Transmission of 16SrV Phytoplasmas by *Scaphoideus titanus* Ball in Northern Italy

PIERO ATTILIO BIANCO[1], ALBERTO ALMA[2], PAOLA CASATI[3], GIORGO SCATTINI[1] and ALESSANDRA ARZONE[2]

[1]*Centro CNR per il Miglioramento Sanitario delle Colture Agrarie, Milano;* [2]*Di.Va.P.R.A. – Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano", Università degli Studi, Torino;* [3]*Istituto di Patologia Vegetale, Università degli Studi, Milano, Italy*

Abstract

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Flavescence dorée (FD) has been defined as the disease that is caused by a phytoplasmas belonging to group 16SrV (elm yellows) and transmitted to plants by the insect *Scaphoideus titanus* Ball. We investigated transmission of FD agent by *S. titanus* in a vineyard located in Veneto region to determine which phytoplasma(s) may be transmitted in this region. Group 16SrV-C phytoplasma was detected and identified in field-collected *S. titanus* adults, in plant of grapevine (*Vitis vinifera* L. cv. Gamay) and broadbean (*Vicia faba* cv. Arlà) that had been fed upon by the insects. No evidence of experimental transmission of phytoplasma belonging to 16SrI-B subgroup phytoplasma by *S. titanus* has been observed.

Keywords: Flavescence dorée; grapevine yellows; *Scaphoideus titanus*; transmission; PCR

Flavescence dorée (FD) disease is considered to be the epidemic form of grapevine yellows (GY), transmitted by the leafhopper *Scaphoideus titanus* Ball and caused by phytoplasmas belonging to the elm yellows (EY) group (ANONYMOUS 1991; CAUDWELL 1993).

After the first record of the disease in France (CAUDWELL 1957) and in Italy (BELLI *et al.* 1973) experimental transmission tests demonstrated the ability of *S. titanus* to transmit FD disease (CAUDWELL *et al.* 1971a; FORTUSINI *et al.* 1989; CARRARO *et al.* 1994). In other experiments the same insect failed to transmit other forms of GY (MAIXNER *et al.* 1993; OSLER *et al.* 1992). Thus, experimental transmission to *Vitis vinifera* L. and *Vicia faba* L. by use of *S. titanus*, has been applied to detect FD and distinguish it from Bois Noir (BN), a less epidemic form of GY (CAUDWELL *et al.* 1971b).

Recently the use of molecular biology tools allowed to detect and classify phytoplasmas on the basis of the analysis of their 16S ribosomal RNA (16S rRNA) gene polymorphism (AHRENS & SEEMÜLLER 1992; LEE *et al.* 1993, 1998; NAMBA *et al.* 1993). So far in Italy, at least five genetically different phytoplasmas were detected and identified in grapevine (DAVIS *et al.* 1993, 1997; BIANCO *et al.* 1993, 1996a; DAIRE *et al.* 1993; ALBANESE *et al.* 1995; BERTACCINI *et al.* 1995; ALMA *et al.* 1996). In particular, a survey, conducted in several provinces of northern Italy including Vicenza, reported the presence of two genetically distinct phytoplasmas belonging to 16SrV (elm yellows and related phytoplasmas); one of these (subgroup C, type strain) was found to be very similar to the causal agent of FD in France (BIANCO *et al.* 1996b).

The beginning of the 1980's, a severe outbreak of FD together with high populations of *S. titanus* were observed in Vicenza province (BELLI *et al.* 1983, 1985). Since phytoplasmas belonging to different groups and subgroups have been identified in grapevines exhibiting symptoms characteristic of FD in northern Italy, this research was initiated to determine which phytoplasma *S. titanus* may harbour and/or transmit in this region.

MATERIAL AND METHODS

Experiments were begun during summer 1996 in a vineyard located in the province of Vicenza where grapevine plants, showing typical symptoms of GY, were widely distributed; in addition previous analyses conducted in the same vineyard revealed the presence of three different phytoplasma groups. Two of them belong to 16SrV group

(16SrV-A and 16SrV-C) (BIANCO *et al.* 1996b; LEE *et al.* 1998), while the third one belongs to the 16SrXII group (subgroup A) (DAVIS *et al.* 1997). No samples were found to be infected by phytoplasmas belonging to 16SrI-B subgroup (BIANCO *et al.* 1996a; BIANCO & CASATI, unpublished). In this vineyard, 150 *S. titanus* adults (60 on July 25th and 90 on August 20th) were collected from symptomatic grapevines using a sweeping net.

