European Journal of Plant Pathology **110:** 193–201, 2004.

Detection of a phytoplasma associated with grapevine *Flavescence dorée* in *Clematis vitalba*

Elisa Angelini, Francesca Squizzato, Gianluca Lucchetta and Michele Borgo
Istituto Sperimentale per la Viticoltura, Viale XXVIII Aprile 26 – 31015 Conegliano (TV), Italy (Fax: +39-0438-64779; E-mail: isvbd@libero.it)

Accepted 11 September 2003

Key words: DNA sequence, elm yellows group, grapevine yellows, PCR, RFLP

Abstract

About 40 different species of wild herbaceous and woody plants were collected in underbrush close to a vineyard where *Flavescence dorée* (FD) has been reported for several years. Polymerase chain reaction assays were carried out using DNA extracted from leaves of each species for the detection of the presence of phytoplasmas. Only samples of *Clematis vitalba* were found to be infected with phytoplasmas. Restriction fragment length polymorphism and sequencing data of the 16S ribosomal RNA gene and of a non-ribosomal DNA fragment FD9 revealed that the phytoplasma isolate was identical to that causing FD in the nearby vineyard. The isolate identified in the clematis is the same as the FD-C phytoplasma found in grapevine in north-east Italy.

Abbreviations: BN – Bois Noir; bp – base pair; cv – cultivar; FD – *Flavescence dorée*; GY – grapevine yellows; PCR – polymerase chain reaction; RFLP – restriction fragment length polymorphism; STOL – stolbur.

Introduction

Phytoplasmas are pathogenic mollicutes which cause several hundred diseases in wild and cultivated plants. Phytoplasmas belonging to different phylogenetic groups (16SrI, 16SrIII, 16SrV, 16SrX and 16SrXII) are associated with grapevine yellows (GY) worldwide. Some phytoplasmas occur in grapevine only sporadically or in particular geographical regions, such as isolates belonging to groups 16SrI-A, 16SrI-B, 16SrIII-I, 16SrX-B and 16SrXII-B (Alma et al., 1996; Davis et al., 1997; 1998; Varga et al., 2000).

Phytoplasmas belonging to ribosomal group 16SrXII-A (stolbur phytoplasmas) occur in many herbaceous and woody plants in several European countries (Seemüller et al., 1998). In grapevine, they are associated with Bois Noir (BN) or Vergilbungskrankheit (VK) disease. This phytoplasma is transmitted to grapevine by *Hyalesthes obsoletus*, a ubiquitous Cixiidae (Maixner et al., 1995; Sforza et al., 1998). The involvement of another Cixiidae as a vector of stolbur–BN phytoplasma to sugar beet has been demonstrated, however transmission to grapevine was not investigated (Gatineau et al., 2001).

Phytoplasmas causing the most serious damage to European viticulture belong to ribosomal group 16SrV, subgroups C and D, and are associated with *Flavescence dorée* (FD) disease (Boudon-Padieu, 2000). They are transmitted from one grapevine to another by *Scaphoideus titanus* (Schvester et al., 1961), a leafhopper of American origin widespread in European vineyards. At least five different phytoplasma isolates associated with FD *sensu stricto* (i.e. transmitted by *S. titanus*) have been identified. Three FD isolates have been found in Italy: FD-D, belonging to subgroup 16SrV-D, FD-C and FD-Lombardia/Piemonte (FD-Lomb/Piem), belonging to subgroup 16SrV-C. FD-D seems to be ubiquitous in north Italy, while FD-C is limited to the Veneto region in north-east Italy, and FD Lomb/Piem is typical of areas of the north-west (Martini et al., 1999; 2002). FD-D isolate is identical to the French FD88 and FD92

ones present in France and Spain (Daire et al., 1997; Angelini et al., 2001). In France, two other FD phytoplasmas belonging to ribosomal subgroup 16SrV-C and denoted FD70 and FD2000, according to the years of their detection, have been isolated from grapevine (Caudwell et al., 1970; E. Angelini et al., unpubl.). So far, none of the FD *sensu stricto* isolates has ever been identified in other wild or cultivated plants except grapevine.

