Vitis 37 (3), 131–137 (1998)

Phytoplasmas associated with grapevine yellows in Virginia belong to group 16SrI, subgroup A (tomato big bud phytoplasma subgroup), and group 16SrIII, new subgroup I

by

R. E. Davis[1], Rasa Jomantiene[1], Ellen L. Dally[1], and T. K. Wolf[2]

[1] Molecular Plant Pathology Laboratory, Agricultural Research Service-USDA, Beltsville, MD, USA
[2] Virginia Agricultural Experiment Station, Winchester, Virginia, USA

Summary: Grapevine yellows disease in Virginia closely resembles flavescence dorée and other grapevine yellows diseases, but the phytoplasmas infecting grapevines in Virginia are distinct from other grapevine yellows pathogens. RFLP analysis of PCR-amplified 16S rDNA indicated that a Virginia grapevine yellows phytoplasma, designated VGYIII, was distinct from all other phytoplasmas studied, but was most closely related to spirea stunt (SP1), walnut witches' broom (WWB), and poinsettia branch-inducing (PoiB1) phytoplasmas in subgroups E, G, and H, respectively, of 16S rRNA group 16SrIII. RFLP analysis also indicated the existence of sequence heterogeneity between the two rRNA operons in the genomes of SP1 and WWB. Based on the results from RFLP and sequence comparisons with other group 16SrIII phytoplasmas, the VGYIII phytoplasma was classified in a new subgroup, designated 16SrIII-I. A second phytoplasma (VGYI) was detected in cultivated grapevines (*Vitis vinifera* L.) and in wild grapevines (*V. riparia* Michx.) and identified as a member of subgroup 16SrI-A. There was no evidence of flavescence dorée, bois noir, or Australian grapevine yellows phytoplasmas in Virginia.

Keywords: Vitis sp., grapevine yellows, phytoplasma, 16S rRNA gene.

Introduction

Grapevine yellows diseases occur in widespread viticultural areas of the world (Uyemoto 1976; Caudwell 1983, 1990, 1993; Pearson *et al.* 1985; Magarey and Wachtel 1986; Prince *et al.* 1993; Wolf *et al.* 1994; Davis *et al.* 1998). Symptoms characteristic of grapevine yellows include yellowing of leaves, fruit abortion, and lack of lignification of canes (Caudwell 1983, 1990; Magarey and Wachtel 1986; Wolf *et al.* 1994). The symptoms of grapevine yellows are very similar in Europe, Israel, Australia, and North America (Uyemoto 1976; Caudwell 1983; Pearson *et al.* 1985; Magarey and Wachtel 1986; Wolf *et al.* 1994; Davis *et al.* 1997 a, b), but the phytoplasmas associated with the diseases can be very different. In Europe, the grapevine yellows disease known as flavescence dorée is attributed to a phytoplasma belonging to 16S rRNA group 16SrV (elm yellows and related phytoplasmas) (Daire *et al.* 1993; Davis and Prince 1993; Prince *et al.* 1993; Bianco *et al.* 1996) that is transmitted by *Scaphoideus titanus* (Caudwell 1983). Bois noir disease and the evidently equivalent grapevine yellows in southern Italy, Greece, Israel, and elsewhere in the Mediterranean region, are attributed to group 16SrXII, subgroup A (stolbur phytoplasma) (Caudwell 1993; Daire *et al.* 1993; Seemüller *et al.* 1994; Davis *et al.* 1997 b, 1998) transmitted by *Hyalesthes obsoletus* (Maixner *et al.* 1995). Australian grapevine yellows is attributed to „*Candidatus Phytoplasma australiense*", the type member of subgroup B in group 16SrXII (Davis *et al.* 1997 a).

In North America, grapevine yellows was first reported in New York in 1977 (Uyemoto *et al.* 1976). Caudwell (1983) proposed that both flavescence dorée phytoplasma and its only known insect vector (*S. titanus*) had been introduced into Europe from North America. Work by Pearson *et al.* (1985) drew further attention to the similarity between grapevine yellows disease in New York and flavescence dorée in France. In a separate study of grapevine yellows in New York, Maixner *et al.* (1993) concluded that their data "support the hypothesis of a common North American origin of flavescence dorée and its vector, *S. titanus*". While these studies reiterated the hypothesis that the flavescence dorée pathogen was introduced into Europe from North America, they did not report the identification and classification of the phytoplasma associated with grapevine yellows in New York.

In Virginia, grapevine yellows disease has been observed since 1987 in cvs. Chardonnay and Riesling growing in the Virginia Piedmont area (Wolf *et al.* 1994; this paper). Although the spread of the disease within vineyards appears to be relatively slow, diseased vines do not recover and often die within several years of symptom onset (Wolf *et al.* 1994). Prince *et al.* (1993) detected the association of two different phytoplasmas with grapevine yellows in Virginia and identified the phytoplasmas as members of 16S rRNA groups 16SrI (aster yellows and related phytoplasmas, including stolbur, *sensu* Lee *et al.* 1993 and Vibio *et al.* 1996) and 16SrIII (peach X-disease and related phytoplasmas), respectively. However, the identification of the phytoplasmas to a 16S rRNA subgroup level was not reported.

