

Role of wild *Prunus* species in the epidemiology of European stone fruit yellows

L. Carraro*†, F. Ferrini, P. Ermacora and N. Loi

Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi di Udine, via delle Scienze 208, 33100 Udine, Italy

Several uncultivated trees of the species *Prunus spinosa*, *P. cerasifera* and *P. domestica*, sampled both adjacent to European stone fruit yellows (ESFY)-infected orchards and in isolation from cultivated stone fruit plants, were found to be infected by ESFY phytoplasma. These species were also colonized by *Cacopsylla pruni*, vector of the ESFY agent. In contrast, uncultivated species of *Prunus avium*, *P. cerasus* and *P. mahaleb* hosted neither the pathogen nor the vector. Insect- and graft-transmission trials of ESFY phytoplasma conducted under controlled conditions confirmed the data obtained in the field. The role played by the wild *Prunus* species is discussed and appears to be fundamental in the epidemic cycle of the disease.

Keywords: *Cacopsylla pruni*, phytoplasma, polymerase chain reaction, transmission, vector

Introduction

Several stone fruit (*Prunus*) species in Europe are affected by severe diseases associated with phytoplasmas. These include apricot chlorotic leaf roll (Morvan, 1977), plum leptonecrosis (Giunchedi *et al.*, 1978) and peach yellows (Poggi Pollini *et al.*, 1993). Lorenz *et al.* (1994) determined the common aetiology of these diseases and proposed the single name 'European stone fruit yellows' (ESFY). Occurrences of ESFY have been reported in Spain (Sanchez Capucino & Forner, 1973), France (Desvignes & Cornaggia, 1982), Italy (Giunchedi *et al.*, 1978), Slovenia (Brzin *et al.*, 2001), Greece (Rumbos & Bosalidis, 1985), Romania (Ionica, 1985), Germany (Lederer & Seemüller, 1992), Czech Republic (Navratil *et al.*, 2001), England (Davies & Adams, 2000) and Austria (Laimer da Camara Machado *et al.*, 2001).

ESFY phytoplasma induces economically important disorders in apricot (Desvignes & Cornaggia, 1982), Japanese plum (Dosba *et al.*, 1991) and peach (Marcone *et al.*, 1996). European plum is generally tolerant to ESFY (Carraro *et al.*, 1998a; Jarausch *et al.*, 2000). The disease also affects almond and various *Prunus* rootstocks (Jarausch *et al.*, 1998; Kison & Seemüller, 2001). Recently, Jarausch *et al.* (2001b) detected ESFY phytoplasma in six different wild-growing *Prunus* species as well as in *Celtis australis*, *Fraxinus excelsior* and *Rosa canina* growing in

the surroundings of ESFY-infected apricot orchards in France.

The presence of wild plants is important for the epidemiology of the disease because the pathogen can survive and spread without the presence of susceptible cultivated plants. Up to now the exact role played by wild *Prunus* and non-*Prunus* species in the epidemiology of ESFY was not clear. For example, it is not known if these plants host *Cacopsylla pruni*, the vector of the disease (Carraro *et al.*, 1998b; Jarausch *et al.*, 2001a), and whether it can infect them. Likewise, it is not known if they are end-hosts for the pathogen or sources of inoculum for transmission of the phytoplasma to cultivated plants. The objectives of the present study were: (i) to determine the presence of ESFY phytoplasma in wild *Prunus* species in an area of north-east Italy with a high infection pressure; (ii) to verify the presence of *C. pruni* on the different *Prunus* species and clarify the capability of *C. pruni* to infect these plants; and (iii) to ascertain the exact role of wild *Prunus* species in the epidemiology of the disease.

Materials and methods

Samplings to determine the presence of ESFY phytoplasma and its vector in wild *Prunus* species

Surveys were carried out in the Friuli-Venezia Giulia (F-VG) region, north-east Italy, where ESFY is epidemic (Carraro *et al.*, 1992). In the years 2000 and 2001, from July to October, repeated samplings were made in five different areas; two (Cavazzo and Spessa) were located in the surroundings of ESFY-infected apricot and Japanese

*To whom correspondence should be addressed.