The aim of this study was to identify woody or herbaceous plant species that harbour phytoplasmas which cause FD disease. It was suggested, by comparing literature data concerning the other grapevine phytoplasmas which cause GY, that plants might exist which could act as FD phytoplasma reservoirs. Furthermore, a characteristic trend of FD epidemics was observed in some vineyards. Indeed, the epidemics began from the border of the vineyard, where symptomatic grapevines were concentrated, only later spreading out to the remaining grapevines (Borgo and Angelini, 2002). The identification of these reservoirs could prove to be very important in protecting vineyards from FD disease, which has been declared a quarantine disease in Europe.

Materials and methods

Samples

From 1996 to 2002, nine samples of symptomatic leaves from grapevine, cv. Riesling italico, infected with GY were collected in a vineyard located in the province of Treviso, in north-east Italy. During September 2001, herbaceous plant species and branches of woody plants were collected in underbrush at the border of the vineyard. None of the plants showed any clear symptoms of yellow disease. For species with only a few individual plants present every plant was sampled, whereas for species with many individual plants present plants were collected randomly. Different samples collected from the same species were pooled together. Only in the case of plants with reddish leaf colour were two distinct aliquots collected. The total number of plants collected was 153. During 2002, 19 samples of other plants of *Clematis vitalba* were collected in the underbrush monthly from July to October.

Species identification was carried out by visual observation with dichotomic keys (Pignatti, 1997). Plant species were labelled and classified as in Table 1.

Table 1. List of wild herbaceous and woody plant species used in this study and the number of individual plants collected for each species. Plants were collected in underbrush close to a vineyard where FD has been reported for several years

Sample number	Plant species	Number of individual plants
1[1]	*Acalypha virginica* L.	3
2[1]	*Acer campestre* L.	2
3	*Amaranthus chlorostachys* Willd.	1
4[1]	*Amaranthus* sp	2
5[1]	*Arctium nemorosum* Lej et Court	1
6	*Artemisia verlotorum* Lamotte	1
7[1]	*Artemisia vulgaris* L.	1
8[1]	*Ballota nigra* L. subsp. *foetida* Hayek	2
9[1]	*Bidens bipinnata* L.	1
10	*Bidens frondosa* L.	1
11	*Calystegia sepium* (L.) R. Br.	4
12	*Celtis australis* L.	1
13	*Chenopodium album* L. subsp. *album* L.	1
14	*Clematis vitalba* L.	1
15	*Clematis vitalba* L.[2]	3
16	*Cornus sanguinea* L.	3
17	*Cucubalus baccifer* L.	3
18	*Datura stramonium* L.	1
19	*Echinochloa crus-galli* (L.) Beauv.	10
20	*Euonymus europaeus* L.	3
21	*Eupatorium cannabinum* L.	1
22	*Fallopia dumetorum* (L.) Holub	2
23	*Geum urbanum* L.	1
24	*Hedera helix* L.	10
25	*Humulus lupulus* L.	10
26	*Lamium album* L.	1
27	*Lonicera japonica* Thumb.	3
28	*Malus domestica* L.	10
29	*Morus alba* L.	10
30	*Oxalis fontana* Bunge	2
31	*Parietaria officinalis* L.	3
32	*Physalis alkekengi* L.	3
33	*Prunus avium* L.	10
34	*Robinia pseudoacacia* L.	10
35	*Rubus sp*	10
36	*Sambucus nigra* L.	3
37	*Solanum nigrum* L. subsp. *nigrum*	1
38	*Taraxacum officinale* Weber	1
39	*Ulmus minor* L.[2]	1
40	*Ulmus minor* L.	7
41	*Urtica dioica* L.	3
42	*Vitis sp* (from seeds)	1
43	*Vitis berlandieri* × *riparia* cv K5BB	5

[1]Sample used in the preliminary inhibition test.
[2]Sample with reddish coloration of the leaves.

Leaf veins were isolated from all samples, split into 1 g aliquots, frozen with liquid nitrogen and stored at −20 °C until DNA extraction.

Phytoplasma reference isolates

The following phytoplasma isolates of the 16SrV group were used as a reference in Polymerase Chain Reaction (PCR)/Restriction Fragment Length Polymorphism (RFLP) experiments: FD-C and FD-D, obtained from leaves of naturally infected field-grown grapevine in the Veneto region (Italy); FD70, a French isolate of FD; EY1, American elm yellows; ALY, Italian alder yellows. The last three were kindly provided by Elisabeth Boudon-Padieu (INRA, Dijon, France) as DNA extracts obtained from experimentally infected periwinkle (*Catharanthus roseus*) (Angelini et al., 2001).