The present study was initiated to determine the subgroup affiliations of Virginia grapevine yellows-associated phytoplasmas. Knowledge of subgroup affiliation is signifi-

Correspondence to: R. E. Davis, Molecular Plant Pathology Laboratory, Agricultural Research Service-USDA, Room 252, Bldg. 011A, BARC West, Beltsville, MD 20705, USA. Fax: +1-301-504-5449. E-mail: rdavis@asrr.arsusda.gov

cant in order to facilitate the future identification of their insect vectors and alternate plant hosts. With this intention, we collected samples from diseased grapevine (*Vitis vinifera* L.) plants in 5 commercial vineyards in Virginia during a 3-year study, from 1994 through 1996. As in the previous study (PRINCE *et al.* 1993), the data did not indicate the presence of any European grapevine yellows phytoplasma in Virginia. The results indicated infection of cultivated grapevine and of wild grapevine (*V. riparia* Michx.) by a phytoplasma belonging to group 16SrI, subgroup A (tomato big bud and related phytoplasmas). The group 16SrIII Virginia grapevine yellows phytoplasma was determined to represent a new subgroup, designated subgroup I, in group 16SrIII. A preliminary report of these findings has been published (DAVIS *et al.* 1998).

Materials and methods

Plant samples and reference phytoplasma strains: During 1994, 1995, and 1996, samples of leaves were collected from naturally diseased cvs Chardonnay, Cabernet franc, and Riesling grapevine (*V. vinifera* L.) plants exhibiting symptoms of grapevine yellows in 5 commercial vineyards located in the Virginia Piedmont area. Leaf samples were also collected from plants of wild grapevine (*V. riparia* Michx.) growing in the vicinity of vineyards containing diseased *V. vinifera* and from healthy seedlings of *V. vinifera* grown in an insect proof greenhouse. The reference phytoplasmas used as controls in this study were maintained by graft transmission in plants of periwinkle (*Catharanthus roseus* [L.] G.Don) maintained in a greenhouse; they are listed in the Table.

Primer pairs and conditions for polymerase chain reaction (PCR): Nucleic acid for use as template in PCR was extracted from fresh tissue by a previously described method (PRINCE *et al.* 1993). After extraction, DNA was purified using GeneCleanIII kit as specified by the manufacturer (Bio101, Vista, CA).

Seven pairs of oligonucleotide primers were used in PCRs. R16mF2/R16R1 and R16F2n/R16R2 are phytoplasma universal primer pairs (GUNDERSEN and LEE 1996). Primer pairs R16(I)F1/R16(I)R1, R16(III)F2/R16(III)R1, and R16(V)F1/R16(V)R1 prime specific amplification of 16S rDNA from phytoplasmas belonging to groups (*sensu* LEE *et al.* 1993) 16SrI, 16SrIII, and 16SrV, respectively (LEE *et al.* 1994 a). Primer pair rpF1/R1 primes amplificaton of phytoplasma ribosomal protein (rp) gene operon sequences (LIM and SEARS 1992; GUNDERSEN *et al.* 1994). Primer pair rp(III)F1/rp(III)R1 primes amplification of rp gene operon sequences from group 16SrIII phytoplasma strains (this paper).

Both, a single (direct, non-nested) PCR and nested PCR were used. In one nested PCR protocol, DNA amplified in PCR primed by R16mF2/R16R1 was diluted 1:50 with sterile distilled water and used as template in PCR primed by R16F2n/R16R2. In a separate nested PCR protocol, DNA amplified in PCR primed by R16F2n/R16R2 was diluted 1:50 with sterile distilled water and used as template in PCR primed by R16(I)F1/R16(I)R1, R16(III)F2/R16(III)R1, or R16(V)F1/R16(V)R1. Primer pairs rpF1/R1 and rp(III)F1/rp(III)R1 were used in direct, non-nested PCR only. All PCR was carried out as previously described (JOMANTIENE *et al.* 1998).

Table

Reference phytoplasmas used in this study and their 16S rRNA group and subgroup classifications[1)]

Phytoplasma strain	16S rRNA group-subgroup
Maryland aster yellows (AY1)	I-B
Peanut witches'-broom	II-A
Western X-disease (WX)	III-A
Canada peach X-disease (CX)	III-A
Clover yellow edge (CYE)	III-B
Vaccinium witches'-broom	III-B
Pecan bunch (PB)	III-C
Goldenrod yellows (GR1)	III-D
Spirea stunt (SP1)	III-E
Milkweed yellows (MW1)	III-F
Walnut witches'-broom(WWB)	III-G
Poinsettia branch-inducing (PoiBI)	III-H
Coconut lethal yellows (LY3)	IV-A
Elm yellows (EY1)	V-A
Clover proliferation (CP)	VI-A
Ash yellows (AshY)	VII-A
Pigeon pea witches'-broom (PPWB)	IX-A
Apple proliferation (AP)	X-A
Rice yellow dwarf (RYD)	XI-A
Stolbur (STOL)	XII-A
Australian grapevine yellows (AUSGY) (*Candidatus Phytoplasma australiense*)	XII-B
Mexican periwinkle virescence (MPV)	XIII-A

[1)] Classifications according to LEE *et al.* 1998.

Restriction fragment length polymorphism (RFLP) analyses of PCR-amplified DNA: Products from nested PCR primed by R16F2n/R16R2 were analyzed by single enzyme digestion, according to manufacturers' instructions, with *Aci* I, *Bam* HI, *Bfa* I, *Dde* I, *Dra* I, *Eco* R2, *Hae* III, *Hha* I, *Hinf* I, *Hpa* I, *Hpa* II, *Kpn* I, *Mse* I, *Msp* I, *Rsa* I, *Sau* 3AI, *Taq* I, and *Tha* I (New England Biolabs, Beverly, Mass.) and *Alu* I (Life Technologies Inc., Gaithersburg, MD). The digested DNAs were analyzed by electrophoresis of the digestion products in a 5 % polyacrylamide gel, followed by staining with ethidium bromide and visualization of DNA bands with a UV transilluminator. The RFLP patterns of Virginia grapevine yellows phytoplasma DNAs were compared with the RFLP patterns obtained using reference phytoplasmas in this study and the RFLP patterns previously published (LEE *et al.* 1993; PRINCE *et al.* 1993; VIBIO *et al.* 1996; DAVIS *et al.* 1997 a, b; LEE

et al. 1998). Phytoplasmas detected in this work were assigned to 16S rRNA gene groups and subgroups as previously described by LEE *et al.* (1993, 1998).