†E-mail: carraro@pldef.uniud.it

Accepted 17 February 2002

Site	Species	Plants with symptoms/ analysed plants	Infected/ analysed plants	Presence of <i>C. pruni</i>
Cavazzo	<i>Prunus avium</i>	0/4	0/4	No
	<i>Prunus cerasifera</i>	1/4	1/4	Yes
	<i>Prunus cerasus</i>	0/1	0/1	No
	<i>Prunus mahaleb</i>	0/4	0/4	No
	<i>Prunus spinosa</i>	0/7	2/7	Yes
	<i>Crataegus</i> spp.	0/2	0/2	No
	<i>Rhamnus catharticus</i>	1/2	0/2	No
Spessa	<i>Rosa canina</i>	0/8	0/8	No
	<i>Prunus avium</i>	0/1	0/1	No
	<i>Prunus cerasifera</i>	2/2	2/2	Yes
	<i>Prunus domestica</i>	0/2	1/2	Yes
	<i>Prunus spinosa</i>	0/4	1/4	Yes
	<i>Crataegus</i> spp.	1/2	0/2	No
	<i>Prunus cerasifera</i>	1/1	1/1	Yes
Cornino	<i>Prunus mahaleb</i>	0/3	0/3	No
	<i>Prunus spinosa</i>	1/12	3/12	Yes
	<i>Prunus cerasifera</i>	1/1	1/1	Yes
Romans	<i>Prunus spinosa</i>	0/8	1/8	Yes
	<i>Prunus cerasifera</i>	3/3	3/3	Yes
Udine	<i>Prunus cerasifera</i>	3/3	3/3	Yes
	<i>Prunus spinosa</i>	0/1	1/1	Yes

Table 1 Nested-PCR analyses for European stone fruit yellows (ESFY) phytoplasma in wild plant species sampled in different locations in the Friuli-Venezia Giulia region, north-east Italy. The same plants were checked for the presence of symptoms similar to those of phytoplasma infection and the vector *Cacopsylla pruni*

plum orchards and three (Cornino, Romans and Udine) were far from any stone fruit orchard. All *Prunus* spp. plants, with or without typical phytoplasma symptoms, were considered and some of them tested for the presence of ESFY phytoplasma by using PCR/RFLP analyses. Particular attention was given to *Prunus* spp. which were common to all the sites. In Cavazzo and Spessa, some non-*Prunus* species with light or faint symptoms of yellows and/or decline were sampled and analysed. The complete list of the sampled plants is given in Table 1. During 2000–01 the presence/absence of *C. pruni* on wild *Prunus* and on other species of plants was checked periodically by shaking the insects onto an underlying net. Three groups of 10 individuals each of the captured *C. pruni* were also analysed for the presence of ESFY phytoplasma.

Transmission trials with *C. pruni*

All the transmission experiments were carried out in an environmentally controlled glasshouse at 23–25°C with supplementary light and 16-h days. In April 2001, groups of overwintering adults of *C. pruni* were captured as above in the stone fruit orchards of Cavazzo and Spessa. Twenty groups, each of five individuals, were immediately tested by PCR/RFLP for ESFY phytoplasma; the other psyllids were confined in groups of 50 individuals on each test plant until their death. Longevity of the insects as well as oviposition on the test plants were checked. The choice of the test plants was based on the surveys carried out during 2000. Consequently, species common to all the analysed sites (*P. spinosa* and *P. cerasifera*) and known to be typical in north-east Italy (*P. mahaleb* and *P. padus*) were selected. For each selected species, five potted seedlings were exposed to the vector. After inoculation, the test plants were held in separate sectors of the glasshouse,

where they were observed for 6 months for symptom expression and then analysed by PCR/RFLP. Seedlings of apricot cv. Canino were used as susceptible controls, either noninoculated or exposed to the vector. Negative controls were unexposed seedlings of the selected *Prunus* species.