Transmission Tests. 31 grapevine seedlings obtained from grapevine seeds of cv. Gamay and 31 seedlings of *V. faba* were used for the experiments.

In July, 18 grapevine plants were inoculated using three *S. titanus* adults for each plant. After one week the alive adults were transferred to 18 broadbean seedlings for a second inoculation period of one week.

In August, separated and parallel tests had been carried out using the remnant 13 grapevine and 13 broadbean seedling plants. Groups of three just collected *S. titanus* adults were used adopting the same protocol as described for the trial conducted in July.

At the end of the two transmission experiments, the living insects were captured, immediately frozen and stored at –30°C until total nucleic acid extraction.

After inoculation, the tested plants were sprayed with an insecticide and maintained in greenhouse at 20–28°C. Disease symptoms were weekly checked.

Healthy *S. titanus* adults, reared on a seedling grapevine (cv. Barbera) in screenhouse, were included in the two experiments as healthy control and put on ten grapevine and ten broadbean seedlings (always three adults per plant). Transmission tests were conducted in greenhouse.

PCR and RFLP Analyses. At the end of every inoculation, total nucleic acids were extracted from each insect collected alive (114 adult insects), according to VEGA *et al.* (1993) with some modifications. The insects were singly placed in 1.7 ml Eppendorf tube and macerated in 400 µl of extraction buffer (100 mM Tris, 50 mM EDTA, 500 mM NaCl, pH 8) plus 2 µl of mercaptoethanol, 20 µl of 20% SDS (sodium dodecyl sulfate) and 40 mg of sterile sand (white quartz). The solution was carefully homogenized with a plastic sterilized minipestle, centrifuged at 550 g for 10 min. The supernatant was collected and transferred into a new tube. The pellet was further centrifuged at 5600 g: the supernatants were combined, heated at 65°C for 5 min, and centrifuged at 15 000 g for 10 min: 200 µl of chloroform-isoamyl alcohol and 200 µl of TE saturated phenol were added to the supernatant. Then, cold absolute ethanol (2.5 volumes) was used to precipitate nucleic acids; after centrifugation at 15 000 g the pellet was washed with 70% ethanol, centrifuged and resuspended in 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8). One µl of 50 folds diluted DNA solution, for each sample, was included in PCR mixture reaction and further analyzed by RFLP tests as below described.

Two months after inoculation, 2 symptomatic grapevine (No. 1, Table 1; No. 1G, Table 2) and 2 broadbean plants were sampled. In addition, on the basis of the results of PCR tests conducted on the insect samples, selected DNA samples extracted from asymptomatic grapevine plants Nos. 2, 7, 8 (Table 1) and asymptomatic broadbean plant No. 3B (Table 2) were tested: 100–200 mg of leaf tissues were used for the total nucleic acid extraction.

PCR tests were conducted as previously reported (SCHAFF *et al.* 1992) in a total volume of 25 µl. The primer pair 16SrF2n/16SrR2 (abbreviated 16SrF2n/R2), designed for universal amplification of 16SrDNA gene from all known phytoplasmas (LEE *et al.* 1993), was used for a first round of PCR tests. A second round of PCR test (nested-PCR) was conducted for all the examined samples, using primer pairs designed for specific amplification of aster yellows and stolbur (R16[I]F1/R1)(DAVIS *et al.* 1997), and elm yellows (R16[V]F1/R1) phytoplasma groups (LEE *et al.* 1994).

Control reference strains of aster yellows (strain AY1, 16SrI-B), elm yellows (strain EY1, 16SrV-A), and 16SrXII-A (Strain Italian Periwinkle) phytoplasmas were as previously described (PRINCE *et al.* 1993). The reference strains were maintained by grafting in a white-flowered clone of periwinkle (*Catharanthus roseus* [L] G. Don) plants.

RFLP analyses using *Bfa*I, *Mse*I (or *Tru*9), and *Taq*I were conducted by single enzyme digestions of products from PCR primed by R16[I]F1/R1, R16[V]F1/R1 or 16SrF2n/R2 primer pairs; the fragments obtained were separated by electrophoresis through 5.5% polyacrylamide gel, DNA bands are stained in ethidium bromide and visualized by means of UV transilluminator.

