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Detection and indentification of European stone fruit yellows and other phytoplasmas in wild plants in the surroundings of apricot chlorotic leaf roll-affected orchards in southern France

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Abstract

Between 1994 and 1998 a field study was conducted to identify plant hosts of the European stone fruit yellows (ESFY) phytoplasma in two apricot growing regions in southern and southwestern France where the incidence of apricot chlorotic leaf roll was high. A total of 431 samples from 51 different plant species were tested for the presence of phytoplasmas by PCR using universal and ESFY-specific primers. ESFY phytoplasma was detected in six different wild growing *Prunus* species exhibiting typical ESFY symptoms as well as in symptomless dog rose bushes (*Rosa canina*), ash trees (*Fraxinus excelsior*) and a declining hackberry (*Celtis australis*). The possible role of these plant species in the spread of ESFY phytoplasma is discussed. PCR-RFLP analysis of ribosomal DNA amplified with the universal primers was carried out to characterize the other phytoplasmas found. Thus, elm yellows phytoplasma, alder yellows phytoplasma and rubus stunt phytoplasma were detected in declining European field elm trees (*Ulmus carpinifolia* Gled), in declining European alder trees (*Alnus glutinosa*) and in proliferating *Rubus* spp. respectively. The presence of rubus stunt phytoplasma in great mallow (*Malva sylvestris*) and dog rose was demonstrated for the first time. Furthermore, the stolbur phytoplasma was detected in proliferating field bindweed (*Convolvulus arvensis)* and a previously undescribed phytoplasma type was detected in red dogwood (*Cornus sanguinea*). According to the 16S rDNA-RFLP pattern this new phytoplasma belongs to the stolbur phytoplasmas group.

Introduction

Apricot chlorotic leaf roll is one of the most important vector-transmitted diseases of apricots in France. The European stone fruit yellows phytoplasma is associated with the disease and has been found in almost all *Prunus* species cultivated in southern Europe (Morvan, 1977; Lorenz et al., 1994; Jarausch et al., 1998). Recently, the psylla *Cacopsylla pruni* has been identified as the ESFY phytoplasma vector in Italy (Carraro et al., 1998) and its role in transmission confirmed (W. Jarausch, unpublished results). The possible role of alternate plant hosts such as bindweed (*Convolvulus arvensis)* and Bermudagrass (*Cynodon dactylon* (L.) Pers.) in the spread of ESFY phytoplasma was suggested by Sanchez-Capuchino et al. (1982), but could not be confirmed as detection and identification methods for phytoplasmas were not available. Since then, RFLP analyses of 16S ribosomal DNA and sequence analyses have been introduced for phytoplasmas characterization and classification (Lee et al., 1993; Schneider et al., 1993; Zreik et al., 1995). As of today, 20 phylogenetic groups have been proposed (Seemüller et al., 1998). Amplification of

16S rDNA is now used for detection of phytoplasmas in plants and insects and for identification of putative reservoir plants. Thus, Marcone et al. (1997a) characterized phytoplasmas of the elm yellows group infecting *Ulmus*, *Alnus* and *Rubus* species. In another study, Marcone et al. (1997b) detected a putative new type of stolbur phytoplasmas in yellows-diseased weeds in Italy although previous reports indicated minor genetic variation in the stolbur group (Daire et al., 1997). Besides the universal primers, we have recently developed ESFY phytoplasma-specific primers. Their use in epidemiological studies confirmed the presence of ESFY-phytoplasmas in 14 different cultivated *Prunus* species (Jarausch et al., 1998, 2000).

In this paper additional plant hosts of the ESFY phytoplasma in the surroundings of orchards with high ESFY phytoplasma-infection have been identified. New phytoplasma plant hosts and a new phytoplasma type were discovered. A partial report on ESFY phytoplasma additional plant hosts has been published (Jarausch et al., 1999).

Materials and methods

Sampling. Surveys were done in the surroundings of highly ESFY phytoplasma-infected apricot orchards in ten different locations of the Pyrénées-Orientales department (Roussillon) and in two locations of the Tarn-et-Garonne department. In the latter, highly ESFY phytoplasma-infected Japanese plum orchards are predominant. A systematic botanical inventory was established for all sites. Sampling was done in summer and autumn from 1994 to 1998. In winter, *Prunus* species were especially surveyed for off-season growth symptoms. All plants with symptoms typical for phytoplasma diseases were sampled and special attention was given to woody plant species which were common to all sites. For the most common woody plant species, plants without symptoms were also sampled. The complete list of the sampled plants is given in Tables 1 and 2.