Transmission trials by grafting

In May 2001, groups of plants of the same *Prunus* species used in the transmission trials with *C. pruni* were graft-inoculated (four plants per species) in the glasshouse. The sources of inoculum were 10 Japanese plum trees infected in previous experiments by vector (Carraro *et al.*, 1998b). Four chip-buds and one approach graft were used for each test plant. In July 2001, five 1-year-old micropropagated Japanese plum trees were graft-inoculated (two chip-buds and one approach-graft) each by using as the source of inoculum one ESFY-infected *P. spinosa* tree from the Romans location.

Testing for the presence of ESFY phytoplasma in plants and in psyllids

Using PCR/RFLP, all the plants listed in Table 1, the sources of inoculum used in the graft-transmission trials, groups of *C. pruni*, the inoculated test plants and a representative number of negative controls were tested for the presence of ESFY phytoplasma.

A modification of the phytoplasma-enrichment procedure developed by Kirkpatrick (Malisano *et al.*, 1996) was adopted for the DNA extraction from plants, using 1 g of leaf petioles and midrib tissues; for extraction of insect DNA the Doyle & Doyle (1990) method was used. The presence of ESFY phytoplasma was determined by nested PCR using the universal phytoplasma primers

P1/P7 (Schneider *et al.*, 1995). After 1:40 dilution, 5 µL of PCR products obtained in the initial amplification were used as a template for further amplification with the nested primers f01/r01 (Lorenz *et al.*, 1995). The primer pair f01/r01 are specific for the fruit-tree phytoplasmas – i.e. ESFY, apple proliferation and pear decline – all belonging to the phylogenetic group ‘apple proliferation’ (Seemüller *et al.*, 1998). The final PCR products (5 µL) were analysed by electrophoresis in 1.5% agarose gel and visualized by staining with ethidium bromide and UV illumination; 10 µL of the PCR products were then digested with *Bsa* AI, according to the manufacturer’s instructions (Bio Laboratories, Beverly, MA, USA). Restriction fragments were resolved in 2% agarose gel.

Results

Presence of ESFY phytoplasma and of *C. pruni* in wild *Prunus* species

Using PCR/RFLP, the ESFY phytoplasma was detected in several wild *Prunus* species (Table 1) growing both near ESFY-infected orchards (Spessa and Cavazzo) and far from stone-fruit orchards (Cornino, Romans and Udine). Considering all the localities, the *Prunus* species that tested positive for ESFY phytoplasma were: *P. cerasifera* (eight trees out of the 11 analysed), *Prunus domestica* (one out of two) and *P. spinosa* (eight out of 32). Among these, ESFY-infected *P. cerasifera* and *P. spinosa* were present at all the sites checked. *Prunus avium*, *P. cerasus* and *P. mahaleb*, as well as the non-*Prunus* species analysed, were found not to be infected by ESFY phytoplasma. The infected plant of *P. domestica* collected in Spessa was symptomless; only one infected *P. spinosa* plant (collected in Cornino) showed symptoms of small leaves and off-season growth in winter; all the infected *P. cerasifera* plants showed slight leaf roll at the end of summer.

The presence of *C. pruni* was confirmed on *P. spinosa*, *P. cerasifera* and *P. domestica*; both overwintering adults in April–May and individuals of the new generation in June–July were observed and captured. The vector was not found on *P. avium*, *P. mahaleb* or *P. cerasus*, or on the non-*Prunus* species examined. Two of the three groups of *C. pruni* analysed by PCR/RFLP were ESFY-infected; one of these groups was captured in Spessa on *P. spinosa* and the other in Udine on *P. cerasifera*.

