huang *et al*., 2011) in *Taxodium distichum* var. *imbricarium* (pond cypress) showing abnormal shoot proliferation, little leaf and leaf necrosis.

'*Ca*. P. pini' is a member of the pine shoot proliferation phytoplasma group or 16SrXXI group, subgroup 16SrXXI-A (Davis *et al*., 2013). This phytoplasma is a homogeneous pathogen at the level of 16S rDNA sequence. Nucleotide sequence comparisons revealed that the 16S rDNA sequences of strains from Europe and China were identical or nearly so, showing identity values between 99.7 and 100% (Schneider *et al*., 2005; Śliwa *et al*., 2008; Huang *et al*., 2011; Kamińska *et al*., 2011). '*Ca*. P. pini' is only distantly related to other phytoplasmas. The closest relatives are members of the coconut lethal yellowing (16SrIV group) and rice yellow dwarf (16SrXI group) phytoplasma groups and '*Ca*. P. castanea' (16SrXIX-A subgroup) (Schneider *et al*., 2005; Davis *et al*., 2013).

Phytoplasmas of other taxonomic groups have also been detected in conifers. A member of the X-disease phytoplasma group (16SrIII group) was identified in Italy in *Cupressus* sp. (cypress) trees showing witches' broom, stunting and fasciation (Paltrinieri *et al*., 1998) and in *Picea abies* (Norway spruce) and *P. glauca* (white spruce) with symptoms similar to those associated with the above mentioned '*Ca*. P. pini' infections in Poland (Kamińska and Berniak, 2011). In the latter country, infections by phytoplasma of the aster yellows group were also detected in diseased *P. pungens* trees, whereas a 16SrI-B phytoplasma was identified in diseased *Larix* sp. (larch) in Ukraine (Jomantiene *et al*., 2011; Kamińska and Berniak, 2011). The affected larch trees showed symptoms of yellowing, dwarfing, proliferation and necrosis of the needles. Hence, the larch-infecting agent was named larch dwarfed needle proliferation (LD-NP) phytoplasma (Jomantiene *et al*., 2011).

Phytoplasmas were also detected by electron microscopy in a few young declining larch trees from a nursery in northern Germany (Nienhaus, 1985). A '*Ca*. P. phoenicium'-related strain, subgroup 16SrIX-F, was reported to be associated with the juniper witches' broom (JunWB) disease of *Juniperus occidentalis* (western juniper) in Oregon (USA). This disease is characterized by abnormal proliferation of shoots, little leaves, shortened internodes, and ball-like structures. The incidence of JunWB disease is about 1% (Davis *et al*., 2010). A '*Ca*. P. trifolii'-related strain, member of the clover proliferation phytoplasma group or 16SrVI group, was identified in diseased trees of *Araucaria heterophylla* (Norfolk Island pine) showing yellowing, little leaves, witches' broom, and shoot proliferation in India. The percentage of affected trees was about 15% (Gupta *et al*., 2010).

The suitability of gymnosperms as phytoplasma hosts has for a long time been questioned mainly because of the small pore sizes in the sieve cells, which may hinder phytoplasma movement within the plant (for review see Seemüller *et al*., 2002). However, data obtained using PCR technology suggest that phytoplasma infections in conifers, which usually occur at low titer, are more common than previously thought. Although detection is often associated with different kinds of symptoms including shoot proliferation and dwarfed needles, the pathological relevance of phytoplasma infections in conifers is not always clear mainly because the same phytoplasmas were sometime observed in symptomless trees. Thus, further research based mainly on graft inoculation studies is needed to validate the effect of phytoplasma infections in conifers.

Sandal spike

Sandal spike (SAS), a serious disease of *Santalum album* (sandal), is widespread in southern India, mainly in the states of Karnataka, Tamil Nadu and Kerala, but does not occur elsewhere (Thomas and Balasundaran, 1998, 1999; Khan *et al*., 2004, 2006, 2008). The disease was first observed in the late 1890s and was thought to be caused by virus. Subsequent fluorescence and electron microscopy observations and symptom remission obtained by tetracycline treatments clearly demonstrated its phytoplasmal etiology (Dijkstra and Ie, 1969; Hull *et al*., 1969; Varma *et al*., 1969; Raychaudhuri *et al*., 1972). SAS has spread progressively over the years, devastating large forest tracts and threatening the sandal industry of southern India, where production of sandalwood oil is of major importance.

The most characteristic symptoms of SAS are shortened internodes, small and extremely narrow leaves, and phyllody. The affected leaves, which are pale-green or yellow, stand out stiffly from the shoots, giving them a spike-like appearance. The most severely affected trees die within two or three years from the appearance of the first symptoms (Fig. 2S and T). A disease incidence reaching up to 55% was recorded in southern Karnataka (Rao and Muniyappa, 1988). SAS is associated with the aster yellows agent '*Ca.* P. asteris', subgroup 16SrI-B (Marcone *et al*., 2000; Lee *et al*., 2004b; Khan *et al*., 2008). SAS phytoplasma was transmitted from diseased sandal trees to periwinkle and from this host back to sandal trees via dodder (*C. subinclusa*) bridges. The dodder-inoculated periwinkle plants developed witches' broom symptoms (Dijkstra and Lee, 1972; Hiruki and Dijkstra, 1973). It was also experimentally transmitted from sandal to sandal by grafing. The leafhopper *Coelidia indica*, originally identified as *Jassus indicus*, was reported as a natural vector of SAS (Rangaswami and Griffith, 1941; Weintraub and Beanland, 2006).

Ash yellows and lilac witches' broom

Ash yellows (AshY) and lilac witches' broom (LWB) are diseases of *Fraxinus* (ash) and *Syringa* (lilac) species known to occur in North America (Sinclair *et al*., 1996). These diseases are caused by the same pathogen, the AshY agent '*Ca*. P. fraxini', a member of the AshY phytoplasma group or 16SrVII group, subgroup 16SrVII-A (Griffiths *et al*., 1999; Davis *et al*., 2013). The relationships between AshY and LWB phytoplasmas was established by: (i) reciprocal graft transmissions that caused typical symptoms of the respective diseases in ash and lilac; (ii) the occurrence of identical symptoms in periwinkle to which the two phytoplasmas were transmitted by dodder; (iii) the concomitant presence of AshY and LWB in several arboreta and (iv) the use of serological and DNA-based assays (Brierley, 1955; Hibben and Wolanski, 1971; Hibben and Franzen, 1989; Hibben *et al*., 1986, 1991; Griffiths *et al*., 1994, 1999). *Fraxinus* and *Syringa* are closely related genera in the family Oleaceae and, as they are graft-compatible, some lilac cultivars have been grown on ash rootstocks.