Nucleotide sequencing, sequence alignment, and putative restriction site analysis: PCR-amplified 16S rRNA gene and rp gene operon products were commercially sequenced using standard procedures. The nucleotide sequence determined for phytoplasma VGYIII rDNA was deposited in the GenBank database (GenBank, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland). Other sequences used in this study were obtained from GenBank. A partial sequence of clover yellow edge (CYE) phytoplasma 16S rDNA (Accession no. L33766) was obtained from GenBank. In the present work, we amplified and sequenced 16S rDNA from clover yellow edge phytoplasma in order to verify the GenBank 16S rDNA sequence from clover yellow edge phytoplasma and to complete nucleotide sequencing of 42 bases missing from GenBank Accession no. L33766. 16S rRNA gene sequences from Virginia grapevine yellows phytoplasma VGYIII and from other group 16SrIII phytoplasmas were analyzed to identify recognition sequences for selected restriction enzymes. Putative restriction site maps were generated by using the DNASTAR program MapDraw option (DNASTAR Inc., Madison, Wisc.). Alignments of sequences were generated and sequence similarities evaluated by using the DNASTAR program Megalign option.

Results

Detection and classification of phytoplasmas in diseased cultivated grapevines and in wild grapevine (*V. riparia*): Phytoplasmas belonging to two groups, 16SrI and 16SrIII, were detected in grapevine yellows-diseased *V. vinifera* in Virginia on the basis of phytoplasma-specific DNA amplification in PCR (data not shown). These results confirmed earlier findings by PRINCE *et al.* (1993).

Preliminary RFLP analysis of amplified 16S rDNA indicated that the group 16SrI phytoplasma was similar to both subgroup I-A and subgroup I-B phytoplasmas; further analysis by digestion of amplified DNA using *Hha* I revealed that this phytoplasma was most closely related to subgroup A (type strain, tomato big bud [BB] phytoplasma). RFLP analysis using 12 different restriction enzymes did not distinguish the group 16SrI phytoplasma, designated VGYI, from BB phytoplasma (Fig. 1). Parallel investigation of wild grapevine (*V. riparia*) plants growing close to infected commercial vineyards indicated that wild grapevine was also infected by a group 16SrI phytoplasma. RFLP analysis distinguished the phytoplasma strains in wild grapevine as members of subgroup I-A (data not shown).

Classification of the group 16SrIII phytoplasma strains in diseased, cultivated grapevines was accomplished using 19 restriction enzymes for RFLP analysis of PCR-amplified 16S rDNA. All strains yielded the same RFLP patterns (data not shown). Of the 8 subgroups previously described in group 16SrIII (LEE *et al.* 1998), the group 16SrIII Virginia grapevine yellows phytoplasma was most closely related to Canada peach X-disease (subgroup III-A), walnut witches'-broom (subgroup III-G), spirea stunt (subgroup III-E), and poinsettia branch-inducing (subgroup III-H) phytoplasmas. The VGYIII phytoplasma could be distinguished from poinsettia branch-inducing phytoplasma by digestion of PCR products with *Hpa* I or with *Dde* I (Fig. 2). VGYIII phytoplasma could be distinguished from Canada peach X-disease phytoplasma by digestion of PCR products with *Mse* I (Fig. 3), from walnut witches'-broom phytoplasma by *Rsa* I and from spirea stunt phytoplasma by *Dde* I (Fig. 2) and *Hha* I (Fig. 3). RFLP patterns of walnut witches'-broom and spirea stunt phytoplasma DNAs were unusual in the presence of „extra" DNA bands; that is, the total of fragment sizes was considerably larger than that expected for the approx. 1.2 kbp PCR product. We explain these results as

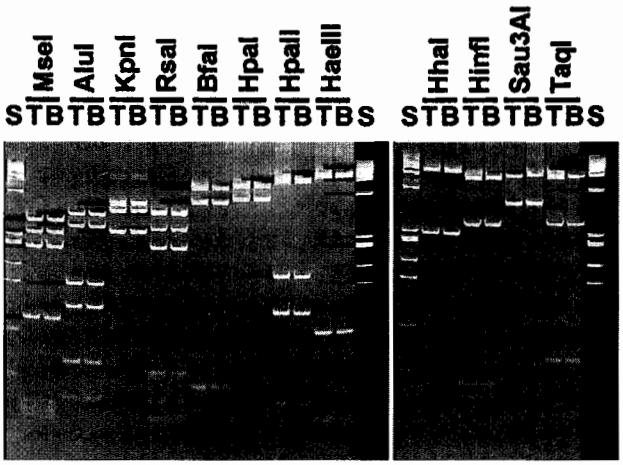

Fig. 1: Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA amplified in nested PCRs primed by oligonucleotide pair R16F2n/R16R2 (F2n/R2) from Virginia grapevine yellows phytoplasma strain VGYI (B) and tomato big bud (T) phytoplasma. DNA products from nested PCR were digested with restriction endonucleases *Mse* I, *Alu* I, *Kpn* I, *Rsa* I, *Bfa* I, *Hpa* I, *Hpa* II, *Hae* III, *Hha* I, *Hinf* I, *Sau* 3AI, and *Taq* I. S, contained PhiX174 *Hae* III digest size standard.