RESULTS

Transmission Tests. Tables 1 and 2 report the results of the transmission tests conducted respectively in July and in August using field collected *S. titanus* adults. Rolling and reddening of the leaves followed by tip necrosis were observed in grapevine two months after inoculation, while leaf roll and shortening of the stems were evident in broadbean forty days after inoculation. These symptoms were observed on 6 grapevine plants and 6 broadbean seedlings within the group inoculated in July (Table 1) and on 5 grapevine plants and 7 broadbean seedlings within the group inoculated in August (Table 2). No symptoms were observed on the ten grapevine and ten broadbean seedlings that were fed upon by healthy *S. titanus* adults.

PCR and RFLP Analyses. Tables 1 and 2 also report the results of the tests conducted on the DNA samples extracted from *S. titanus* adults: 19 of them were found to be infected by phytoplasmas in July and 25 in August. In particular phytoplasmas in a 16SrV group were found in 16 of 44 insects used in July and in 24 of 70 insects in

Table 1. Results of the transmission tests conducted in July using *Scaphoideus titanus* adults

Experiment	Phytoplasmas detected in *S. titanus*	Symptoms on		Experiment	Phytoplasmas detected in *S. titanus*	Symptoms on	
		grapevine	broadbean			grapevine	broadbean
1	EY-FD(a) EY-FD – (dead)	+ (b)	+	10	0 0 0	0	0
2	AY-B EY-FD 0	0	0	11	0 0 0	0	0
3	EY-FD EY-FD – (dead)	+	+	12	EY-FD – (dead) – (dead)	+	+
4	EY-FD 0 0	0	0	13	0 0 0	0	0
5	0 0 0	0	0	14	EY-FD EY-FD – (dead)	+	+
6	0 0 0	0	0	15	0 0 – (dead)	0	0
7	AY-B EY-FD 0	0	0	16	EY-FD 0 – (dead)	+	+
8	AY-B EY-FD EY-FD	0	0	17	EY-FD 0 – (dead)	+	–
9	0 0 – (dead)	0	0	18	EY-FD EY-FD 0	0	+

(a) EY-FD = 16SrV-C phytoplasma subgroup; AY-B = 16SrI-B phytoplasma subgroup
(b) + = evident symptoms; 0 = no phytoplasmas detected or no symptoms observed; – = not tested

August; a representative example of PCR tests is reported in Fig. 1. In the same tests, 4 DNA insect samples (3 samples in July and 1 sample in August) positively reacted in PCR tests using R16[I]F1/R1 primer pair.

Concerning the PCR tests conducted on the inoculated plants, 2 grapevine and 2 broadbean seedlings were infected by phytoplasmas belonging to the EY group (Fig. 2).

No positive results were obtained with DNA samples extracted from plants inoculated by *S. titanus* adults containing phytoplasmas belonging to 16SrI group (exp. No. 2, No. 7 and No. 8, Table 1; exp. No. 3B, Table 2): the same results were obtained when additional PCR tests were conducted, on the grapevine plants, six months and twelve months after the inoculation trial.

No amplification, also, was obtained with DNA samples from grapevine and broadbean plants inoculated by healthy *S. titanus*.

The subsequent enzymatic digestion tests, conducted with *Taq*I and *Mse*I restriction enzymes on all the 16SrV amplicons, revealed the identity between the pattern profiles of the reference strain EY1 and those ones obtained from grapevine and broadbean samples (Fig. 3). Moreover further digestion tests, conducted with *Bfa*I restriction enzyme, showed that the DNA profiles of grapevine, *S. titanus* and broadbean samples were identical from each other and different from that one obtained after digestion of EY1 amplicon (Figs. 3 and 4).

Subgroup identification of these samples was conducted on the basis of the putative restriction enzyme analyses conducted on the 16S rDNA sequence gene (Mycoplasma, MLOFDNA transmitted from *V. vinifera* to *V. faba*; sequence accession No. X765607) (BIANCO *et al.* 1996b). Moreover we have not detected in *S. titanus* and in the inoculated plants, phytoplasmas belonging to 16SrV-A subgroup.