Although AshY may have occurred in the north-eastern USA since the 1930s, it was not considered as a major forest disease until the early 1980s (for review see Sinclair

and Griffiths, 1994). This late recognition was due to the difficulties met in identifying phytoplasma diseases and to the inconsistency with which infected trees show specific symptoms. LWB was first reported in 1951 and was fully described several years later (Hibben *et al*., 1986; Hibben and Franzen, 1989). The natural host range of '*Ca*. P. fraxini' includes 12 ash species and 19 lilac species and numerous infraspecific taxa and interspecific hybrids: *F. americana* (white ash), *F. pennsylvanica* (green ash), *F. velutina* (velvet ash), *F. angustifolia* (syn. *F. oxyacarpa*), *F. bungeana*, *F. excelsior* (European ash), *F. nigra* (black ash), *F. latifolia* (syn. *F. oregona*, Oregon ash), *F. ornus* (flowering ash), *F. potamophila*, *F. profunda* (syn. *F. tomentosa*), *F. quadrangulata* (blue ash), *S.* x *diversifolia*, *S.* x *henryi*, *S.* x *josiflexa*, *S. josikaea*, *S. julianae*, *S. komarowii*, *S. laciniata*, *S. meyeri*, *S. microphylla*, *S.* x *nanceiana*, *S. oblata*, *S. patula*, *S.* x *persica*, *S.* x *prestoniae*, *S. sweginzowii*, *S. tomentella*, *S. villosa*, *S. vulgaris*, and *S. yunnanensis*. Experimental hosts are dodder, periwinkle, *Daucus carota* (carrot) and *Trifolium pratense* (red clover) (Hibben *et al*., 1986; Hibben and Franzen, 1989; Sinclair *et al*., 1996).

Infections by a subgroup 16SrVII-A phytoplasma have also been detected in diseased plants of *Ugni molinae* (murta), *Paeonia lactiflora* (peony) and grapevine in Chile, and peach in Canada (Gajardo *et al*., 2009; Zunnoon-Khan *et al*., 2010; Arismendi *et al*., 2011), whereas 16SrI phytoplasma infections were identified in declining trees of *F. excelsior* in Poland and *F. uhdei* in Colombia (Kamińska and Berniak, 2009; Perilla-Henao *et al*., 2012). In the latter country, dieback-affected *F. uhdei* trees proved to be infected by a phytoplasma that is closely related to the AshY agent (Filgueira *et al*., 2004).

AshY and LWB cause reduced apical and radial growth, loss of apical dominance or deliquescent branching (the branch bears abnormally short twigs that are all of similar length), suppressed root development, premature flowering and shoot growth, and witches' brooms. Subnormal greenness of the foliage and leaf malformations are common while chlorosis may occur occasionally (Fig. 2C through K). Highly susceptible hosts show dieback of branches and root shoot proliferation, stunted deliquescent branches, and die prematurely. Affected white ash trees produce abnormally short, bushy roots or show necrosis of the rootlets that may lead to sudden wilting and death.

Histological symptoms of both diseases, which are more readily observed in the roots than in the aerial parts of affected plants, include autofluorescent sieve tubes and pathological sclerenchyma. Autofluorescent sieve tubes are sieve tubes that fluoresce without the addition of any reagent when exposed to UV radiation. Such sieve tubes are presumed to be non-functional and often collapse (Dyer and Sinclair, 1991). Pathological sclerenchyma is a distinct parenchyma tissue adjacent to infected sieve tubes, characterized by prominently thickened and lignified cell walls, which greatly differs from the sclerotic parenchyma that is often seen in healthy phloem (Dyer and Sinclair, 1991). Stomatal closure and high diffusive resistance are also known to occur in the leaves of affected white ash trees (Matteoni and Sinclair, 1983).

Infected ash and lilac plants are highly sensitive to frost injuries. Lilac stands can be killed in winter, and in white ash cambial damage has been observed, appearing as vertical cracks of the bark (Fig. 2E) and/or tangential separation of bark and wood at the base of the trunk (Matteoni and Sinclair, 1985; Hibben and Franzen, 1989). Witches' brooms are regarded as specific symptoms of the diseases, whereas other symptoms are non-specific since they can be induced by several other biotic and abiotic factors. AshY incidence greater than 50% has been recorded in some white ash populations in northern US states and in a velvet ash population in Utah. Incidence rates of 3 to 27% were found in green ash shade trees in Iowa and Wisconsin cities (for review see Sinclair *et al*., 1996). '*Ca*. P. fraxini' infections were also detected in healthy-appearing plants of ash and lilac (Sinclair and Griffiths, 1995; Sinclair *et al*., 1996). Possible explanations provided for these observations are either that the plants were tolerant, or harboured strains of low virulence, or were in the early stages of colonization by strains of normal virulence (Sinclair and Griffiths, 1995; Sinclair *et al*., 1996).

Sinclair and Griffiths (2000) investigated the virulence of 12 '*Ca*. P. fraxini' strains, from across the known range of AshY disease and representing six host species, three of each *Fraxinus* and *Syringa* genera, by graft-inoculating green ash seedlings and periwinkle plants and monitoring symptom development under greenhouse conditions. In graft-inoculated plants, different strains caused significantly different degrees of growth suppression and loss of foliar greenness, ranging from slight or imperceptible to severe. The same study (Sinclair and Griffiths, 2000) revealed the occurrence of interference among strains of the same taxon. When periwinkle plants were co-inoculated with two strains of the AshY phytoplasma that differed greatly in aggressiveness, the most aggressive strain appeared sooner and more frequently than the less aggressive strain in the leaves located at a distance from the inoculation sites. Thus, aggressiveness was associated either with a faster movement or higher multiplication rate of the most aggressive strain compared to the less aggressive one. However, when either strain was inoculated 11 weeks before the other into the same plant, only the initial strain could be detected after a further 12 week of incubation. Therefore, the initial strain or its effect on the host may have interfered with long-distance movement or multiplication of the second strain. Thus, a concept of pre-emptive dominance was proposed by Sinclair and Griffiths (2000) to explain the dominance of the first strain that colonized a plant, regardless of its aggressiveness.