Phytoplasma reference strains. The ACLR-isolate ECA-G32, maintained in *in vitro* propagated *Prunus marianna* GF 8-1 (Jarausch et al., 1994a), was used as a reference strain for ESFY phytoplasma. Other phytoplasma reference strains were maintained in periwinkle plants (*Catharanthus roseus* (L.) G. Don) by periodic graft-inoculation to healthy plants. These were: EAY (European aster yellows from France), STOL (stolbur from France), MOL (a stolbur-like strain of Molières disease from France), AYA (an aster yellows-like strain from Spain formerly called ACLR-L), AshY (ash yellows from the USA), ULW (elm yellows from France), EY (elm yellows from USA) and AP (apple proliferation from Italy) (Schneider et al., 1993). Additional description and references about the periwinkle-maintained isolates used in this work are given in Jarausch et al. (1994b).

Nucleic acid extraction. Crude phloem tissue fractions were prepared from field-collected samples, either from woody branches or from the stems of herbaceous plants. DNA from phytoplasma reference strains was obtained from petioles of infected periwinkle plants or infected *in vitro* propagated plants. DNA extraction was carried out with approximately 1.0 g of freshly prepared plant material either using the phytoplasma enrichment procedure described by Ahrens and Seemüller (1992) or the simplified protocol of Maixner et al. (1995).

Primers and PCR amplification. ESFY phytoplasma-specific primers ECA1 and ECA2 (Jarausch et al., 1998) were used for direct identification of ESFY phytoplasma. For the detection and characterization of other phytoplasmas, universal phytoplasma ribosomal primers were used. These were fU5 (Lorenz et al., 1995) in combination with either rU4 (Ahrens and Seemüller, 1992) or P7 (Schneider et al., 1995). For nested PCR assays, primers fD1 (Weisburg et al., 1989) and P7 were used for the first amplification round and the primer pair fU5/P7 was used for the second amplification step.

All PCR were of 40 cycles in a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, USA), preceded by a 1-min denaturation step at 95 °C and followed by an elongation step for 4 min at 72 °C. Cycle conditions were as follows: primer pair ECA1/ECA2, 15 s at 95 °C, 15 s at 55 °C and 30 s at 72 °C; ribosomal primer pairs fU5/rU4 and fU5/P7, 15 s at 95 °C, 15 s at 55 °C and 60 s at 72 °C; primer pair fD1/P7, 15 s at 95 °C, 15 s at 55 °C and 90 s at 72 °C. Reaction mixtures of 40 µl contained 10–100 ng of DNA, 0.5 µM of each primer, 125 µM dNTP, 1.5 mM $MgCl_2$, and 0.5 U Taq polymerase (BRL-Life Technologies, Cergy Pontoise, France) in the reaction buffer supplied by the manufacturer. For nested PCR, 1 µM of PCR product from the first amplification was subjected to 20 additional cycles under the conditions described above.

Table 1. Detection by PCR and characterization by PCR-RFLP of the phytoplasmas found in common wild plant species growing in the surroundings of ESFY-infected apricot orchards in southern and southwestern France

Family	Species	nb. infected/ tested samples	Phytoplasma type[1]
Betulaceae	*Alnus glutinosa* L.	5/8	ALY
Convolvulaceae	*Convolvulus arvensis* L.	4/17	STOL
Cornaceae	*Cornus sanguinea* L.	1/10	COR
Malvaceae	*Malva sylvestris* L.	1/4	RS
Oleaceae	*Fraxinus excelsior* L.	2/33	ESFY
Rosaceae	*Prunus* spp.	4/35	ESFY
	Prunus amygdalus Batsch.	6/31	ESFY
	Prunus armeniaca L.	6/27	ESFY
	Prunus domestica L.	0/3	—
	Prunus cerasifera Ehrh.	1/9	ESFY
	Prunus spinosa L.	2/8	ESFY
	Rosa canina L.	7/21	3 ESFY/4 RS
	Rubus spp.	9/37	RS
	Rubus caesius L.	6/8	RS
	Rubus fructicosus L.	12/13	RS
	Rubus ulmifolius Schott	3/3	RS
	Crataegus monogyna Jacq.	0/11	—
Salicaceae	*Populus nigra* L.	0/34	—
	Populus alba L.	0/1	—
	Salix alba L.	0/4	—
Ulmaceae	*Ulmus carpinifolia* Gled.	17/49	EY
Vitaceae	*Vitis vinifera* L.	0/6	—
Total		86/372	

[1]Abbreviations of phytoplasma types are: ALY = alder yellows; STOL = stolbur; COR = new phytoplasma of Cornus sanguinea L.; RS = rubus stunt; ESFY = European stone fruit yellows; EY = elm yellows.