Table 2. Results of the transmission tests conducted in August using *Scaphoideus titanus* adults

Experiment	Phytoplasmas detected in *S. titanus*	Symptoms on grapevine	Experiment	Phytoplasmas detected in *S. titanus*	Symptoms on broadbean
1G	EY-FD(a) EY-FD EY-FD	+ (b)	1B	0 0 0	0
2G	0 EY-FD 0	+	2B	EY-FD 0 0	+
3G	0 0 0	0	3B	AY-B 0 0	0
4G	EY-FD EY-FD – (dead)	0	4B	0 0 – (dead)	0
5G	0 0 0	0	5B	0 EY-FD EY-FD	+
6G	EY-FD EY-FD EY-FD	+	6B	0 0 0	0
7G	0 0 – (dead)	+	7B	0 0 0	0
8G	0 0 0	0	8B	EY-FD 0 0	+
9G	0 – (dead) – (dead)	0	9B	EY-FD 0 0	+
10G	EY-FD 0 0	0	10B	EY-FD EY-FD EY-FD	+
11G	EY-FD EY-FD – (dead)	+	11B	0 0 – (dead)	+
12G	EY-FD 0 0	0	12B	0 0 0	0
13G	0 0 – (dead)	0	13B	EY-FD EY-FD EY-FD	+

(a) EY-FD = 16SrV-C phytoplasma subgroup; AY-B = 16SrI-B phytoplasma subgroup
(b) + = evident symptoms; 0 = no phytoplasmas detected or no symptoms observed; – = not tested

In addition, when digested with *Mse*I restriction enzyme, the four 16SrI amplicons, obtained by group-specific amplification of *S. titanus* extracted DNAs, showed characteristic RFLP patterns of DNA from 16SrI-B phytoplasma subgroup (data not shown).

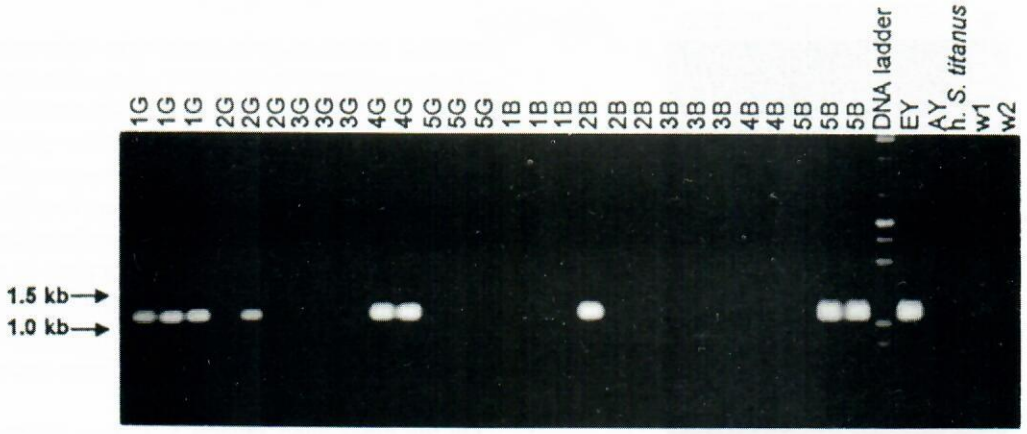

Fig. 1. Agarose gel of the nested-PCR amplification of phytoplasma 16S rDNA sequences of DNA templates extracted from *Scaphoideus titanus* adults used for transmission tests in August as reported in Table 2:

The 16SrF2n/16SrR2 (abbreviated 16SrF2n/R2) primer pair was used for a first round of PCR tests. A second round of group specific amplification (nested-PCR) was primed by R16[V]F1/R1 oligonucleotide pair.

Insect samples indicated as 1G, 2G, 3G, 4G and 5G represent DNA templates extracted from each *S. titanus* used for inoculation tests to grapevine plants: insect samples indicated as 1B, 2B, 3B, 4B and 5B represent DNA templates extracted from each *S. titanus* used for inoculation tests to broadbean plants. EY and AY are respectively the reference strains for elm yellows (EY1) and aster yellows (AY1) phytoplasma groups. Healthy *S. titanus* (h *S. titanus*) was included as healthy control; w1 and w2 are the controls of first round PCR (w1) and nested-PCR (w2)