Differences in virulence among strains of '*Ca*. P. fraxini' have also been observed under natural infection conditions in which six cultivars of green ash and five cultivars

of white ash were graf-inoculated with six distinctly different strains at two different locations of Iowa and New York, where the inoculated plants were maintained under observations for three years (Sinclair *et al*., 2000b). The strains greatly differed in aggressiveness as indicated by host growth suppression, reduction of foliar greenness and frequency of witches' brooms. However, strain-cultivar interactions were not identified (Sinclair *et al*., 2000b). The differences in aggressiveness of two strains of the AshY phytoplasma were correlated with the level of photosynthesis inhibition and the occurrence of photoinhibition (Tan and Whitlow, 2001).

Several studies based on field observations and graft-inoculation experiments have shown that green ash and velvet ash are more tolerant than white ash to '*Ca*. P. fraxini' infections and that heritable intraspecific variation in tolerance occurs in green ash (Sinclair *et al*., 1994, 1997a, 1997b). Sinclair *et al*. (1997b) reported that in graft-inoculated plants '*Ca*. P. fraxini' suppressed shoot growth of white ash and green ash at the onset of bud break, but not until 60 days of growth post inoculation in velvet ash. AshY-associated growth losses in height, stem diameter, and root volume were 80, 93, and 98%, respectively in white ash; 60, 57, and 79% in green ash; and 23, 0, and 12% in velvet ash. Also, the growth of diseased velvet ash on white ash rootstock was severely suppressed in comparison with that of diseased own-rooted velvet ash, whereas diseased white ash scions on velvet ash rootstocks registered a significantly lower growth suppression than did diseased own-rooted white ash. White ash witches' brooms grafted on velvet ash rootstocks continued to retain their original form but did not produce vigorous shoots. These findings indicate that although tolerant rootstocks mitigate the impact of '*Ca*. P. fraxini' infections on scions, management of AshY disease through the use of tolerant genotypes may require tolerance in both scion and rootstock (Sinclair *et al*., 1997b).

The vectors of AshY and LWB are unknown. Field-collected *Paraphlepsius irroratus* and *Philaenus spumarius* transmitted naturally acquired phytoplasmas to caged ash seedlings (Matteoni and Sinclair, 1988). However, the identity of the associated phytoplasma(s) was unknown, and laboratory colonies of these insect species failed to transmit '*Ca*. P. fraxini' under controlled conditions. In the work by Hill and Sinclair (2000), 33 taxa of leafhoppers, including members of 13 genera known to comprise phytoplasma vectors, were collected in two sites of high AshY incidence in New York state and tested by PCR assays for the presence of phytoplasmas. The most abundant genus was *Scaphoideus*, with numbers about six times greater than any other genus. '*Ca*. P. fraxini' presence was detected in 19 of the 812 individuals of *Scaphoideus* spp. and in 1 of 87 of *Colladonus clitellarius*. Phytoplasmas of the X-disease group were detected in one *Scaphoideus* sp., four *C. clitellarius*, and 4 of 83 *Scaphytopius acutus* individuals. Phytoplasmas of the aster yellows group were identified in 1 of 68 individuals of *Gyponana* spp. and five *S. acutus*. Therefore, '*Ca*. P. fraxini'-infected leafhoppers should be regarded as potential vectors of this pathogen and should be checked experimentally for their transmission ability (Hill and Sinclair, 2000).

Paulownia witches' broom

Paulownia witches' broom (PaWB), one of the first described phytoplasma diseases (Doi *et al*., 1967), affects several *Paulownia* spp. (paulownia) and is widespread only in East Asia. In PaWB-affected paulownia trees, growth and vigor are greatly reduced, the leaves on affected shoots are yellowish, malformed and reduced in size, while flower clusters, when they are produced, show different degrees of distortion and virescence, along with sterility. Proliferation of slender shoots which arise from the main branches gives rise to typical witches' brooms (Fig. 2L, M). Affected shoots show phloem necrosis and an irregular cell arrangement in the woody cylinder, mainly around the vessel. Severely affected trees die prematurely. The wood of infected trees is of poor quality and often commercially unfit. In China, the expansion of paulownia plantations during 1970s contributed significantly to the rapid spread of PaWB, due to the large use of PaWB-infected root cuttings for propagation (for review see Hiruki, 1999). In northern China, the disease incidence was 10-20% in the early 1970s and more than 70% in the 1980s. A separate survey in China registered an incidence of 5-10% at the seedling stage, 10-20% in one-year-old saplings, 50% at the middle age stage, and 70-100% in aged trees.

PaWB is caused by a distinct member of the aster yellows phytoplasma group, subgroup 16SrI-D (Marcone *et al*., 2000; Lee *et al*., 2004b). This phytoplasma seems to infect in nature only paulownia and was experimentally transmitted to periwinkle by dodder (Doi and Asuyama, 1981). Three species of heteropteran insects of the family Pentatomidae (stinkbugs), namely *Halyomorpha mista*, *H. halys* and *H. picus* are reported as vectors of the PaWB agent (for reviews see Hiruki, 1999; Weintraub and Beanland, 2006). A recent work based on high-throughput mRNA sequencing (RNA-Seq) and digital gene expression (DGE) analyses has shown that dramatic changes occur in the gene expression profile of paulownia plants upon infection with the PaWB phytoplasma (Mou *et al*., 2013). Genes encoding key enzymes in cytokinin biosynthesis, such as isopentenyl diphosphate isomerase and isopentenyltransferase, were significantly induced in the infected plants, while genes involved in cell wall biosynthesis and degradation were largely up-regulated and genes related to photosynthesis were down-regulated (Mou *et al*., 2013). Previous studies using *in vitro* plant tissue cultures showed that the total free indole-3-acetic acid (IAA) content of stems and leaves of diseased paulownia plantlets was lower than that of healthy plantlets while the peroxidase activity

in the extract of diseased plantlets was much higher than in healthy tissues (Tian *et al*., 1995). Transgenic resistance to PaWB phytoplasma has been obtained by expressing an antibacterial peptide encoded by the *shiva-1* gene in *Paulownia tomentosa* x *P. fortunei* plants. Both symptom development and phytoplasma titer were significantly reduced in transgenic plants (Du *et al*., 2005).