PCR amplification products (10 µl) were analysed by electrophoresis on 1 or 2% agarose gel. DNA was stained with ethidium bromide and visualized on a UV transilluminator.

RFLP analyses of ribosomal PCR products. RFLP analyses were done on the PCR products of approximately 1430 bp obtained with the primer pair fU5/P7 which comprised about 75% of the 16S rRNA gene and the complete 16S/23S intergenic region. RFLP patterns obtained from phytoplasma reference strains were used as standards. Fifteen µl of PCR product were digested with *Alu*I, *Hin*fI, *Hpa*II, *Kpn*I, *Rsa*I or *Taq*I according to the manufacturer's instructions (BRL-Life Technologies, Cergy Pontoise, France; Eurogentec, Seraing, Belgium). Restriction enzyme digests were analysed by agarose gel electrophoresis using a mixture of 2% NuSieve GTG agarose (FMC, Rockland, Maine, USA) and 1% agarose and were visualized as above.

RFLP patterns were used to calculate similarity coefficients (F) according to Nei and Li (1979). In the equation $F = 2N_{xy}/(N_x + N_y)$ N_x and N_y are the number of fragments in strains x and y and N_{xy} is the number of common fragments shared by strains x and y.

Results

The ESFY phytoplasma was detected with primer pair ECA1/ECA2 in several wild growing *Prunus* species (Figure 1 and Table 1) in the surroundings of highly ACLR-affected apricot orchards in the south (Roussillon) and in the southwest (Tarn-et-Garonne) of France.

These were *P. spinosa* L., *P. amygdalus* Batsch. (almond), *P. cerasifera* Ehrh. and undetermined *Prunus* species (Figure 1). The ESFY phytoplasma was also detected in apricots (*P. armeniaca* L.) and peach trees (*P. persica* L.) grown from seeds dispersed outside the orchards (Tables 1 and 2). The symptoms

Table 2. Phytoplasma detection and characterization in wild plant species occasionally found in the surroundings of ESFY-infected apricot orchards in southern and southwestern France

Family	Species	nb. infected/ tested samples	Phytoplasma type[1]
Aristolochiaceae	*Aristolochia clematitis* L.[2]	0/1	—
Anacardiaceae	*Pistacia lentiscus* L.	0/2	—
Caprifoliaceae	*Sambucus nigra* L.	0/5	—
	Sambucus racemosa L.	0/6	—
Caryophyllaceae	*Cucubalus baccifer* L.	0/2	—
	Saponaria officinalis L.	0/1	—
Cistaceae	*Cistus monspeliensis* L.[2]	0/3	—
	Cistus spp.	0/3	—
Compositae	*Inula viscosa* L.[2]	0/4	—
	Chondrilla juncea L.	0/1	—
Corylaceae	*Corylus avellana* L.	0/5	—
Fagaceae	*Quercus ilex* L.[2]	0/1	—
Gramineae	*Arundo donax* L.[2]	0/2	—
Labiatae	*Mentha* spp.	0/1	—
Lauraceae	*Laurus nobilis* L.	0/3	—
Oleaceae	*Ligustrum lucidum* Ait.	0/3	—
	Olea europea L.	0/1	—
Papilionaceae	*Ulex parviflorus* Pourret[2]	0/1	—
	Spatium junceum L.[2]	0/1	—
	Medicago sativa L.	0/1	—
Platanaceae	*Platanus acerifolia* L.	0/1	—
Polygonaceae	*Rumex* spp.	0/2	—
Rosaceae	*Prunus avium* L.	0/1	—
	Prunus persica L.	1/1	ESFY
	Pyrus communis L.	0/1	—
	Cydonia oblonga Mill.	0/1	—
Salicaceae	*Salix caprea* L.	0/1	—
Ulmaceae	*Celtis australis* L.	1/3	ESFY
Urticaceae	*Urtica dioica* L.	0/1	—
Total		2/59	

[1]Abbreviation of phytoplasma types: ESFY = European stone fruit yellows.
[2]Frequent species only at the Mediterranean coast.