Transmission trials with *C. pruni* and by grafting

Eight of the 20 groups of *C. pruni*, each of five individuals, tested by PCR/RFLP immediately after their capture, were ESFY-infected. In terms of single insects, the minimum percentage of psyllids carrying phytoplasmas was 8%. The results of the transmission trials, both with the vector *C. pruni* and by grafting, are reported in Table 2. *Cacopsylla pruni* transmitted ESFY agent to four of the five *P. spinosa* and *P. cerasifera* test plants and to all of the apricot control plants. In contrast, the exposed *P. mahaleb* and *P. padus* plants were found to be healthy by PCR/RFLP analyses. The infected *P. spinosa* and *P. cerasifera* test plants never showed symptoms of phytoplasma infection; on the other hand, the infected apricot plants showed the typical leaf roll and decline symptoms. Under glasshouse conditions, the mortality of *C. pruni* (Table 2) was high on *P. mahaleb* and *P. padus*, intermediate on apricot and low on *P. spinosa* and *P. cerasifera*. Eggs and individuals of the new generation, born under controlled conditions, were observed only on apricot, *P. spinosa* and *P. cerasifera*.

All the graft-inoculated apricots, *P. spinosa* and *P. cerasifera* and none of the *P. mahaleb* and *P. padus* test plants became ESFY-infected; symptoms were observed only on apricot.

Three of the five Japanese plum trees graft-inoculated using *P. spinosa* as the source of inoculum became infected and developed typical symptoms: leaf reddening, small leaves, leaf roll and thickening of the leaf blade.

Phytoplasma detection in plants and psyllids

In nested PCR, using the primer pair P1/P7 followed by nested primers f01/r01, ESFY phytoplasma DNA was amplified from all the sources of inoculum used in the transmission trials by grafting as well as from some of the wild and test plants and groups of psyllids analysed (Fig. 1). No DNA amplification was obtained from the negative controls. After digestion with *Bsa* AI, the PCR products obtained showed the same ESFY restriction profile (Fig. 2).

Discussion

European stone fruit yellows is one of the most important pathological problems of stone fruit in Europe. The typically epidemic disease spreads quickly and can compromise

Table 2 Transmission of European stone fruit yellows (ESFY) phytoplasma to *Prunus* spp. test plants by groups of *Cacopsylla pruni* and by grafting; mortality after the beginning of inoculation, oviposition and the presence of individuals of the new generation of the vector on the test plants

Species	Transmission by (infected/ inoculated plants)		Mortality (%) of <i>C. pruni</i> after		Oviposition and new generation of <i>C. pruni</i>
	<i>C. pruni</i>	Grafting	7 days	14 days	
<i>P. cerasifera</i>	4/5	4/4	0	20	Yes
<i>P. mahaleb</i>	0/5	0/4	50	100	No
<i>P. padus</i>	0/5	0/4	50	100	No
<i>P. spinosa</i>	4/5	4/4	0	20	Yes
Apricot	4/4	4/4	10	40	Yes

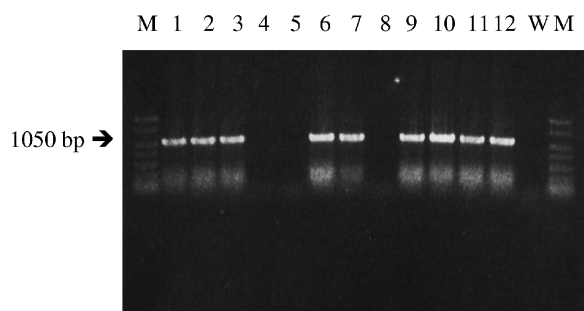


Figure 1 Agarose gel electrophoresis of PCR products from wild *Prunus* spp. and groups of *Cacopsylla pruni* amplified in nested PCR with primer pair f01/r01. Lanes 1–5, *Prunus spinosa*; 6–8, *P. cerasifera*; 9, *P. domestica*; 10–11, *C. pruni*; 12, European stone fruit yellows (ESFY) positive control; W, water control; M, marker (P9577, Sigma, St Louis, MO, USA).