Other phytoplasma isolates belonging to different ribosomal groups were used as outgroups: AAY, American aster yellows (Florida), belonging to aster yellows group (16SrI); STOLC, stolbur of tomato (France), belonging to stolbur group (16SrXII). These isolates were kindly provided by Elisabeth Boudon-Padieu (INRA, Dijon, France) as DNA extracts obtained from experimentally-infected periwinkle.

DNA extraction and amplification

DNA extraction was performed using the CTAB method (Angelini et al., 2001). Healthy periwinkle samples grown from seedling under greenhouse conditions were used as negative controls.

Preliminary direct PCR assays with primer pair P1/P7 (Deng and Hiruki, 1991; Smart et al., 1996) were performed to test inhibition of polymerase reaction by extracted material different from grapevine. At first, 1 µl of extracted DNA from periwinkle experimentally infected with EY1 was added to the PCR mixture, containing 1 µl of DNA extract of seven samples on trial (Table 1) diluted on a scale of 1 : 1, 1 : 10 or 1 : 100 with deionized water. The other samples were subsequently amplified using 1 µl of the 1 : 10 dilution from the plant extracts, added to 1 µl of EY1 DNA extract. Samples giving no positive signal were further diluted until they yielded a PCR product corresponding to the EY1 amplimer.

PCR assays for detection of phytoplasmas in wild plant samples were carried out using a nested procedure and suitable dilution of extracted DNA estimated in the previous inhibition tests. The following primer pairs were used: P1/P7, followed by R16F2n/R2 (Gundersen and Lee, 1996), 16r758f/M23Sr (Gibb et al., 1995; Padovan et al., 1995), R16(V)F1/R1 or R16(I)F1/R1 (Lee et al., 1994); FD9f/r (Daire et al., 1997), followed by FD9f3/r2 or FD9f/r2 (Angelini et al., 2001).

The first set of primer pairs amplified a fragment of the 16S-23S ribosomal gene region of phytoplasmal DNA: P1/P7 of about 1800 bp, R16F2n/R2 of about 1200 bp, 16r758f/M23Sr of about 1050 bp, R16(V)F1/R1 and R16(I)F1/R1 of about 1100 bp. Primer pairs P1/P7, R16F2n/R2 and 16r758f/M23Sr are universal primer pairs for phytoplasmas, while R16(V)F1/R1 yields PCR products only for 16SrV-group phytoplasmas and R16(I)F1/R1 for phytoplasmas belonging to ribosomal groups 16SrI and 16SrXII.

The second set of primer pairs amplified a DNA fragment, denoted FD9, which corresponds to a part of the *spc* operon of mycoplasmas, coding for the translocase protein SecY (Reinert, 1999). Primer pair FD9f/r amplified a product of about 1300 bp, FD9f/r2 of about 1200 bp and FD9f3/r2 of about 1150 bp. These primer pairs are specific for DNA amplification of 16SrV-group phytoplasmas.

PCRs were performed using a Biometra T-personal apparatus according to Angelini et al. (2001). Reaction mixtures with de-ionized water in place of plant nucleic acid were used as negative controls together with healthy periwinkles. AAY and EY1 phytoplasmal DNA extracts were used as positive controls. PCR products (5 µl) were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV.

RFLP analyses

Aliquots of the PCR products obtained from the positive samples were digested with restriction enzymes in amounts of 5–20 µl depending on the intensity of the DNA bands in agarose gels. R16F2n/R2, 16r758f/M23Sr and FD9f3/r2 fragments were separately processed using *Taq*I and *Mse*I (New England Biolabs), according to the manufacturer's instructions. EY1, ALY, FD70, FD-D and FD-C phytoplasmas were used as a reference to compare the restriction patterns of both fragments. AAY and STOLC isolates were added as a reference in RFLP analysis of PCR-amplified 16Sr DNA fragments.

Restriction products were separated by 10% polyacrylamide gel electrophoresis in TBE buffer (Tris–borate 90 mM, EDTA 1 mM), stained with ethidium bromide and visualized under an UV transilluminator.