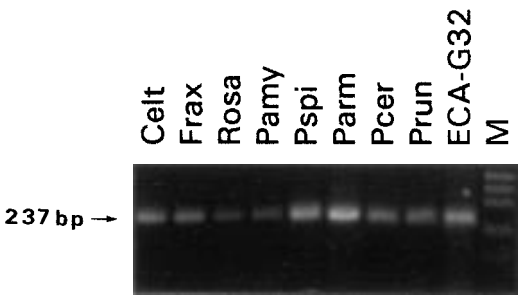

Figure 1. Amplification of the European stone fruit yellows phytoplasma DNA by the specific primer pair ECA1/ECA2 from total DNA extracted from wild plants and from the ESFY phytoplasma reference strain ECA-G32. See Table 3 for abbreviations of the phytoplasma isolates. $M = 1$ kb-ladder molecular weight marker.

on the wild *Prunus* species consisted in more or less pronounced off-season growth in winter. Old trees also showed decline. In summer and autumn, wild apricot trees showed typical chlorotic leaf roll whereas peach and certain almond trees had chlorotic leaves of reduced size.

To look for further additional plant hosts a botanical inventory was established in the orchard's environment. Woody species which were common to most sites were tested systematically. In this way, the ESFY phytoplasma was detected in plants which exhibited no symptoms, such as European ash (*Fraxinus excelsior* L.) and dog rose (*Rosa canina* L.) (Table 1, Figure 1). One older ash did show some die-back of branches but dog roses were symptomless. No

ESFY-infections could be detected in other frequently found plant species in the surroundings of the studied orchards (Table 1). The ESFY phytoplasma was also found in one declining European hackberry (*Celtis australis* L.) which showed leaf chlorosis. This is a species which grows locally on the Mediterranean coast (Table 2, Figure 1). No phytoplasmas were found, even using nested PCR, in symptomless hackberry. PCR and nested PCR also failed to detect phytoplasmas in the few European hazel trees (*Corylus avellana* L.) found in the surveys.

All plants with suspicious phytoplasma symptoms which were found during the surveys were also analysed by PCR using universal phytoplasma primers. A total of 431 samples from 51 different plant species were tested. Phytoplasma DNA was amplified from samples of European alder (*Alnus glutinosa* L.), field bindweed (*Convolvulus arvensis* L.), red dogwood (*Cornus sanguinea* L.) great mallow (*Malva sylvestris* L.), dog rose and European field elm (*Ulmus carpinifolia* L.) (Table 1). However, nested PCR was required to obtain PCR products from proliferating *Rubus*. RFLP analysis of the fU5/P7-amplified DNA products was carried out to identify the phytoplasma. The ESFY phytoplasmas found on wild plant species were included in these analyses to confirm the data obtained with the ESFY-specific PCR. After digestion with restriction endonucleases *Alu*I (Figure 2a), *Rsa*I (Figure 2b), *Kpn*I (Figure 2c), *Hin*fI (Figure 2d), *Hpa*II (Figure 2e), or *Taq*I (Figure 2f and g) all ESFY phytoplasma isolates