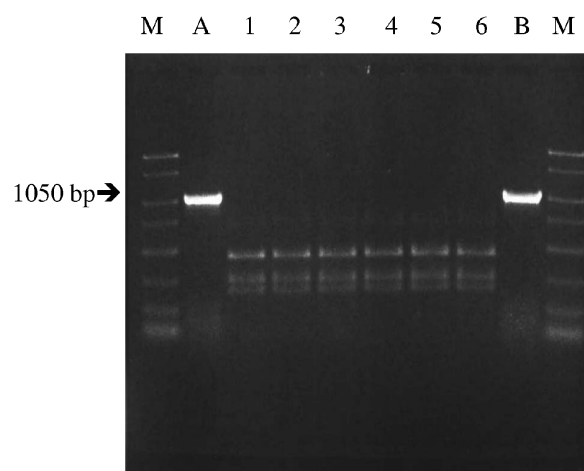


Figure 2 *Bsa* AI restriction profiles of phytoplasma 16S rDNA amplified in nested PCR with primer pair f01/r01. The template DNA was from European stone fruit yellows (ESFY)-infected: 1, *C. pruni*; 2–3, *P. spinosa*; 4–5, *P. cerasifera*; 6, ESFY positive control; A and B, undigested amplified ESFY-16S rDNA (controls); M, marker (P9577, Sigma).

the cultivation of the more susceptible stone-fruit species, apricot and Japanese plum (Carraro *et al.*, 1992; Jarausch *et al.*, 1998). ESFY is also commonly epidemic in areas like the F-VG region, Italy, where stone-fruit trees are rarely cultivated. In this area, Carraro *et al.* (1998a) hypothesized the passage of early natural infections from wild to cultivated *Prunus* spp. This hypothesis was later reinforced after the verification that *C. pruni* is the vector of ESFY phytoplasma (Carraro *et al.*, 1998b). The insect, whose recorded presence in F-VG dates from 1888, is in fact strictly oligophagous on *Prunus* spp. (Conci *et al.*, 1992). Bearing in mind that the disease is epidemic but also spreads in scarcely cultivated areas, and the vector is restricted to *Prunus* spp., the role played by wild *Prunus* spp. in the epidemic cycle of ESFY is explained in the present work. First, the diffuse presence of the ESFY agent in *P. spinosa*, a species native to Europe and Caucasic

region (Pignatti, 1982), was shown and the agent was also detected in wild *P. cerasifera* and *P. domestica* plants. Infected wild *Prunus* spp. plants were present both in the surroundings and far from cultivated stone-fruit orchards, confirming the established presence of the disease in the F-VG region. In other wild plants – *P. avium*, *P. cerasus* and *P. mahaleb* – the ESFY agent was not detected. There was a correlation between the vector, the agent of the disease and the host plants: only the *Prunus* species hosting the vector also hosted the agent. The non-*Prunus* plants analysed – in particular *R. canina*, reported to be ESFY-infected in France (Jarausch *et al.*, 2001b) – were not confirmed as hosts of either *C. pruni* or ESFY phytoplasma.

The transmission trials conducted in the glasshouse confirmed the data obtained in the field. Groups of naturally infected *C. pruni* (Carraro *et al.*, 2001) transmitted ESFY phytoplasma to *P. cerasifera* and *P. spinosa* test plants, as well as to apricot controls. Furthermore, these plants also hosted the vector, on which it lay eggs that hatched. The susceptibility of *P. cerasifera* and *P. spinosa* to ESFY phytoplasma was also confirmed by graft transmission. *Prunus mahaleb* and *P. padus* did not seem to be hosts either for the vector or for the phytoplasma, as proved by insect and graft-transmission trials. Besides, the ESFY agent was graft-transmitted from one naturally infected *P. spinosa* plant to Japanese plum test plants, thus further confirming that this species is an important natural host and a widespread potential source of inoculum for ESFY and infection of cultivated *Prunus* spp.