Mulberry dwarf

Mulberry dwarf (MD), one of the most serious diseases of *Morus* (mulberry) species, including *M. alba*, *M. bombycis* and *M. multicaulis*, occurs in Japan and Korea (Sato *et al*., 1996; Ji *et al*., 2009). The phytoplasmal etiology of this disease was established in 1967 when Doi *et al*. (1967) recognized in the phloem sieve tube elements of yellows-diseased mulberry plants numerous wall-less, pleomorphic bodies which resembled morphologically and ultrastructurally mycoplasmas known to cause animal and human diseases. At the same time, Ishiie *et al*. (1967) applied tetracyclines to diseased mulberry plants obtaining a remission of symptoms. The most characteristic symptoms of MD are yellowing of the leaves, phyllody, stunting, proliferation, and witches' brooms (Fig. 2N and U). The disease is caused by the aster yellows agent '*Ca.* P. asteris', subgroup 16SrI-B (Namba *et al*., 1993; Lee *et al*., 2004b; Ji *et al*., 2009), and its causal agent is spread in nature by the leafhoppers *Hishimonoides sellatiformis* and *Hishimonus sellatus*, the first of these two species being a more efficient vector (Kawakita *et al*., 2000).

Although phytoplasmas were not believed to be vertically transmitted to the progeny of the vectors for many years, PCR-based and electron microscopy investigations provided indications for transovarial transmission of MD phytoplasma by *H. sellatiformis* (Kawakita *et al*., 2000). *H. sellatus* experimentally transmitted the MD phytoplasma to five herbaceous species, *i.e.* periwinkle, white clover, Ladino clover, red clover and Chinese milk vetch (Kim *et al*., 1985). Natural resistance to the MD phytoplasma identified in some *M. alba* cultivars is associated with the phytoalexin concentration of the mulberry tree cortex. The amount of a small group of compounds isolated by thin-layer chromatography was four times higher in the resistant than in susceptible cultivars (Kuai *et al*., 1999).

Recent studies have shown that an increase in soluble carbohydrate and starch contents and a decrease in the net photosynthesis rate, carboxylation efficiency, and pigment content occurr in MD phytoplasma-infected leaves of mulberry. Also, damages to the chloroplast ultrastructure were observed in infected leaves (Ji *et al*., 2009). Several proteins that were differentially expressed upon MD phytoplasma infections were identified in affected mulberry leaves. These proteins are involved in photosynthesis, amino acid biosynthesis, nucleotide metabolism, transcription, defense response, signal transduction, and regulation. Of these, the large subunit of the photosynthetic proteins rubisco, rubisco activase, and sedoheptulose-1,7-bisphosphatase showed enhanced degradation (Ji *et al*., 2009).

Characterization of the expressed proteome of MD phytoplasma-infected mulberry plants using a shotgun proteomic approach has also been reported (Ji *et al*., 2010). Metabolomic changes of mulberry plants in response to MD phytoplasma infections were examined using gas chromatography-mass spectrometry (GC-MS) techniques and biochemical methods (Gai *et al*., 2014a). This study revealed that in diseased plants carbohydrate transportation and metabolism are affected, as well as the amino acid and phytohormone contents. In particular, a repression of starch degradation due to a marked reduced activity of α-amylase and β-amylase occurs. In infected plants, the metabolome of stem phloem tissues was more severely affected than that of the leaves. Thus, it was concluded that leaves and stem phloem tissues have complex and different metabolic responses to MD phytoplasma infections (Gai *et al*., 2014a). By next-generation sequencing technology microRNAs (miRNAs) and small interfering RNAs (siRNAs) were identified that are differentially expressed in mulberry plants in response to MD phytoplasma infections (Gai *et al*., 2014b). Some responsive miRNAs target genes are involved in hormone signaling pathways, plant growth and development, protein metabolism, lipid metabolism, and carbohydrate metabolism, while high numbers of responsive siRNAs target genes are associated with a wide range of functions in hormone networks, development and metabolism and, thus, play major role in symptom development (Gai *et al*., 2014b).

Black locust witches' broom

Black locust witches' broom (BLWB), one of the earliest recorded yellows diseases of forest trees, was first described on *Robinia pseudoacacia* (black locust) in Maryland (USA) in 1898 (Waters, 1898). Since then, the disease has been found throughout the eastern US as well as in some European countries, including Italy, Bulgaria and the Czech Republic, and China (Atanasoff, 1935; Ciferri and Corte, 1959, 1960; Seliskar *et al*., 1973; Seliskar and Wilson, 1981; Seemüller, 1992; Chapman *et al*., 2001; Ren *et al*., 2014). The disease is characterized by the presence of erect brooms on root and stump sprouts and, less frequently, in the crown of affected trees. Brooms usually result from the abnormal proliferation of normally dormant axillary buds in the leaf axils of affected shoots. Leaves in the brooms are small and show vein clearing, particularly in the early stages. Because brooms continue to grow into late fall or early winter, they frequently dieback during winter. Brooming may also occur on the roots of infected trees. A tree producing broomed shoots one year may produce normal-looking shoots the following year. Sometimes, infected trees exhibit no symptoms unless the trees

are cut to a stump level; then, witches' brooms develop from stump and roots. No insect vector is known.

Phytoplasma infections in BLWB-affected black locust trees in the USA have been detected by light and transmission electron microscope observations and PCR assays (Seliskar *et al*., 1973; Chapman *et al*., 2001). Based on RFLP and sequence analyses of PCR-amplified rDNA, the phytoplasma detected in diseased black locust trees growing in Maryland was identified as a member of the X-disease phytoplasma group and proved to be closely related to a phytoplasma infecting grapevine in Virginia (Davis and Dally, 2000; Chapman *et al*., 2001). In China, BLWB is associated with the jujube witches' broom (JWB) agent '*Ca*. P. ziziphi', subgroup 16SrV-B (Ren *et al*., 2014). However, all reports of BLWB disease from European countries are based only on field observations, with no microscopical and molecular data, which call further for investigations aimed at determining the etiology of the European BLWB.