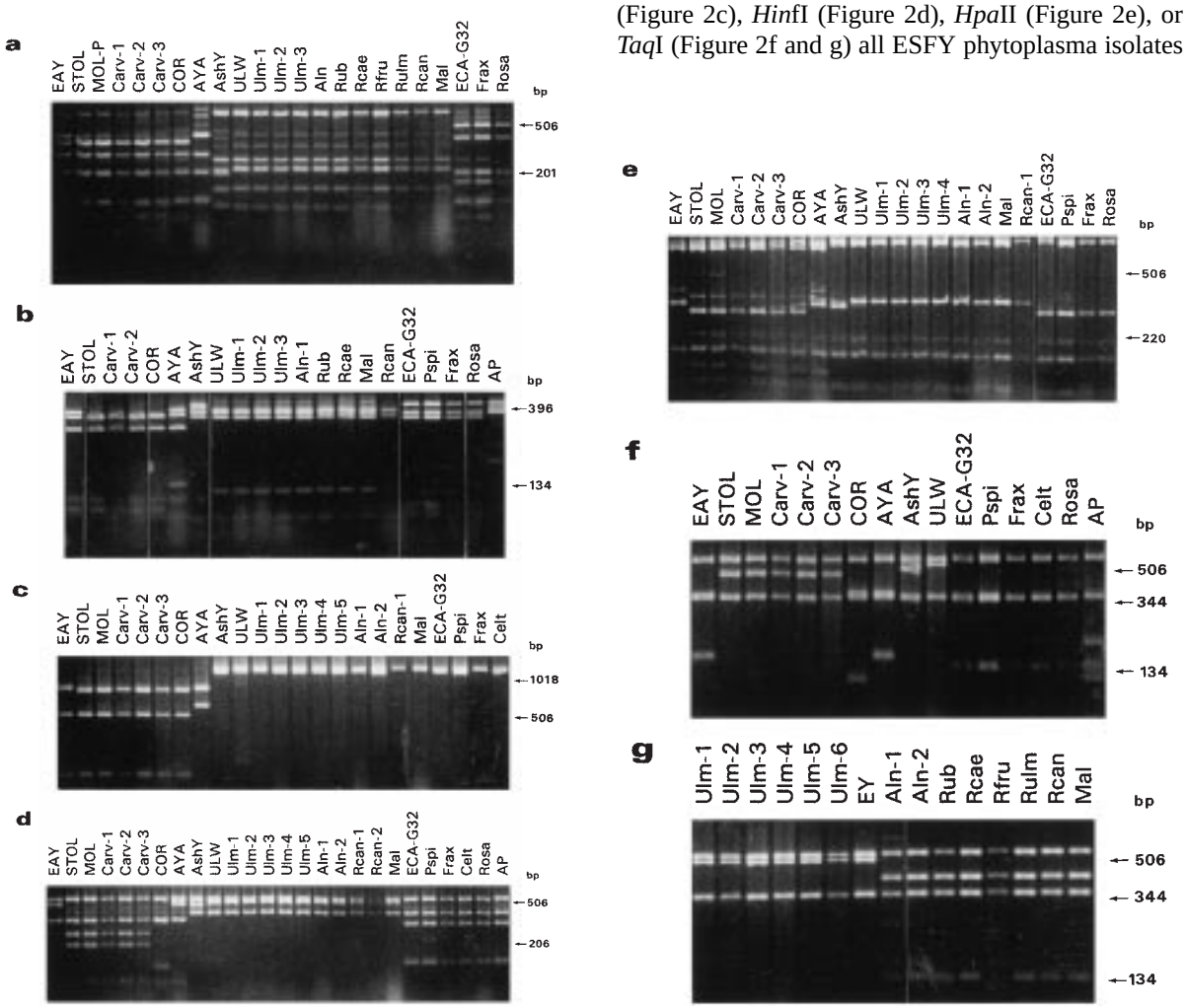

Figure 2. *Alu*I (a), *Rsa*I (b), *Kpn*I (c), *Hin*fI (d), *Hpa*II (e) and *Taq*I (f and g) restriction profiles of ribosomal phytoplasma DNA amplified by PCR with primer pair fU5/P7. See Table 3 for abbreviations of the phytoplasma isolates.

Table 3. Amplification with primers fU5/P7 and PCR-RFLP analysis of phytoplasmas from wild plants in the surroundings of ESFY-infected apricot orchards in southern and southwestern France