In conclusion, the role of some wild *Prunus* species in the epidemiology of European stone fruit yellows is now clarified: wild *P. spinosa*, *P. cerasifera* and *P. domestica* plants are natural hosts for both ESFY phytoplasma and the vector *C. pruni*. Therefore, the cycle of ESFY can be completed independently from the presence of ESFY-infected cultivated stone-fruit trees. In areas like this part of north-east Italy, where both the vector and the agent are present on wild *Prunus* species, the disease appears to be endemic. Other wild *Prunus* spp. – *P. avium*, *P. cerasus*, *P. mahaleb* and *P. padus* – do not play an important epidemiological role because they host neither the vector nor the pathogen.

Research is continuing with the main aims of explaining the reported presence of ESFY phytoplasma in plants other than *Prunus* spp. (Jarausch *et al.*, 2001a,b) and its sporadic detection in *P. avium* (Jarausch *et al.*, 1999) and *P. cerasus* (Navratil *et al.*, 2001). The presence of secondary vector(s) of ESFY can be hypothesized, as can the natural distribution of different strains of the phytoplasma.

References

- Brzin J, Petrovic N, Seljak G, Osler R, Ermacora P, Loi N, Carraro L, Ferrini F, Refatti E, Ravnikar M, 2001. *First Results on Laboratory Analyses of Phytoplasmas on Fruit Trees. Fifth Slovenian Conference on Plant Protection*. Ljubljana, Slovenia: Planprint, 217–21.
- Carraro L, Loi N, Ermacora P, 2001. Transmission characteristics of the European stone fruit yellows