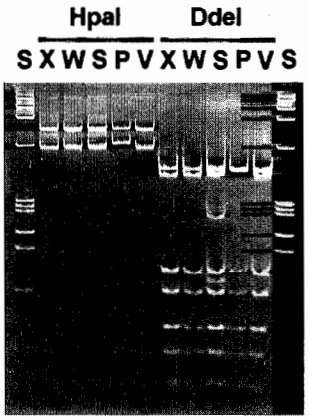

Fig. 2: *Dde* I and *Hpa* I RFLP profiles of phytoplasma 16S rDNA amplified in nested PCRs primed by oligonucleotide pair F2n/R2 from Canada peach X-disease (X), walnut witches'-broom (W), spirea stunt (S), poinsettia branch-inducing strain PoinB1 (P), and Virginia grapevine yellows strain VGYIII (V) phytoplasmas. S, PhiX174 *Hae* III digest size standard.

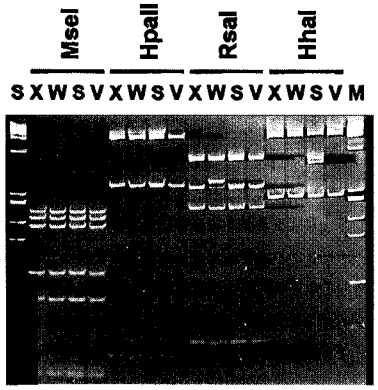

Fig. 3: *Mse* I, *Hpa* II, *Rsa* I, and *Hha* I RFLP profiles of phytoplasma 16S rDNA amplified in nested PCRs primed by oligonucleotide pair F2n/R2 from Canada peach X-disease, walnut witches'-broom, spirea stunt, and Virginia grapevine yellows VGYIII phytoplasmas. Abbreviations see Fig. 2.

composite patterns of 16S rDNA amplified from two rRNA operons, exhibiting interoperon sequence heterogeneity, in both walnut witches'-broom and spirea stunt phytoplasmas. We did not obtain RFLP evidence of two rRNA operons of different sequence in VGYIII, Canada peach X-disease, or poinsettia branch-inducing phytoplasmas. Since the collective RFLP patterns of 16S rDNA from Virginia grapevine yellows phytoplasma VGYIII were unique, this phytoplasma was assigned to a new 16S rRNA subgroup, designated I, in group 16SrIII.

In order to confirm the presence of group 16SrIII phytoplasmas in some grapevine plants, an oligonucleotide pair was designed to prime PCR-mediated amplification of ribosomal protein gene operon sequences from group 16SrIII phytoplasmas. The primers have the following designations and sequences: rp(III)F1, 5"-CTATTGCAAATCAG ATGTCTG-3" and rp(III)R1, 5"-AATAAACGAGTTAAA CGAAGACC-3". Conditions of PCR using these primers were the same as those described previously (JOMANTIENE *et al.* 1998.). A group 16SrIII-specific DNA fragment of 244 bp was amplified in direct (non-nested) PCR from template DNAs derived from all group 16SrIII phytoplasmas in this study, including the VGYIII Virginia grapevine yellows phytoplasma. No group III-specific DNA amplification was observed when template DNAs were derived from the remaining phytoplasmas listed in the Table. RFLP analysis of amplified rp gene operon sequences using *Alu* I and *Mse* I did not distinguish VGYIII from those of Canada peach X-disease, walnut witches'-broom, or spirea stunt phytoplasmas (data not shown). The amplified 244 bp fragments of the ribosomal protein gene operons from VGYIII and SP1 phytoplasmas were sequenced, and the sequences were aligned with those from group 16SrIII phytoplasmas associated with Canada peach X-disease, western X-disease, and clover yellow edge (Fig. 4). The VGYIII rp gene operon sequence was identical to that of Canada peach X-disease phytoplasma and was differentiated from those of spirea stunt, western X-disease, and clover yellow edge phytoplasmas by base substitutions in from one to three positions.

Nucleotide sequence and putative restriction sites in a 16S rRNA gene sequence from Virginia grapevine yellows phytoplasma VGYIII: The nucleotide sequence determined for the 16S rDNA amplified from VGYIII phytoplasma in PCR primed by F2n/R2 was deposited in the GenBank database as Accession number AFO60875. The nucleotide sequence of 16S rDNA from VGYIII phytoplasma was compared with the corresponding sequences from western X-disease phytoplasma (Accession no. L04682), clover yellow edge phytoplasma (Accession no. L33766), and Vaccinium witches'-broom phytoplasma (Accession no. X76430). Results from comparative analyses of putative restriction sites in the sequenced DNA are shown in Fig. 5. Expected fragment sizes based on analysis of putative re-