Host plant	Phytoplasma isolate	RFLP pattern[1]					
		*Alu*I	*Rsa*I	*Kpn*I	*Hinf*I	*Hpa*II	*Taq*I
P. armeniaca L.	ECA-G32	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
P. armeniaca L.[2]	Parm	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
P. amygdalus Batsch.[2]	Pamy	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
P. persica L.[2]	Pper	ESFY/AP	ESFY	nd	nd	nd	nd
P. cerasifera Ehrh.[2]	Pcer	ESFY/AP	ESFY	nd	nd	nd	nd
P. spinosa L.[2]	Pspi	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
Prunus spp.[2]	Prun	ESFY/AP	ESFY	nd	nd	nd	nd
F. excelsior L.	Frax	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
R. canina L.	Rosa	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
C. australis L.	Celt	ESFY/AP	ESFY	ESFY/AP	ESFY/AP	ESFY/AP	ESFY
Malus pumila Mill.[3]	AP	ESFY/AP	AP	nd[4]	ESFY/AP	nd[4]	AP
C. roseus (L.) G. Don[3]	EAY	EAY	EAY	EAY	EAY/AYA	EAY/AYA	EAY/AYA
C. roseus (L.) G. Don[3]	AYA	AYA	AYA	AYA	EAY/AYA	EAY/AYA	EAY/AYA
L. esculentum L.[3]	STOL	STOL	STOL	STOL	STOL	STOL	STOL
P. mahaleb L.[3]	MOL	STOL	STOL	STOL	STOL	STOL	STOL
C. arvensis L.	Carv	STOL	STOL	STOL	STOL	STOL	STOL
C. sanguinea L.	COR	STOL	STOL	STOL	COR	STOL	COR
F. americana L.[3]	AshY	AshY	AshY	AshY	AshY	AshY	AshY
U. carpinifolia Gled.[3]	ULW	EY	EY	EY	EY	EY	EY
U. americana L.[3]	EY	EY	EY	EY	EY	EY	EY
U. carpinifolia Gled.	Ulm	EY	EY	EY	EY	EY	EY
A. glutinosa L.	Aln	EY	EY	EY	EY	EY	ALY/RS
Rubus spp.	Rub	EY	EY	nd	nd	nd	RS
R. caesius L.	Rcae	EY	EY	nd	nd	nd	RS
R. fructicosus L.	Rfru	EY	EY	nd	nd	nd	RS
R. ulmifolius Schott	Rulm	EY	EY	nd	nd	nd	RS
R. canina L.	Rcan	EY	EY	EY	EY	EY	RS
M. sylvestris L.	Mal	EY	EY	EY	EY	EY	RS

[1] Abbreviations see Materials and methods and Table 1.
[2] *Prunus* trees from dispersed seeds outside the orchards.
[3] Periwinkle-maintained reference strains.
[4] Identical RFLP profiles for AP and ESFY phytoplasmas have been reported by Lee et al. (1998).
nd = not determined.

showed patterns identical to the reference strain ECA-G32 (Table 3).

Phytoplasmas from elm (Figure 2, Ulm), alder (Figure 2, Aln), mallow (Figure 2, Mal) and *Rubus* (Figure 2, Rub; Rcae; Rfru; Rulm) as well as four isolates from dog rose, designated Rcan, showed the same restriction profiles as the elm yellows reference strains ULW and EY after digestion with *Alu*I, *Hin*fI, *Hpa*II, *Kpn*I and *Rsa*I. After *Taq*I-digestion (Table 3, Figure 2f and g) all isolates from elm (Ulm) showed patterns identical to those of the ULW and EY reference strains, but all other isolates had a different profile corresponding to that published by Marcone et al. (1997a) for rubus stunt (RS) and alder yellows (ALY) phytoplasmas. The phytoplasmas found on bindweed with typical stolbur proliferation symptoms gave RFLP profiles identical to those of the stolbur reference strain (Table 3, Figure 2, Carv). The phytoplasma detected on red dogwood showed unreported restriction profiles (COR) after *Hin*fI- and *Taq*I-digestion but the pattern obtained with restriction endonucleases *Alu*I, *Hpa*II, *Kpn*I and *Rsa*I were identical to the stolbur phytoplasma pattern (Table 3, Figure 2).

The analysis of similarity coefficients of the examined phytoplasmas indicated that the phytoplasma amplified from red dogwood named COR is a new member in the stolbur-group (16SrXII) (Lee et al., 1998) as it showed the highest coefficient (0.90) with the stolbur-type phytoplasmas STOL and MOL-P. The tree, which showed no symptoms, was sampled in

Tarn-et-Garonne department. No phytoplasmas could be detected, even by nested PCR, in other red dogwood trees of the same area.

Discussion

Apricot chlorotic leaf roll, a disease associated with the ESFY phytoplasma, is the one of the most important pathological problem of apricot cultures in southern and southwestern France. Epidemiological studies on ACLR were concentrated in these regions from 1994 and the results have shown that cultivated *Prunus* species were highly infected with the ESFY agent in both regions (Jarausch et al., 1998, 1999, 2000).

The existence and the identity of additional plant hosts of the ESFY phytoplasma has long been uncertain. In Spain, Sanchez-Capuchino et al. (1982) explained the failure of the phytoplasma eradication trial on [re]infection from secondary hosts such as field bindweed and Bermuda-grass. Our epidemiological observations in Roussillon also indicated that wild plants in the orchard surroundings were likely to play a role in the spread of the disease because isolated orchards were as severely affected as those located in highly affected areas.