Chestnut witches' broom and Chinese chestnut yellow crinkle

A graft-transmissible yellows disease of *Castanea crenata* (Japanese chestnut), supposed to be caused by a virus, was first described in Japan in 1954 and was named chestnut yellows (Shimada and Kouda, 1954). Later, transmission electron microscope observations determined that this disease is associated with phytoplasma infections (Okuda *et al*., 1974). In 1993, Japanese chestnut trees from Korea showing mainly symptoms of yellowing and little leaf were found to be infected by phytoplasmas using fluorescence and electron microscopy techniques (Han *et al*., 1997). In 2002, the occurrence in Korea of a phytoplasma disease of Japanese chestnut was reported by Jung *et al.* (2002). This disease, which was denoted chestnut witches' broom (CnWB), caused severe crop losses, particularly in the Kyongnam and Chonbuk provinces. Affected trees showed symptoms of witches' brooms, small leaves and yellowing. CnWB disease is caused by a distinct phytoplasma, '*Ca*. P. castaneae', a member of the Japanese chestnut witches' broom phytoplasma group or 16SrXIX group, subgroup 16SrXIX-A (Jung *et al*., 2002; Davis *et al*., 2013).

A disease of *C. mollissima* (Chinese chestnut), the Chinese chestnut yellow crinkle (CnYC), has been reported from China (Lin *et al*., 2011). The phytoplasmal etiology of this disease, which had previously been supposed to have a virus origin or be induced by abiotic factors, was ascertained by symptoms remission following oxytetracycline treatments, transmission electron microscopy and PCR assays (Lin *et al*., 2011). In recent years, CnYC has spread to several Chinese chestnut-growing areas causing substantial economic losses (Lin *et al*., 2011). Major symptoms are yellowing and leaf crinkling. However, symptoms of small leaf, shortened internodes, shoot proliferation and empty burrs are also common. The phytoplasma associated with CnYC disease is a strain of '*Ca*. P. castaneae', and shares 99.72% 16S rDNA sequence identity with the reference strain CnWB (GenBank accession No. AB054986) of the mentioned taxon (Lin *et al*., 2011). Insect vectors involved in the natural spread of CnWB and CnYC phytoplasmas are unknown.

A yellows disease of *C. sativa* (European chestnut) has been observed in central and northern Italy (Mittempergher and Sfalanga, 1998). Affected trees show pronounced yellowing, leaf curl and necrosis, small leaves, shortened internodes, rosetting and premature defoliation. However, no phytoplasma infections could be detected in the diseased trees by PCR. Thus, the etiology of this disease remains unknown (Mittempergher and Sfalanga, 1998).

Willow witches' broom

Willow witches' broom (WWB) was first reported in *Salix rigida* (wand willow) from widely scattered areas of southern New Hampshire, New York and Massachusetts (Holmes *et al*., 1972). The disease has also been observed in Virginia on *S. nigra* (black willow) (Seliskar and Wilson, 1981). WWB is characterized by the appearance of witches' brooms in the crowns of affected trees, consisting of numerous, spindly, upright shoots bearing small leaves. Shoot proliferation results from the abnormal growth of dormant axillary buds. The disease was experimentally transmitted by grafting to *S. caprea* (European pussy willow) and its etiology established by electron microscopy (Holmes *et al*., 1972). However, the molecular identity of the infecting phytoplasma(s) has not been determined and the vector has not been identified.

A disease similar to WWB was observed in the Edmonton area (Canada) in 1994 on *S. bebbiana*, *S. discolor*, *S. exigua* and *S. petiolaris*. The causal agent was identified as a member of the clover proliferation phytoplasma group (Khadhair and Hiruki, 1995). Further studies showed that the disease is widespread in the Saskatchewan River valley in the central area of Alberta (Canada). However, the phytoplasma detected during these investigations proved to be a member of 16SrI group, subgroup 16SrI-B (Hiruki and Wang, 1999; Wang and Hiruki, 2005). A subgroup 16SrI-B phytoplasma was also identified in declining *Salix* sp. trees showing proliferation symptoms in Lithuania and in *S. tetradenia* (black mountain willow) trees with witches' broom and flower abnormalities in China (Lee *et al*., 2004b; Mou *et al*., 2014). In the latter country, the incidence of the disease affecting black mountain willow was 30-40% (Mou *et al*., 2014). In the Shaanxi province of China, *S. babylonica* (weeping willow) trees showing yellowing and decline were found to be infected by a 16SrI-C phytoplasma. The percentage of diseased plants was less than 10% (Wei *et al*., 2009). However, in Erdos (inner Mongolia, China) weeping willow trees with proliferation symptoms were reported to be affected by a phytoplasma of the 16SrVI

group, subgroup 16SrVI-A, with an incidence of *ca.* 36% (Zhang *et al*., 2012).

Phytoplasma infections have also been detected in weeping willow trees showing ball-like structures in southern Italy (Ragozzino *et al*., 1977) and Spain, where the infecting phytoplasma was identified as a member of the 16SrXII group (Alfaro-Fernández *et al*., 2011).

Walnut witches' broom

Walnut witches' broom (WalWB), a disease affecting *Juglans nigra* (black walnut), one of the most valuable American forest trees, was first reported in 1932 in Delaware (USA) and presumed to be induced by a virus. Later, it was associated with phytoplasma infections on the basis of electron microscope observations (for review see Seliskar and Wilson, 1981). WalWB is distributed throughout the eastern and central USA, but has not been reported elsewhere (Chen *et al*., 1992a). WalWB-affected trees show witches' brooms with dwarfed and chlorotic leaves. Brooms produced late in the growing season are often killed by early freezes. Nut production may be severely affected and the wood becomes brittle, making limbs highly susceptible to storm damage. Dieback is also common. The disease is efficiently transmitted by grafting but its vector is unknown. The WalWB phytoplasma is a '*Ca.* P. pruni'-related strain, member of the subgroup 16SrIII-G (Chen *et al*., 1992b; Davis *et al*., 2013).

Salt cedar witches' broom

Tamarix chinensis (salt cedar) is an ornamental shrub or moderate size tree native to Asia and East Europe. A phytoplasma disease affecting it, the salt cedar witches' broom (SCWB), is known to occur in China (Zhao *et al*., 2005; 2009). Diseased plants develop dense clusters of thin and highly proliferating twigs, with shortened internodes and scale-like leaves greatly reduced in size. These growth abnormalities confer the affected twigs a ball-like appearance. The causative agent is '*Ca.* P. tamaricis', a member of the SCWB phytoplasma group or 16SrXXX group (Zhao *et al*., 2009). '*Ca.* P. tamaricis' is most closely related to apple proliferation (AP) fruit tree phytoplasmas, sharing 96.6% 16S rDNA sequence identity with '*Ca.* P. prunorum' (Zhao *et al*., 2009).