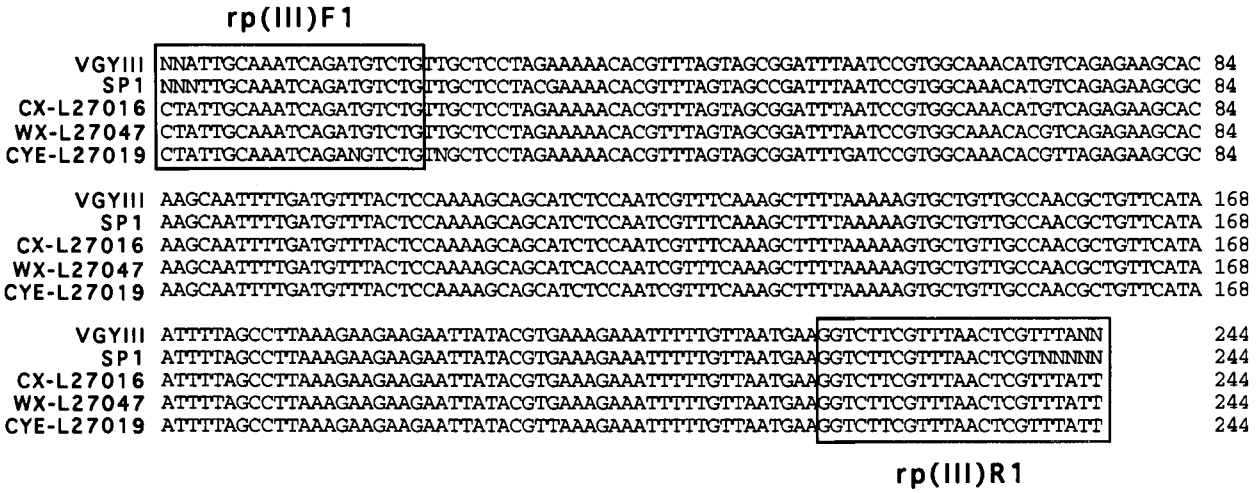

Fig. 4: Alignment of ribosomal protein (rp) gene operon sequences amplified in direct (non-nested) polymerase chain reactions (PCRs) primed by group 16SrIII-specific oligonucleotide pair rp(III)F1/rp(III)R1 in PCRs containing template DNA derived from Virginia grapevine yellows phytoplasma VGYIII (VGYIII), or from spirea stunt (SP1), Canada peach X-disease (CX) (GenBank Accession no. L27016), western X-disease (WX) (GenBank Accession no. L27047), or clover yellow edge (CYE) (GenBank Accession no. L27019) phytoplasmas. Boxes indicate positions of rp(III)F1 and rp(III)R1.

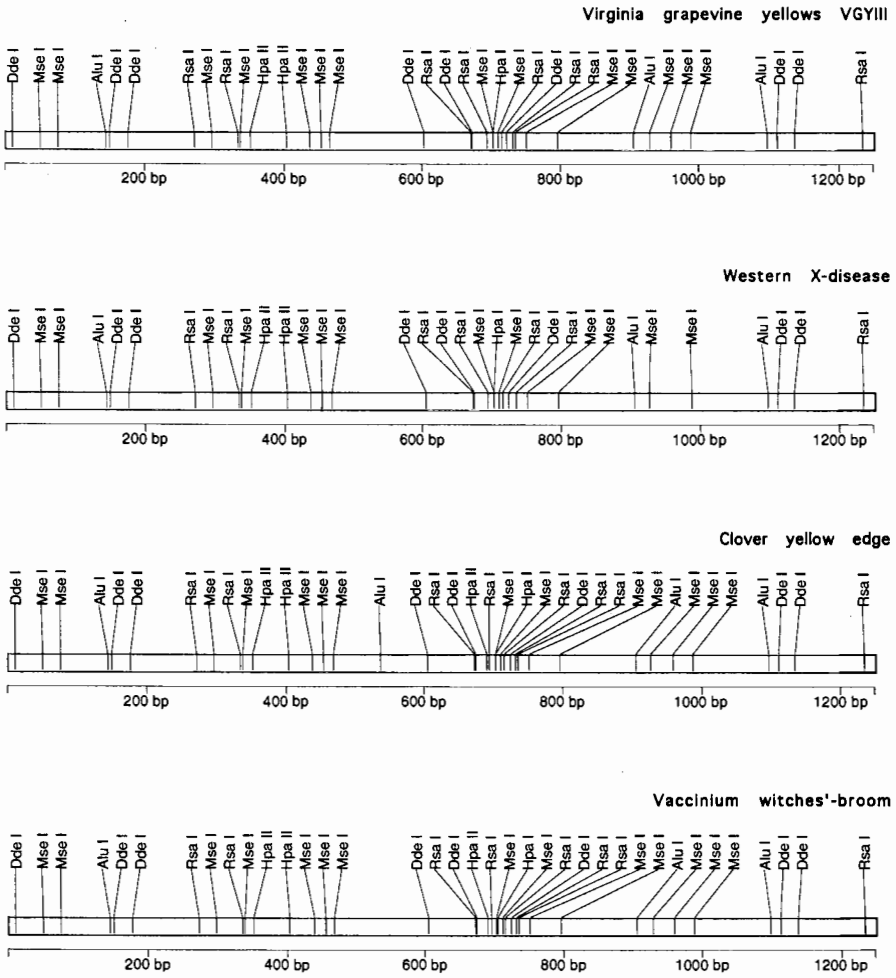

Fig. 5: Analysis of putative restriction sites of phytoplasma 16S rRNA gene sequences. Maps were generated by using the MapDraw option of the DNASTAR program (DNASTAR Inc., Madison, Wis.) and were manually aligned for comparison of recognition sites for restriction endonucleases *Alu* I, *Dde* I, *Hha* I, *Hpa* I, *Hpa* II, *Mse* I, and *Rsa* I. Virginia grapevine yellows phytoplasma VGYIII (GenBank Accession no. AFO60875), Western X-disease phytoplasma (GenBank Accession no. L04682), Clover yellow edge phytoplasma (GenBank Accession no. L33766), Vaccinium witches'-broom phytoplasma (GenBank Accession no. X76430).

striction sites were in excellent agreement with fragment sizes obtained by enzymic RFLP analysis of amplified 16S rDNA. Sequences from Western X-disease and clover yellow edge phytoplasmas, representatives of subgroups 16SrIII-A and 16SrIII-B, respectively, and of Vaccinium witches'-broom phytoplasma, a member of subgroup 16SrIII-B, were distinguished from Virginia grapevine yellows phytoplasma VGYIII by the restriction site analysis.