- phytoplasma and its vector *Cacopsylla pruni*. *European Journal of Plant Pathology* **107**, 695–700.
- Carraro L, Loi N, Ermacora P, Osler R, 1998a. High tolerance of European plum varieties to plum leptonecrosis. *European Journal of Plant Pathology* **104**, 141–5.
- Carraro L, Osler R, Loi N, Ermacora P, Refatti E, 1998b. Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni*. *Journal of Plant Pathology* **80**, 233–9.
- Carraro L, Osler R, Refatti E, Favali MA, 1992. Natural diffusion and experimental transmission of plum leptonecrosis. *Acta Horticulturae* **309**, 285–90.
- Conci C, Rapisarda C, Tamanini L, 1992. Annotated catalogue of Italian *Psylloideae*. *Atti Dell'accademia Roveretana Degli Agiati II B (ser. VII)*, 107–8.
- Davies DL, Adams AN, 2000. European stone fruit yellows phytoplasmas associated with a decline disease of apricot in southern England. *Plant Pathology* **49**, 635–9.
- Desvignes JC, Cornaggia D, 1982. Observation on apricot chlorotic leaf roll (ACLR): sensitiveness of different *Prunus* species, detection, spread in plum orchards. *Acta Horticulturae* **130**, 249–56.
- Dosba F, Lansac M, Mazy K, Garnier M, Eyquard JP, 1991. Incidence of different diseases associated with mycoplasma-like organisms in different species of *Prunus*. *Acta Horticulturae* **283**, 311–20.
- Doyle JJ, Doyle JB, 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–5.
- Giunchedi L, Marani F, Credi R, 1978. Mycoplasma-like bodies associated with plum decline (leptonecrosis). *Phytopathologia Mediterranea* **17**, 205–9.
- Ionica M, 1985. Investigations on the role of mycoplasmas in peach decline in Romania. *Analele Institutului de Cercetari Pentru Protectia Planteor* **18**, 11–7.
- Jarausch W, Danet JL, Labonne G, Dosba F, Broquaire JM, Saillard C, Garnier M, 2001a. Mapping the spread of apricot chlorotic leaf roll (ACLR) in southern France and implication of *Cacopsylla pruni* as a vector of European stone fruit yellows (ESFY) phytoplasmas. *Plant Pathology* **50**, 782–90.
- Jarausch W, Eyquard JP, Lansac M, Mohns M, Dosba F, 2000. Susceptibility and tolerance of new French *Prunus domestica* cultivars to European stone fruit yellows phytoplasmas. *Journal of Phytopathology* **148**, 489–93.
- Jarausch W, Eyquard JP, Mazy K, Lansac M, Dosba F, 1999. High level of resistance of sweet cherry (*Prunus avium* L.) towards European stone fruit yellows phytoplasmas. *Advances in Horticultural Science* **13**, 108–12.
- Jarausch W, Jarausch Wehrheim B, Danet JL, Broquaire JM, Dosba F, Saillard C, Garnier M, 2001b. Detection and identification of European stone fruit yellows and other phytoplasmas in wild plants in the surroundings of apricot chlorotic leaf roll-affected orchards in southern France. *European Journal of Plant Pathology* **107**, 209–17.
- Jarausch W, Lansac M, Saillard C, Broquaire JM, Dosba F, 1998. PCR assay for specific detection of European stone fruit yellows phytoplasmas and its use for epidemiological studies in France. *European Journal of Plant Pathology* **104**, 17–27.
- Kison H, Seemüller E, 2001. Differences in strain virulence of the European stone fruit yellows phytoplasma and susceptibility of stone fruit trees on various rootstocks to this pathogen. *Journal of Phytopathology* **149**, 533–41.
- Laimer da Camara Machado M, Paltrinieri S, Hanzer V, Arthofer W, Strommer S, Martini M, Pondrelli M, Bertaccini A, 2001. Presence of European stone fruit yellows (ESFY or 16SrX-B) phytoplasmas in apricots in Austria. *Plant Pathology* **50**, 130–5.
- Lederer W, Seemüller E, 1992. Demonstration of mycoplasmas in *Prunus* species in Germany. *Journal of Phytopathology* **134**, 89–96.
- Lorenz KH, Dosba F, Poggi Pollini C, Llacer G, Seemüller E, 1994. Phytoplasma diseases of *Prunus* species in Europe are caused by genetically similar organisms. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **101**, 567–75.
- Lorenz KH, Schneider B, Ahrens U, Seemüller E, 1995. Detection of apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non ribosomal DNA. *Phytopathology* **85**, 771–6.
- Malisano G, Firrao G, Locci R, 1996. 16S rDNA-derived oligonucleotide probes for the differential diagnosis of plum leptonecrosis and apple proliferation phytoplasmas. *EPPO Bulletin* **26**, 421–8.
- Marcone C, Ragozzino A, Seemüller E, 1996. European stone fruit yellows phytoplasma as the cause of peach vein enlargement and other decline diseases of stone fruits in southern Italy. *Journal of Phytopathology* **144**, 559–64.
- Morvan G, 1977. Apricot chlorotic leaf roll. *EPPO Bulletin* **7**, 37–55.
- Navratil M, Valova P, Fialova R, Petrova K, Poncarova Vorackova Z, Franova J, Nebesarova J, Karesova R, 2001. Survey for stone fruit phytoplasmas in the Czech republic. *Acta Horticulturae* **550**, 377–82.
- Pignatti S, 1982. *Flora D'Italia I*. Bologna, Italy: Edagricole, 616.
- Poggi Pollini C, Giunchedi L, Gambin E, 1993. Presence of mycoplasma-like organisms in peach trees in Northern Central Italy. *Phytopathologia Mediterranea* **32**, 188–92.
- Rumbos I, Bosalidis AM, 1985. Mycoplasma-like organisms associated with decline of plum tree in Greece. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **92**, 47–54.
- Sanchez Capucino JA, Forner JB, 1973. Vegetative disorders in the Japanese plum trees on Myrobalan rootstocks in the province of Valencia (Spain). *Acta Horticulturae* **44**, 93–7.
- Schneider B, Seemüller E, Smart CD, Kirkpatrick B, 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG, eds. *Molecular and Diagnostic Procedures in Mycoplasmaology*. San Diego, CA: Academic Press, 369–80.
- Seemüller E, Marcone C, Lauer U, Ragozzino A, Göschl M, 1998. Current status of molecular classification of the phytoplasmas. *Journal of Plant Pathology* **80**, 3–26.