DNA sequencing

Primer pair FD9f/r2 (about 1200 bp) and 16r758f/M23Sr (about 1050 bp) PCR products, obtained from infected wild plants, were double-strand-sequenced using automated equipment (CRIBI Service, Padova). Intermediate primers were used when necessary to allow the sequences to overlap. Sequences from each DNA region were assembled after each nucleotidic position have been covered 2–4 times by sequencing. Only 165 nucleotides at the beginning of the FD9 amplicon were single-strand-sequenced. The LFASTA program was used for comparison (Chao et al., 1992). Alignment with 16S ribosomal RNA gene sequences from FD-C (GeneBank accession number AF458378, Angelini et al., 2003), FD70 (GeneBank accession number AF176319), FD-D (GeneBank accession number AF458380, Angelini et al., 2003) and ALY (GeneBank accession number Y16387) and alignment with non-ribosomal FD9 sequences from FD-C (GeneBank accession number AF458382, Angelini et al., 2003), FD70 (GeneBank accession number AF458383, Angelini et al., 2003), FD-D (GeneBank accession number AF458384, Angelini et al., 2003) and ALY (GeneBank accession number AY093580, Angelini et al., 2003) was carried out using CLUSTAL W (Thompson et al., 1994).

Results

PCR assays carried out using symptomatic grapevine samples collected in the vineyard infected with GY yielded a PCR product from all the samples. RFLP analysis of the PCR-amplified 16S rDNA fragment showed that two grapevines, sampled before 2000, were infected with BN (Table 2). Samples collected in 2000–2001 were all infected with FD-C; in 2002, three samples were found to be infected with FD-C and one with BN.

DNA amplification for detecting phytoplasmal DNA on wild plants was performed using each sample at the lowest dilution giving positive signals estimated by the preliminary inhibition assay, which was 1 : 10 dilution for all the samples. One sample of *C. vitalba* (number 15, see Table 1) yielded a PCR product only at 1 : 100 and 1 : 1000 dilutions in the preliminary inhibition PCR assay.

Only two samples out of 43 collected in 2001, both corresponding to DNA extracts from *C. vitalba*, showed positive signals with primer pairs R16F2n/R2, R16(V)F1/R1, 16r758f/M23Sr and FD9f3/r2 (Figure 1). No PCR product was amplified with primer

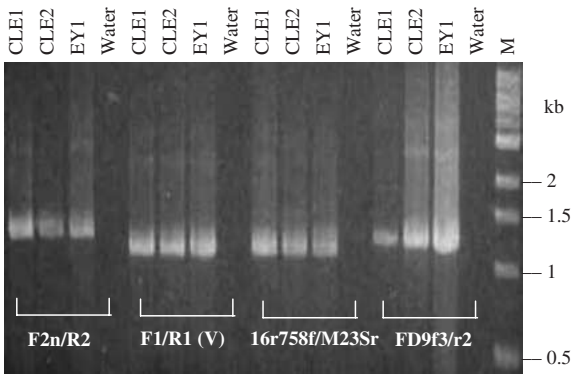

Figure 1. Agarose gel (1%) of PCR-amplified final DNA products from *C. vitalba* samples collected in 2001. Amplimers were obtained in nested-PCR using ribosomal primer pair P1/P7 followed by R16F2n/R2, R16(V)F1/R1 and 16S758f/M23Sr and using non-ribosomal primer pair FD9f/r followed by FD9f3/r2. CLE1 and CLE2: samples from clematis; water: negative control; EY1: positive control. M: marker, 1 kb ladder (New England Biolabs).

Table 2. Results of PCR/RFLP analyses on symptomatic grapevine samples cv Riesling italico collected from 1996 to 2002 in a vineyard located in the province of Treviso in north-east Italy. Primer pairs P1/P7 and 16r758f/M23Sr were used in nested-PCR assays. The ribosomal 16r758f/M23Sr fragment was subsequently digested with restriction endonuclease *Taq*I in order to identify the phytoplasma isolate

	1996	1997	1998	1999	2000	2001	2002
Number of samples	1	0	0	1	1	2	4
Phytoplasma type	BN	—	—	BN	FD-C	FD-C	FD-C and BN

Table 3. Results of PCR/RFLP detection of FD-C phytoplasma in 19 clematis samples, collected from July to October 2002 in underbrush close to a FD-C infected vineyard in the province of Treviso in north-east Italy. Primer pairs P1/P7 and 16r758f/M23Sr were used in nested-PCR assays. The ribosomal 16r758f/M23Sr fragment was subsequently digested with restriction endonuclease *Taq*I in order to identify the FD isolate

	1 July	2 August	28 August	30 September	14 October
Positive samples	0	0	0	6	3
Negative samples	2	2	2	0	4
Total samples	2	2	2	6	7

pair R16(I)F1/R1 from any samples, with the sole exception of the positive test (data not shown).