Discussion

The use of molecular criteria, particularly the analysis of conserved gene sequences, has permitted the development of a comprehensive phytoplasma classification system (Lee *et al.* 1993, 1998; Gundersen *et al.* 1994; Seemüller *et al.* 1994) and an emerging phytoplasma taxonomy. The recognition of 16S rRNA gene groups and some subgroups as distinct species is giving rise to the designation of „*Candidatus Phytoplasma* species". Thus far, two have been named, „*Candidatus Phytoplasma aurantifolia*" (Zreik *et al.* 1995) and „*Candidatus Phytoplasma australiense*" (Davis *et al.* 1997 a). Most phytoplasmas have not yet been named as „*Candidatus* species", and group and subgroup designations continue to be highly useful in the current molecular systematics. In particular, subgroup classification of the phytoplasma strains infecting *Vitis* species may have significance for understanding the epidemiology of grapevine yellows. This communication reports detection, identification, and classification of subgroup 16SrI-A and 16SrIII-I phytoplasmas infecting diseased grapevine plants in Virginia. Subgroup 16SrI-A has been previously described (Lee *et al.* 1993). Subgroup 16SrIII-I (III-I) is a newly described subgroup. Neither of these subgroups has been previously determined to infect *Vitis* species.

Our classification of the group 16SrI Virginia grapevine yellows phytoplasma in subgroup I-A expands the known plant host range of members of this subgroup and sheds new light on grapevine yellows in North America. While some phytoplasma strains infecting cultivated grapevine (*V. vinifera*) and wild grapevine (*Vitis* sp.) plants in Virginia were previously identified as members of group 16SrI, their

subgroup affiliation was not reported (PRINCE *et al.* 1993, 1994). Subgroup 16SrI-A (tomato big bud [BB] and related phytoplasmas) is known only in North America and is distinct from subgroup 16SrI-B (aster yellows and related phytoplasmas) (LEE *et al.* 1993). While subgroup 16SrI-B is known in North America, it has not been reported in *Vitis* spp. in North America, although it has been reported in *V. vinifera* in Europe (ALMA *et al.* 1996). The present communication reports the first work to document that both cultivated and wild grapevine plants are infected by subgroup 16SrI-A strains. Distinction of the group 16SrI Virginia grapevine yellows phytoplasma at 16S rRNA subgroup level distinguishes this phytoplasma from others known to infect *Vitis* species and provides new data that should aid the search for insect vectors and alternate plant hosts that play a role in its spread in Virginia. Subgroup 16SrI-A has a wide plant host range, including wild species native to Virginia and the surrounding region. Subgroup 16SrI-A (I-A) phytoplasmal infections of wild grapevine, gray dogwood (*Cornus racemosa* Lam.), fleabane (*Erigeron canadensis* L.), goldenrod (*Solidago* spp.), and other plants (LEE *et al.* 1993, 1994 a, b; GRIFFITHS *et al.* 1994; this paper) point to several possible sources of subgroup I-A phytoplasma strains that may be transmitted to cultivated grapevines.

While group 16SrIII phytoplasma strains have been reported in *V. vinifera* and *V. riparia* in Virginia (PRINCE *et al.* 1993, 1994), subgroup affiliation of such strains has not previously been reported. Although other natural plant hosts of group 16SrIII phytoplasmas are known in the region of eastern North America including Virginia (LEE *et al.* 1993; GUNDERSEN *et al.* 1996), no plant host other than *V. vinifera* (this paper) has been shown to harbor phytoplasma strains belonging to subgroup III-I.

Other work has also indicated *V. riparia* as an alternate plant host of phytoplasmas that may infect cultivated *V. vinifera*. CHEN *et al.* (1993) detected a phytoplasma in *V. riparia* growing near yellows-diseased cultivated grapevines in New York. On the basis of serological reactions and dot blot hybridizations, they found that this phytoplasma was related to an unidentified grapevine yellows phytoplasma from northern Italy. Based on available data, it is not known whether the phytoplasma reported by CHEN *et al.* (1993) in *V. riparia* in New York is related to either of the phytoplasmas in *Vitis* species in Virginia (this paper). MAIXNER *et al.* (1993) reported that *S. titanus* collected from *V. riparia* and *V. vinifera* in New York contained an antigen that was serologically related to flavescence dorée phytoplasma on the basis of results from enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy using polyclonal antiserum. Plants of *Vicia faba* L., but not plants of *V. vinifera*, that had been fed upon by such insects developed symptoms typical of infection by a phytoplasma. Although MAIXNER *et al.* (1993) did not identify the phytoplasma antigen presumably acquired by *S. titanus* from *V. riparia* and *V. vinifera*, their work also points to wild *V. riparia* as a possible source of phytoplasma(s) that may infect cultivated grapevine.

In addressing the question of possible relationships between grapevine yellows in North America and grapevine yellows diseases in other parts of the world, it is important to note that neither of the grapevine-infecting phytoplasmas in Virginia has been reported outside of North America. Conversely, no phytoplasma associated with flavescence dorée disease or bois noir disease in Europe, or with Australian grapevine yellows disease, has been reported in North America. Although *S. titanus* in New York was reported to carry a phytoplasma serologically related to the flavescence dorée phytoplasma in France, the published data (MAIXNER *et al.* 1993) do not make it possible to identify the phytoplasma or to classify it in any 16S rRNA group.