Japanese maple witches' broom

Japanese maple witches' broom (JMWB) is a disease of *Acer palmatum* (Japanese maple), observed in the Shaanxi province of China (Li *et al*., 2012). JMWB-affected trees, show witches' brooms, shortened internodes and leaf necrosis. The presence of phytoplasma infections in diseased trees was shown by transmission electron microscope observations and PCR assays. On the basis of RFLP and sequence analyses of PCR-amplified 16S rDNA sequences, the JMWB phytoplasma was identified as a member 16SrI group, subgroup 16SrI-D (Li *et al*., 2012). In Poland, declining *A. negundo* (ashleaf maple) trees with symptoms of reduced apical growth, leaf malformation and necrosis and witches' brooms were found to be infected by '*Ca*. P. asteris', subgroup 16SrI-B (Kamińska and Śliwa, 2006).

Japanese raisin witches' broom

Hovenia dulcis (Japanese raisin) is an ornamental tree widely grown in Japan, Korea and eastern China. Diseased trees showing yellowing, small and malformed leaves, and witches' brooms observed in South Korea (Kamala-Kannan *et al*., 2011) were infected by a phytoplasma, as shown by electron microscopy and PCR assays. Successful transmission from diseased to healthy trees was obtained by graft inoculations. Phylogenetic studies based on 16S rDNA and 16S-23S rDNA spacer region, and *rpsV* (*rpl22*) and *secY* gene sequences showed that the phytoplasma infecting Japanese raisin trees, the Japanese raisin witches' broom (JRWB) agent, is a member of the 16SrV group, subgroup 16SrV-B and is most closely related to the JWB agent '*Ca.* P. ziziphi' (Kamala-Kannan *et al*., 2011).

Chinese tallow tree yellows

Sapium sebiferum (Chinese tallow tree) is a deciduous tree with high landscape value native to eastern Asia. The Chinese tallow tree yellows (CTTY) has recently been reported from the Shandong province of China (Gao *et al*., 2015). The most characteristic symptoms include foliar yellowing, small leaves, stunted growth and branch dieback. The causative agent, the CTTY phytoplasma, is a member of the 16SrIII group, and represents a new subgroup, denoted 16SrIII-Y (Gao *et al*., 2015).

goldenrain tree little leaf

Koelreuteria paniculata (goldenrain tree) is a very popular landscape tree in Asian countries. A yellows and decline disease of this species has been reported from South Korea (Kamala-Kannan *et al*., 2010). Affected trees show small leaves, malformation of vegetative and floral parts and general decline. On the basis of phylogenetic analysis of 16S rDNA, ribosomal protein (*rp*) and *tuf* gene sequences, the phytoplasma detected in diseased trees was identified as a member of the 16SrI group, subgroup B (Kamala-Kannan *et al*., 2010).

China tree decline, chinaberry yellows, and melia witches' broom

Melia azedarach (China tree also known as Chinaberry) is affected by various yellows and decline diseases, including China tree decline, Chinaberry yellows, and melia witches' broom. These diseases are characterized by similar symptoms, which include foliar yellowing, small leaves, shoot proliferation, shortened internodes and death of the trees, but differ for their geographic distribution and nature of the associated phytoplasma(s).

China tree decline was first described in the late 1970s in Argentina on the basis of field observations (for review see Galdeano *et al*., 2004). It spread throughout the Argentinian China tree-growing areas and seriously damaged the newly implanted China tree forests. Later, the phytoplasma etiology of this disease was elucidated by serological and molecular-based studies (Gómez *et al*., 1996; Galdeano *et al*., 2004, 2013). Extensive survey conducted by PCR assays using group-specific primer pairs and sequence and RFLP analyses of PCR-amplified rDNA sequences, revealed that China tree decline disease is associated with a subgroup 16SrIII-B phytoplasma in almost all Argentinian China tree-growing areas. However, in the north-east of the country a second phytoplasma which is a member of the Mexican periwinkle virescence (MPV) phytoplasma group or 16SrXIII group, subgroup C, has also been identified in diseased trees. In these areas, the two China tree-infecting phytoplasmas occurr in about the same proportion, some trees being doubly infected with the two agents (Arneodo *et al*., 2007).

China tree decline associated with subgroup 16SrIII-B phytoplasma infections has also been reported from Brazil where the incidence and severity of this disease prevents further use of China tree for landscape purposes (Duarte *et al*., 2009). Subgroup 16SrIII-B phytoplasmas are particularly widespread in Argentina and Brazil. They are known to occur in several other hosts including peach, tomato, eggplant and cassava (Montano *et al*., 2011; Fernández *et al*., 2013; Galdeano *et al*., 2013). Single or double infections with subgroup 16SrIII-B and 16SrXIII-C phytoplasmas have also been reported to be associated with China tree decline disease in Paraguay (Arneodo *et al*., 2005).

Chinaberry yellows occurs in Bolivia and Vietnam. In Bolivia, the disease is associated with two genetically different phytoplasmas, namely a subgroup 16SrIII-J agent and a member of the 16SrXIII-C subgroup (Harrison *et al*., 2003). In some diseased trees, double infections with the two phytoplasmas have also been detected (Harrison *et al*., 2003). Nucleotide sequence comparisons revealed that the subgroup 16SrXIII-C phytoplasma strain CbY1 infecting China tree in Bolivia is identical or nearly identical to subgroup 16SrXIII-C phytoplasma strains from Argentina, sharing 99.3-99.6% 16S rDNA sequence identity with them (Arneodo *et al*., 2007). In Vietnam, a subgroup 16SrI-B phytoplasma is associated with Chinaberry yellows disease (Harrison *et al*., 2006).