PCR assays carried out using clematis plants sampled in 2002 showed nine were positive out of 19 (Table 3). DNA extracts from clematis collected from July to August did not yield any PCR amplification products, whereas all six samples obtained from clematis plants collected at the end of September exhibited positive signals with primer pairs R16F2n/R2, 16r758f/M23Sr, R16(V)F1/R1 and FD9f3/r2. In October, only three samples out of seven tested positive.

R16F2n/R2, 16r758f/M23Sr and FD9f3/r2 products from all 11 infected clematis were separately digested using *Taq*I and *Mse*I, together with amplimers from phytoplasma reference isolates. RFLP patterns in all clematis samples were similar to each other in every RFLP analysis of PCR-amplified ribosomal and non-ribosomal gene fragments. Enzymatic digestion of the R16F2n/R2 ribosomal amplimers allowed clematis phytoplasma to be classified as a member of the 16SrV ribosomal group (data not shown). The RFLP pattern for the 16r758f/M23Sr ribosomal fragments showed that clematis phytoplasma was similar to FD-C and FD70 isolates and different from EY1 and FD-D phytoplasmas (Figure 2). The RFLP profile for the non-ribosomal FD9 amplicons demonstrated that clematis isolate was different from FD70 isolate and similar to grapevine FD-C phytoplasma (Figure 3). Ribosomal and non-ribosomal RFLP patterns for FD-C, FD-D, FD70, ALY, EY1, AAY and STOLC isolates were according to literature (Lee et al., 1998; Martini et al., 1999; Angelini et al., 2001).

Sequencing was carried out using the two DNA fragments 16r758f/M23Sr and FD9f/r2 for clematis phytoplasma. The comparison of the sequences with the LFASTA and CLUSTAL W programs showed that both DNA sequences were identical to those for FD-C isolate and different from all other FD phytoplasma isolates sequenced up to that point, especially in the more variable region corresponding to the *secY* gene. Five nucleotides were not clearly identified at the beginning of the FD9 amplimer, where the sequencing was single-strand; a nucleotide at the end of the same amplicon was also uncertain, though the sequencing was double-strand.[1]

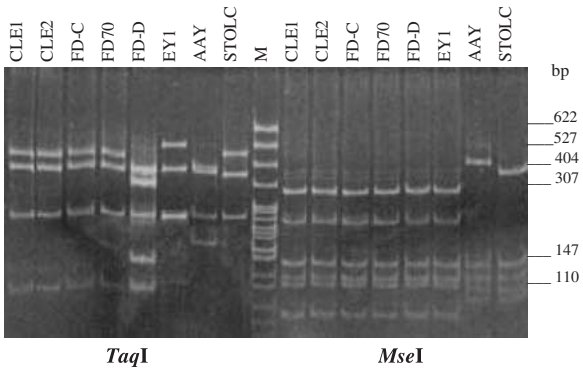

Figure 2. Polyacrylamide gel (10%) showing RFLP patterns in ribosomal nested-PCR products for clematis samples and reference phytoplasma isolates obtained from grapevine or periwinkle. The 16r758f/M23Sr fragment was digested using restriction endonucleases *Taq*I (left of the panel) and *Mse*I (right of the panel). Phytoplasmas: CLE1 and CLE2, samples from clematis; FD-C, FD70, FD-D, FD isolates; EY1, American elm yellows; AAY, American aster yellows; STOLC, tomato STOL from France. M: marker of molecular weight, pBR322/*Msp*I digested (New England Biolabs).

Discussion

Preliminary inhibition PCR assays were performed in order to decrease the risk of obtaining false negative results, due to the possible presence of host inhibitors

[1] Analyses of further sequencing data from FD clematis phytoplasma revealed that the FD9 fragment, corresponding to a part of the *secY* gene, shared 100% nucleotide identity with grapevine FD-C phytoplasma isolate (unpublished data).