While the available data do not refute the hypothesis of a North American origin of flavescence dorée phytoplasma as originally proposed by CAUDWELL (1983), there is no evidence that this phytoplasma, which is a member of group 16SrV (elm yellows and related phytoplasmas, *sensu* LEE *et al.* 1993; PRINCE *et al.* 1993), occurs anywhere in North America. To date, subgroup 16SrV-A (V-A) is the only subgroup of group 16SrV thus far reported in North America, although differences in ribosomal and nonribosomal DNA have been noted among group V strains (GRIFFITHS *et al.* 1993). By contrast, subgroups V-A (elm yellows), V-C (Rubus stunt), and V-D (flavescence dorée) phytoplasmas have been reported in Europe (PRINCE *et al.* 1993; BIANCO *et al.* 1996; GUNDERSEN and LEE 1996), and subgroup V-B (cherry lethal yellows and related phytoplasmas) has been reported in China (LEE *et al.* 1995). We suggest that the known lethality of elm yellows phytoplasma for American elm (*Ulmus americana* L.; SINCLAIR 1981) favors the hypothesis that, although variants of the phytoplasma may have arisen on this continent, elm yellows phytoplasma and its American elm host did not co-evolve and that an elm yellows phytoplasma was introduced into the North American elm ecosystem. It is feasible that other group 16SrV phytoplasmas also originated in geographical regions outside the range of the native North American elm, *U. americana*. In view of the apparent diversity of group 16SrV phytoplasmas on the European and Asian continents, it is possible that the epicenter of group 16SrV phytoplasma evolution is in Europe or Asia. These hypotheses and the available data make it reasonable to seek the origin of group 16SrV phytoplasmas, including flavescence dorée phytoplasma, outside of North America.

Acknowledgements

This work was supported by grant no. US-2335-93 from BARD, the United States-Israel Binational Research and Development Fund.

References

ALMA, A.; DAVIS, R. E.; VIBIO, M.; DANIELLI, A.; BOSCO, D.; BERTACCINI, A.; 1996: Mixed infection of grapevines in northern Italy by phytoplasmas including 16S rRNA RFLP subgroup 16SrI-B strains previously unreported in this host. Plant Dis. **80**, 418-421.

BIANCO, P. A.; DAVIS, R. E.; CASATI, P.; FORTUSINI, A.; 1996: Prevalence of aster yellows (AY) and elm yellows (EY) group phytoplasmas in symptomatic grapevines in three areas of northern Italy. Vitis **35**, 195-199.

CAUDWELL, A.; 1983: L'origine des jaunisses à mycoplasmes (MLO) des plantes et l'exemple des jaunisses de la vigne. Agronomie **3**, 103-111.

- -; 1990: Epidemiology and characterization of Flavescence dorée (FD) and other grapevine yellows. Agronomie **10**, 655-663.

- -; 1993: Advances in grapevine research since 1990. 11th Meet. Council Study of Viruses and Virus Dis. Grapevine, 79-83, 6-9 September 1993. Montreux, Switzerland.

CHEN, K. H.; GUO, J. R.; WU, X. Y.; LOI, N.; CARRARO, L.; GUO, Y. H.; CHEN, Y. D.; OSLER, R., PEARSON, R.; CHEN, T. A.; 1993: Comparison of monoclonal antibodies, DNA probes, and PCR for detection of the grapevine yellows disease agent. Phytopathology **83**, 915-922.

DAIRE, X.; CLAIR, D.; LARRUE, J.; BOUDON-PADIEU, E.; CAUDWELL, A.; 1993: Diversity among mycoplasma-like organisms inducing grapevine yellows in France. Vitis **32**, 159-163.

DAVIS, R. E.; DALLY, E. L.; GUNDERSEN, D. E.; LEE, I. M.; HABILI, N.; 1997 a: „*Candidatus Phytoplasma australiense*", a new phytoplasma taxon associated with Australian grapevine yellows. Intern. J. System. Bacteriol. **47**, 262-269.

- -; - -; TANNE, E.; RUMBOS, I. C.; 1997 b: Phytoplasmas associated with grapevine yellows in Israel and Greece belong to the stolbur phytoplasma subgroup, 16SrXII-A. J. Plant Pathol. **79**, 181-187.

- -; JOMANTIENE, R.; DALLY, E. L.; WOLF, T. K.; 1998: Grapevine yellows in Virginia: Phytoplasma subgroups III-I and I-A in cultivated grapevines and subgroup I-A in wild grapevines. Phytopathology (in press).

- -; PRINCE, J. P.; 1993: Grapevine yellows diseases: Diverse etiologies indicated by new DNA-based methods for pathogen detection and identification-implications for epidemiology. 11th ICVG Meeting, 93-94. Ort ??.

GRIFFITHS, H. M.; SINCLAIR, W. A.; DAVIS, R. E.; LEE, I. M.; DALLY, E. L.; GUO, Y. H.; CHEN, T. A.; HIBBEN, C. R.; 1994: Characterization of mycoplasmalike organisms from Fraxinus, Syringa, and associated plants from geographically diverse sites. Phytopathology **84**, 119-126.

- -; - -; LEE, I. M; BOUDON-PADIEU, E.; DAIRE, X.; SMART, C.; 1993: Implications of differences between elm-inhabiting phytoplasmas and others in 16S RNA group V. Europ. J. Forest. Pathol. **28**, 79.

- -; - -; - -; DAVIS, R. E.; 1994: Characterization of mycoplasmalike organisms detected in plants in the vicinity of natural outbreaks of ash yellows and elm yellows in North America. IOM Letters 3, 259-260.