Melia witches' broom has been reported from China and Korea (Jin *et al*., 1982; Zhang *et al*., 1985; Han *et al*., 2014). In China, the melia witches' broom phytoplasma was identified as a member of the 16SrI group based on RFLP analysis of PCR-amplified 16S rDNA sequences (Qui *et al*., 1998), whereas in Korea affected *M. azedarach* var. *japonica* trees proved to infected by a subgroup 16SrI-B phytoplasma, as revealed by sequence and phylogenetic analyses of 16S rDNA and 16S-23S rDNA spacer region, and *secY*, *rp* and *tuf* gene sequences (Han *et al*., 2014). No information is available on insect vectors of phytoplasmas infecting China tree.

Sophora japonica witches' broom

Sophora japonica witches' broom (SJWB) is a disease affecting *Sophora japonica* (Chinese scholar tree), a widely grown and ecologically relevant ornamental and shade tree in China. This disease has been observed in the Shandong province and Haidian district of Beijing, China (Yu *et al*., 2012; Ren *et al*., 2014). Affected trees show symptoms of yelloying, small leaves, shortened internodes and excessive shoot proliferation. The occurrence of phytoplasma infections in diseased trees has been confirmed by transmission electron microscopy and PCR assays (Yu *et al*., 2012; Ren *et al*., 2014). On the basis of RFLP, computer-simulated RFLP and sequence analyses of 16S rDNA, *rp* and *tuf* gene sequences, the phytoplasma of SJWB-affected Chinese scholar trees was identified as a strain of '*Ca*. P. ziziphi' and proved to be most closely related to the strain infecting black locust in China (Yu *et al*., 2012; Ren *et al*., 2014). Work by Duduk *et al*. (2010) also revealed the occurrence of phytoplasma infections in four Chinese scholar trees showing symptoms of yellowing in the Shaanxi province of China. Of the diseased trees examined, two proved to be infected by a subgroup 16SrXII-A phytoplasma, whereas the remaining trees harboured a '*Ca*. P. japonicum'-related agent, as shown by RFLP and sequences analyses of PCR-amplified 16S rDNA sequences (Duduk *et al*., 2010).

Linden laciniated leaf witches' broom

Tilia platyphyllos var. *laciniata* (large-leaved linden) trees showing narrow and laciniated leaves with intense yellowing of the veins and undulating margins were observed in 2009 in Kaunas, Vilnius and Belvederis (Lithuania). Trees in Belvederis showed also witches' brooms (Jomantiene *et al*., 2013). Phytoplasma infections were detected in all symptomatic trees examined. However, diseased trees from Kaunas and Vilnius proved to be infected by a subgroup 16SrI-B phytoplasma, named linden laciniated leaf (LindLL) agent, whereas the trees from Belvederis

Fig. 2. A and B, symptoms of small leaves, yellowing and shortened internodes on *Eucalyptus* spp. (eucalypt) affected by eucalyptus little leaf. In A, healthy shoot on the left. C-G, ash yellows. C, young diseased trees of *Fraxinus americana* (white ash) in an old-field stand showing thin crowns, chlorosis, and dieback. One tree at left center appears healthy. D, deliquescent sprouts on white ash sapling. E, witches' brooms and split bark due to freezing injuries at trunk base of a white ash tree. F, stunted and dead rootlets of a diseased white ash tree (left) in contrast with roots of a healthy tree. G, witches' brooms at the root collar of a declining *Fraxinus velutina* (velvet ash) tree. H-K, lilac witches' broom. H, chlorosis and stunted growth of *Syringa* x *prestoniae* 'Hiawatha' (lilac). I, small witches' brooms on *S.* x *prestoniae* 'Hiawatha'. J, dieback and brooms on *S.* x *josiflexa* 'Royalty'. K, dead and dying twigs in a broom on *S. josikaea*. Green-tipped buds survived winter but failed to open in spring. L and M, witches' brooms on paulownia witches' broom-affected *Paulownia fortunei* x *P. tomentosa* (paulownia). In L, healthy control on the left. N and U, dwarfed and proliferated growth on *Morus multicaulis* (mulberry) affected by mulberry dwarf. O-Q, apple proliferation. O, beginning of witches' broom formation on *Malus denticulata* (ornamental apple) (left, healthy). P and Q, severely chlorotic and dwarfed leaves of *M. toringoides* (right, healthy). R, European stone fruit yellows-affected *Prunus serrulata* 'Kuanzan' (flowering cherry) showing terminal rosetting and rolling of chlorotic leaves (right, healthy). S and T, symptoms of small leaves and decline in sandal spike-infected *Santalum album* (sandal). In S and T healthy leaves and trees are shown on the left end site. (C, D, E, F, G, H, I and K, courtesy W.A. Sinclair, Department of Plant Pathology, Cornell University, Ithaca, New York, USA; J, courtesy C.R. Hibben, Brooklyn Botanic Garden Research Center, Ossining, New York, USA; L and M, courtesy W.-J. Zhao, Institute of Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing, China; N and U, courtesy X.-L. Ji, State Key Laboratory of Crop Biology and College of Forestry, Shandong Agricultural University, Taian, Shandong, China; O, P, Q and R, courtesy E. Seemüller, Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim, Germany; S and T, courtesy J.A. Khan, Department of Biosciences, Jamia Millia Islamia University, New Delhi, India).

harboured a phytoplasma assigned to a new subgroup (16SrI-(L/L)V) denoted linden laciniated leaf witches' broom (LindLL-WB) phytoplasma (Jomantiene *et al*., 2013). Further studies are needed to clarify whether the prized *T. platyphyllos* var. *laciniata* displaying the unusual morphotype is a valid botanical variety or simply a plant that displays an abnormal leaf morphology caused by phytoplasma infections (Jomantiene *et al*., 2013).

Allocasuarina yellows

Allocasuarina yellows (AlloY) is a disease that affects *Allocasuarina muelleriana* (Slaty she-oak) in Australia (Gibb *et al*., 2003). The most characteristic symptoms are yellowing and decline, occasionally combined with shortened internodes and curled leaves. The disease is associated with the presence of a distinct phytoplasma, '*Ca.* P. allocasuarinae' which is most closely related to '*Ca.* P. rhamni', with which it shares 96% 16S rDNA sequence identity (Marcone *et al*., 2004).

Balanites witches' broom

Balanites witches' broom (BltWB) is a recently described disease of *Balanites triflora* occurring in Myanmar (Win *et al*., 2013). Symptoms include witches' broom, yellowing and small leaves. The causative agent, '*Ca.* P. balanitae', is phylogenetically most closely related to '*Ca.* P. ziziphi', with a share of 98.2% 16S rDNA sequence identity (Win *et al*., 2013).