Our results demonstrate for the first time that wild *Prunus* species are infected by the ESFY phytoplasma. Few trees were found infected but they occurred in the surroundings of all examined apricot orchards in Roussillon and Tarn-et-Garonne, including isolated orchards. PCR signals obtained with samples from wild *Prunus* were generally weak indicating that the phytoplasma titers were low. Nevertheless, wild *Prunus* species represent a reservoir of the phytoplasma and may explain why ACLR is endemic in southern France. They might be the starting point for new infections in orchards planted with healthy material. For this reason, eradication programmes of diseased trees, including *Prunus* outside the orchards, have now started in southern France.

The ESFY phytoplasma has also been detected for the first time on European ash, dog rose and European hackberry. The infection of European hackberry appears as an isolated case because this species was present near apricot orchards on Mediterranean coast of Roussillon only. In Italy, this species has been shown to harbour different types of phytoplasmas belonging to the aster yellows (16SrI), X-disease (16SrIII) and elm yellows (16SrV) group (Bertaccini et al., 1996). Thus, European hackberry seems to be susceptible to several phytoplasmas types. European ash and dog rose were found to be symptomless carriers of ESFY phytoplasma. This may explain why only very few diseased plants were detected although these species were systematically found in the surroundings of diseased orchards. The role of these various plant species in the spread of ESFY phytoplasma has now to be further documented. The ESFY infection of European hazel discovered by Marcone et al. (1996) in Italy could not be confirmed in France.

Proliferation disease of wild *Rubus* was not the result of ESFY phytoplasma infection as hypothesized by Morvan (1991), but of rubus stunt phytoplasma infection. Although RS phytoplasmas have been identified by Mäurer and Seemüller (1994) on some *Rubus* bramble in the Rhone valley, our study demonstrates for the first time the presence of RS on different wild *Rubus* species in southern France. RS on *R. ulmifolius* Schott had not been reported before. PCR-RFLP analysis of ribosomal DNA also enabled the identification of RS phytoplasmas on great mallow and dog rose. Interestingly, nested PCR was often needed to detect RS phytoplasmas in *Rubus* with clear proliferation symptoms whereas single universal PCR readily amplified target DNA from samples of mallow with proliferation symptoms or symptomless dog rose.The vector of the RS phytoplasma, the leafhopper *Macropsis fuscula* (van der Meer, 1987), was found in the same area where RS-infected *Rubus*, dog rose and mallow were detected (W. Jarausch, unpublished results). Thus, this species may be responsible for RS transmission to dog rose and mallow. The susceptibility of woody plants such as European hackberry (Bertaccini et al., 1996) or European hazel (Marcone et al., 1996) to different types of phytoplasmas, seems to be more widespread than previously thought. ESFY- and RS-infection of dog rose is an additional example.

Elm yellows phytoplasma was found on European field elm showing witches' brooms, leaf chlorosis and a general decline. Elm yellows disease was rather common in the Roussillon where it was occurred at four different locations throughout the department. As reported by Marcone et al. (1997a) for Italian EY isolates, no genetic heterogeneity has been found between French isolates and periwinkle-maintained reference strains from Europe and North America. Alder yellows, however, has been identified for the first time in France. Although the ALY isolates gave identical ribosomal RFLP pattern to RS phytoplasmas as published by Marcone et al. (1997a) both phytoplasma types have

been previously distinguished by Southern blot analysis (Mäurer and Seemüller, 1994).

A new phytoplasma isolate designated COR, belonging to the stolbur group (16SrXII), was found on red dogwood. Sequence analysis will be necessary to further elucidate the phylogenetic position of this new isolate. Recently, Marcone et al. (1997b) discovered, in bindweed in Italy, a new phytoplasma which was genetically slightly different from the stolbur phytoplasma and is also distinct from the COR phytoplasma. All phytoplasmas which we detected on field bindweed were indistinguishable from the stolbur reference strain. Genome sizes among the stolbur phytoplasma group vary from 860 to 1350 kb (Marcone et al., 1999), indicating a greater genetic variation in the stolbur group than previously stated by Daire et al. (1997). Minucci and Boccardo (1997) also found genetic variation among stolbur isolates from tomato by Southern blot analysis. It is worth noting that the COR phytoplasma was found in the same area where grapevine is also commonly infected by the stolbur phytoplasma inducing bois noir (Daire et al., 1997). As the diseased red dogwood tree exhibited no symptoms other symptomless carriers of stolbur-type phytoplasmas may exist.

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