GUNDERSEN, D. E.; LEE, I. M.; 1996: Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol. Medit. **35**, 144-151.

- -; - -; REHNER, S. A.; DAVIS, R. E.; KINGSBURY, D. T.; 1994: Phylogeny of mycoplasmalike organisms (phytoplasmas): A basis for their classification. J. Bacteriol. **176**, 5244-5254.

- -; - -; SCHAFF, D. A.; HARRISON, N. A.; CHANG, C. J.; DAVIS, R. E.; KINGSBURY, D. T.; 1996: Genomic diversity and differentiation among phytoplasma strains in 16S rRNA groups I (aster yellows and related phytoplasmas) and III (X-disease and related phytoplasmas). Intern. J. System. Bacteriol. **46**, 64-75.

JOMANTIENE, R.; DAVIS, R. E.; MAAS, J.; DALLY, E. L.; 1998: Classification of new phytoplasmas associated with diseases of strawberry in Florida, based on analysis of 16S rRNA and ribosomal protein gene operon sequences. Intern. J. Syst. Bacteriol. **48**, 269-277.

LEE, I. M.; GUNDERSEN-RINDAL, D. E.; DAVIS, R. E.; BARTOSZYK, I. M.; 1998: Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Intern. J. System. Bacteriol. (in press).

- -; GUNDERSEN, D. E.; HAMMOND, R. W.; DAVIS, R. E.; 1994 a: Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology **84**, 559-566.

- -; - -; HAMMOND, R. W.; DAVIS, R. E.; BERTACCINI, A.; VIBIO, M.; 1994 b: Nested-PCR assays detect mixed-MLO infections in a single host plant. IOM Letters **3**, 263-264.

- -; HAMMOND, R. W.; DAVIS, R. E.; GUNDERSEN, D. E.; 1993: Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology **83**, 834-842.

- -; ZHU, S.; GUNDERSEN, D. E.; ZHANG, C.; HADIDI, A.; 1995: Detection and identification of a new phytoplasma associated with cherry lethal yellows in China. Phytopathology **85**, 1179.

LIM, P. O.; SEARS, B. B.; 1992: Evolutionary relationships of a plant pathogenic mycoplasmalike organism and Acholeplasma laidlawii deduced from two ribosomal protein gene sequences. J. Bacteriol. **174**, 2606-2611.

MAGAREY, P. A.; WACHTEL, M. F.; 1986: Australian grapevine yellows. Intern. J. Trop. Plant Dis. **4**, 1-14.

MAIXNER, M.; AHRENS, U.; SEEMÜLLER, E.; 1995: Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Europ. J. Plant Pathol. **101**, 241-250.

- -; PEARSON, R. C.; BOUDON-PADIEU, E.; CAUDWELL, A.; 1993: *Scaphoideus titanus*, a possible vector of grapevine yellows in New York. Plant Dis. **77**, 408-413.

- -; RÜDEL, M.; DAIRE, X.; BOUDON-PADIEU, E.; 1995: Diversity of grapevine yellows in Germany. Vitis **34**, 235-236.

PEARSON, R. C.; POOL, R. M.; GONSALVES, D.; GOFFINET, M. C.; 1985: Occurrence of flavescence dorée-like symptoms on 'White Riesling' grapevines in New York, U.S.A. Phytopathol. Medit. **24**, 82-87.

PRINCE, J. P.; DAVIS, R. E.; WOLF, T. K.; LEE, I. M.; DALLY, E. L.; 1994: Genomic diversity and possible wild plant sources of mycoplasmalike organisms (MLOs) infecting grapevines: Implications for epidemiology. IOM Letters **3**, 288.

- -; - -; - -; - -; MOGEN, B. D.; DALLY, E. L.; BERTACCINI, A.; CREDI, R.; BARBA, M.; 1993: Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. Phytopathology **83**, 1130-1137.

SEEMÜLLER, E.; SCHNEIDER, B.; MÄURER, R.; AHRENS, U.; DAIRE, X.; KISON, H.; LORENZ, K. H.; FIRRAO, G.; AVINENT, L.; SEARS, B. B.; STACKEBRANDT, E.; 1994: Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. Intern. J. System. Bacteriol. **44**, 440-446.

SINCLAIR, W. A.; 1981: Elm yellows. In: STIPES, R. J.; CAMPANA, R. J. (Eds.): Compendium of Elm Diseases, 25-31. American Phytopathological Society, St. Paul, MN, USA.

TANNE, E.; ORENSTEIN, S.; 1997: Identification and typing of grapevine phytoplasma amplified by graft transmission to periwinkle. Vitis **36**, 35-38.

UYEMOTO, J. K.; 1976: A new disease affecting the grapevine variety de Chaunac. Proc. Amer. Phytopathol. Soc. **1**, 146.

VIBIO, M.; BERTACCINI, A.; LEE, I. M.; DAVIS, R. E.; CLARK, M. F.; 1996: Differentiation and classification of aster yellows and related European phytoplasmas. Phytopathol. Medit. **35**, 33-42.

WOLF, T. K.; PRINCE, J. P.; DAVIS, R. E.; 1994: Occurrence of grapevine yellows in Virginia vineyards. Plant Dis. **78**, 208.

ZREIK, L.; CARLE, P.; BOVÉ, J. M.; GARNIER, M.; 1995: Characterization of the mycoplasmalike organism associated with witches'-broom disease of lime and proposition of a Candidatus taxon for the organism, „*Candidatus Phytoplasma aurantifolia*." Intern. J. System. Bacteriol. **45**, 449-453.

Received May 29, 1998