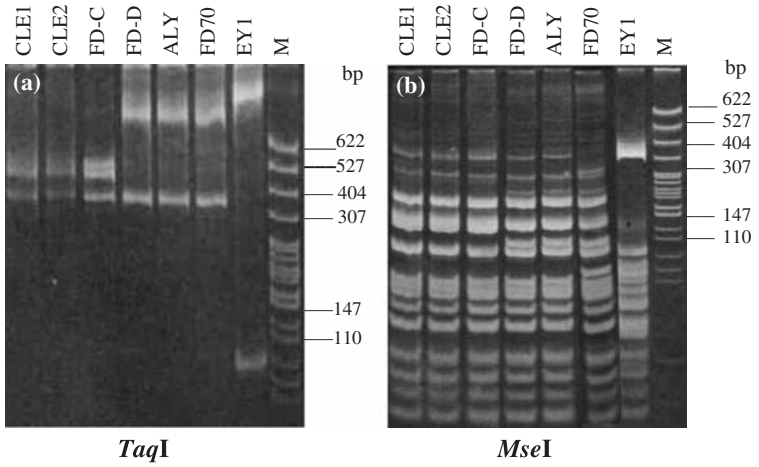

Figure 3. Polyacrylamide gel (10%) showing RFLP patterns in non-ribosomal nested-PCR products for clematis samples and 16SrV-group phytoplasma isolates obtained from grapevine or periwinkle. The FD9f3/r2 fragment was digested using restriction endonucleases *Taq*I (a) and *Mse*I (b). Phytoplasmas: CLE1 and CLE2, samples from clematis; FD-C, FD-D, FD70, FD isolates; ALY, Italian alder yellows; EY1, American elm yellows. M: marker of molecular weight, pBR322/*Msp*I digested (New England Biolabs).

in the DNA extracts. In fact, as reported in grapevine (Daire et al., 1992; Gibb et al., 1999), DNA extracts from herbaceous plants can inhibit the polymerase reaction and require the use of plant-specific DNA extraction kits (Alma et al., 2000). In our trials, an inhibitory effect was found at no dilution of the sample for most of the plants and for one sample at 1 : 10 dilution. The fact that few steps are involved in DNA purification could explain these results.

Among the 40 different species of wild herbaceous and woody plants tested, the only species infected with phytoplasmas was *C. vitalba*, which had never previously been reported to host a phytoplasma. PCR detection showed positive results only in DNA extracts for clematis plants sampled in autumn 2001 and 2002 and never in samples collected in July and August. This could reflect a low concentration or, indeed, absence of the pathogen early in the season. Moreover, there was no evidence of a clear relationship between the presence of phytoplasma and leaf symptoms in clematis plants.

RFLP analysis of the PCR-amplified 16S rDNA gene fragment and of the non-ribosomal FD9 fragment showed that all infected clematis examined in both years contained the same phytoplasma isolate. This was indistinguishable from the Italian FD-C isolate detected in grapevine in the nearby vineyard. Indeed, nucleotide sequencing of the 16S-26S DNA gene fragment and of the non-ribosomal FD9 fragment confirmed that clematis phytoplasma was identical to the FD-C strain found in grapevine. Phytoplasma isolates belonging to the 16SrV ribosomal group were identified in many wild and cultivated plants worldwide (Lee et al., 1998; Seemüller et al., 1998; Griffith et al., 1999; Harrison et al., 2002). Some 16SrV-group phytoplasmas, such as elm yellows phytoplasma and Palatinate Grapevine Yellows (PGY) phytoplasmas, usually occurring in non-grapevine plants, were also found sporadically in grapevine (Maixner and Reinert, 1999; Borgo et al., 2002; Bertaccini, 2002). In any case, to our knowledge no FD phytoplasma has so far been identified in plants other than grapevine. This is, indeed, the first report of a FD phytoplasma in a plant host different from *Vitis* spp.

The detection of a FD isolate in *C. vitalba* demonstrates that FD phytoplasma reservoirs different from grapevine do exist and this raises questions regarding FD epidemics. It can be assumed that the elimination of infected grapevines is not sufficient to stop the epidemics when there are other plant species hosting FD phytoplasmas close to the vineyards. Likewise, control strategies aimed at eliminating the vector in the vineyards could prove to be insufficient. As matters stand, the presence of FD reservoirs should not be undervalued.