Magnolia stunt

Magnolia stunt is a decline disease of *Magnolia* spp. (magnolia) observed in some gardens and nurseries of Poland (Kamińska *et al*., 2001). Symptoms include a progressive loss of vigor, stunting, browning and severe malformation of apical leaves, apical shoot necrosis, leaf necrosis and premature leaf fall. Some of the most severely affected plants show also shoot proliferation and an upright growth habit. The disease is common in plants of *Magnolia liliiflora* 'Nigra'. However, diseased plants of *M. stellata*, *M. soulangeana* 'Alexandrina', 'Lennei' and 'Lennei Alba', and *Magnolia* x *loebneri* 'Leonard Messel', were also recorded (Kamińska *et al*., 2001). A subgroup 16SrI-B phytoplasma was identified in all diseased plants. Double infections with the 16SrI-B agent and a phytoplasma of 16SrX group were also detected in a few magnolia plants (Kamińska *et al*., 2001; Kamińska and Śliwa, 2003). The magnolia-infecting 16SrI-B phytoplasma was transmitted from diseased magnolia to periwinkle through dodder bridges (Kamińska *et al*., 2001). Treatments of diseased magnolias with antibiotics, including oxytetracyclines, promoted temporary shoot growth and development of asymptomatic leaves and flower buds, thereby supporting the phytoplasmal etiology of this disease (Kamińska and Śliwa, 2003).

Apple proliferation

Apple proliferation (AP) is one of the most important phytoplasma diseases in Europe. It seriously impairs fruit quality and productivity of *Malus* x *domestica* (domestic apple) trees. However, ornamental apples can also be affected. AP-diseased ornamental apples are very common in Germany (Kartte and Seemüller, 1988; Seemüller *et al*., 2011b). In *Malus toringoides* and *M. denticulata* the symptoms are nearly the same as in the cultivated apple. They include yellowing, stunting, dwarfed leaves, witches' brooms, reddening of the foliage, rosetting and enlarged stipules (Fig. 2O, P and Q). In other taxa such as *M. coronaria* and *M.* x *platycarpa* the prevalent symptoms are rolling and curling of the leaves, premature defoliation, browning of the veins, yellowish bands along the veins, wilting and death. These symptoms are not obseved in cultivated apples. '*Ca.* P. mali' (subgroup 16SrX-A) has been identified in all diseased ornamental apple trees examined (Seemüller *et al*., 2011b).

European stone fruit yellows and flowering cherry decline

Among the several *Prunus* species affected by European stone fruit yellows (ESFY) in Europe there are *Prunus serrulata* (flowering cherry), *P. cerasifera* (myrobalan) and *P. subhirtella* (spring cherry), which are also used for ornamental purposes. The occurrence of ESFY on flowering cherry has been reported from Germany (Lederer and Seemüller, 1992). The most characteristic symptoms of diseased flowering cherries are sparse foliage, reduced vigor, shortened terminal growth resulting in a marked rosetting of the leaves, yellowing, dieback of individual branches which slowly spreads to the rest of the crown (Fig. 2R) (Lederer and Seemüller, 1992). In graft-inoculation experiments, ESFY-infected flowering cherry 'Kuanzan' was used as inoculum source and grown as scion on several cherry rootstocks (Kison and Seemüller, 2001). With the exception of Gisela 3 (*P. fructicosa* x *P. avium*), the inoculation resulted in death of the trees on all rootstocks, with Gisela 5 (*P. fructicosa* x *P. cerasus*) being the most affected. Very often, the mortality of scions was higher than that of rootstocks. However, reduced vigor and foliar symptoms were also common.

P. cerasifera and *P. subhirtella* are little affected under experimental inoculation and natural infection conditions. Infected trees of these taxa never develop clear-cut symptoms and only rarely, or temporarily, mild symptoms such

as slight yellowing and slightly reduced vigor and terminal growth. In some instances, they are tolerant (Marcone *et al*., 2010, 2011). The causative agent of ESFY is '*Ca.* P. prunorum' (subgroup 16SrX-F).

A subgroup 16SrV-B phytoplasma is associated with flowering cherry decline in the Shandong province of China. Affected trees show excessive axillary growth, stunting, shortened internodes and decline (Wang *et al*., 2014).

X-DISEASE

X-disease is a major stone fruit disorder which is widespread in North America but does not occur elsewhere in the world. It is caused by '*Ca.* P. pruni', a member of the X-disease phytoplasma group or 16SrIII group, subgroup 16SrIII-A (Uyemoto and Kirkpatrick, 2011; Davis *et al*., 2013). The host range of the X-disease agent includes also the ornamental species *P. virginiana* (chokecherry) and *P. pensylvanica* (pincherry). X-disease-infected chokecherry trees are common in northern USA and eastern Canada where they are characterized by a pronounced reddening of the foliage. By contrast, in western Canada, infected trees develop only mild reddening. Other symptoms include shortened internodes, sterility of flowers and decline (Hiruki and Wang, 1999). In diseased pincherry trees, witches' brooms develop after breaking of dormancy of the axillary buds, due to the growth of erect and spindly axillary shoots. The witches' broom formations often die during winter (Hiruki and Wang, 1999).

CONCLUDING REMARKS

This review summarizes the significant advances made over the last two decades in the detection of phytoplasma diseases of forest and landscape trees and shrubs and in the molecular characterization and classification of the associated agents. It is likely that there are still many unknown phytoplasma diseases of such kind of trees and shrubs and that their economic and ecological significance is greater than currently known. However, for some of the reviewed diseases information is still lacking on vectors, phytoplasma-vector relationships, phytoplasma-host interactions, strain virulence, strain interference, host tolerance, host range, and impact of the infections on the growth and yield of affected plants.

Latent phytoplasma infections, which are common in nature in some forest and landscape trees and shrubs, can serve as inoculum reservoirs for further spread, and may have subtle effects on infected plants, perhaps rendering them more susceptible to damage by other biotic and abiotic factors. However, no such interactions were observed by Ferris *et al.* (1989) who investigated the potential synergistic or additive effects of viruses and the AshY phytoplasma on the growth and symptom development of white ash and green ash seedlings.

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