The farm which is the subject of this study has been under observation for many years. GY symptoms have been observed since 1996 on 2–3% of the grapevines and early molecular analyses showed the presence of BN, as this paper reported. A most

serious epidemic started in 1999, right from the border where the infected clematis plants were discovered, and spread out progressively: the phytoplasma causing the epidemic was the FD-C isolate, as all the further analyses showed. Indeed, the discovery in clematis of the FD-C isolate leads us to argue that there is a strict relationship between vineyard and underbrush and poses the question of where this phytoplasma originates: from grapevine or clematis?

FD-C phytoplasma appeared to be quite different from the other FD strains identified in grapevine. Previous papers reported phylogenetic trees of 16SrV-group phytoplasmas based on sequencing data for three different DNA fragments: the 16S-23S intergenic spacer and the 16S rRNA gene; the ribosomal protein gene fragment coding for the 3′ end of *rpl*22 and the entire *rps*3 genes; the non-ribosomal DNA FD9 fragment coding for the translocase protein SecY (Martini et al., 2002; Angelini et al., 2003). In all these dendrograms FD-C phytoplasma, corresponding to the FD strain identified in clematis, proved to be the most divergent among FD isolates; on the other hand, FD-D (alias FD92), FD70, FD2000 and FD-Lomb/Piem were very similar to each other and were grouped in a homogeneous cluster. FD-C from clematis was, indeed, less related to the FD-group than ALY phytoplasma, an isolate found in southern Italian alder, never detected in grapevine, and different from FD *sensu stricto* (Marcone et al., 1997). Indeed, alder could also be regarded as a wild reservoir of a potential grapevine pathogen, as suggested previously (Angelini et al., 2003). In any case, only successful transmission trials with *S. titanus* could definitely include ALY isolate among FD *sensu stricto* phytoplasmas.

One way to understand the relationship between grapevine FD disease and FD-C phytoplasma identified in clematis involves the study of potential vectors. The ampelophagus *S. titanus* is the vector of FD phytoplasmas from one grapevine to another. Although *Vitis* sp. is its preferred host, in experimental conditions this leafhopper was able to survive and feed on many plant species, such as *Vicia faba*, *Cineraria* spp. and *Chrysanthemum* spp., even if rates of survival were lower than in grapevine (Caudwell et al., 1970; Alma et al., 2001). *Scaphoideus* sp. was detected in the USA on peach, apple and grapevine, but also in thin woods on different herbaceous and woody species, such as *Salix* spp., *Polygonium* spp., *Ulmus* spp., *Juniperus virginiana* and *Onoclea sensibilis* (Barnett, 1976). In addition, the sporadic presence of *S. titanus* individuals on clematis in the underbrush was observed in September 2002 (E. Angelini et al., unpubl.). This leads us to investigate its ecology more deeply, also with regard to clematis.

It is worth remembering, moreover, that other phytoplasma vectors have been identified which are able to transmit GY phytoplasmas from woody or herbaceous hosts to grapevine. Transmission of stolbur–BN phytoplasma from herbaceous species to grapevine is carried out by *H. obsoletus*, which feeds on grapevine only sporadically (Maixner et al., 1995). In Germany, it has been demonstrated that the leafhopper *Oncopsis alni* transmits PGY phytoplasmas from one alder to another and on to grapevine, where it feeds only occasionally (Maixner and Reinert, 1999; Maixner et al., 2000).

In conclusion, a FD phytoplasma was identified in north-east Italy on wild plants of *C. vitalba,* in the underbrush close to a vineyard infected with GY. Analyses of the sequencing data for the 16S-23S rRNA gene fragment and for the *secY* gene fragment identified the phytoplasma as the FD-C strain that occurs in the nearby vineyard and in some areas of the Veneto region. Further research is needed to identify the vector involved in the transmission of FD-C and to understand whether the isolate originates from clematis or grapevine. The hypothesis that *C. vitalba* can serve as a reservoir for FD or other phytoplasmas must be considered.

Acknowledgements

Part of this study was supported by a grant from the *Servizio Fitosanitario* for the Veneto region. We are very grateful to Elisabeth Boudon-Padieu and Maurizio Conti for a critical review of the manuscript.

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