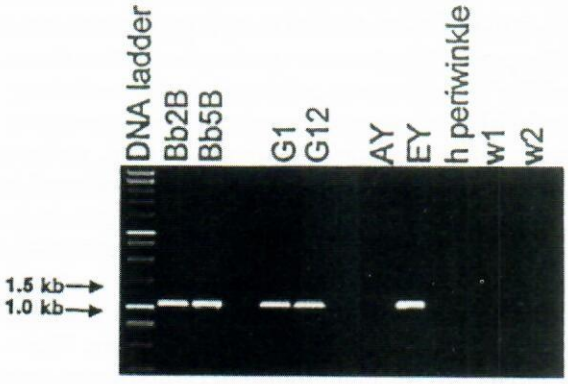

Fig. 2. Agarose gel of the nested-PCR amplification of phytoplasma 16S rDNA sequences of DNA templates extracted from inoculated broadbean (Bb2B and Bb5B) and grapevine (G1 and G12) plants (Table 2). First round PCR was conducted using 16SrF2n/R2 primer pair followed by nested-PCR primed by group specific primer pair (R16[V]F1/R1) for amplification of 16S rDNA sequences from phytoplasmas belonging to the 16SrV group. As controls, AY1 (aster yellows), EY1 (elm yellows), and healthy periwinkle (h periwinkle) were used; w1 and w2 were the water controls respectively for direct PCR (w1) and nested-PCR (w2)

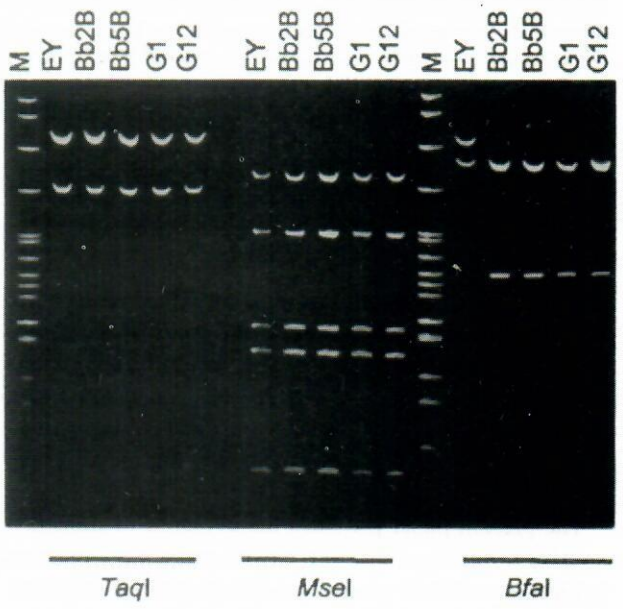

Fig. 3. Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA amplified of DNA templates extracted from inoculated broadbeans (Bb2B and Bb5B) and inoculated grapevine plants (G1 and G12) (Fig. 2). 16SrF2n/R2 primer pair was included in the reaction mixture for direct PCR. Group specific primer pair R16[V]F1/R1 was used in nested-PCR test. DNA products, obtained after the second round cycles of amplification, were separately digested with three different restriction endonucleases: *Taq*I, *Mse*I, and *Bfa*I; pBR322 DNA *Msp*I digest (M) was used as molecular marker weight (fragment sizes in base pairs from top to bottom: 622, 527, 404, 307, 242, 238, 217, 201, 190, 180, 147, 123, 110, 90, 76, 67, 34, 26, 15, 9)

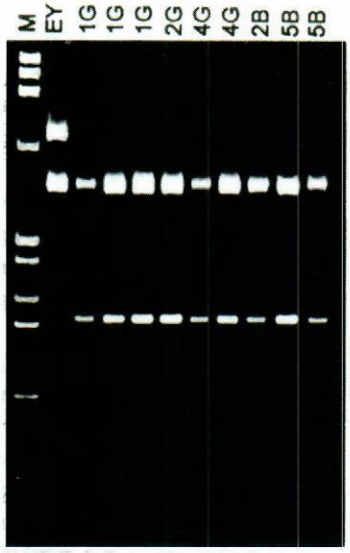

Fig. 4. Restriction fragment length polymorphism (RFLP) analysis of 16S rDNA amplified from DNA templates of the *Scaphoideus titanus* samples resulted positive by using 16S rDNA group-specific primer pair R16 [V]F1/R1 in nested-PCR after direct PCR of total DNA templates using R16SrF2n/R2 primer pair (Fig. 1). DNA products were separately digested with restriction endonuclease *Bfa*I; ØX174 RF 1 DNA *Hae*III digest (M) was used as molecular marker weight (fragment sizes in base pairs from top to bottom are 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72)

DISCUSSION

The ability of *S. titanus* to transmit FD disease pathogen was previously reported on the basis of the symptomatology observed on the inoculated plants (CAUDWELL *et al.* 1971a; FORTUSINI *et al.* 1989). Later, CARRARO *et al.* (1994), showed that this insect can transmit to grapevine phytoplasmas belonging to the group 16SrV (PRINCE *et al.* 1993).

The present paper provides the evidence of transmission by *S. titanus* of one of the two known phytoplasmas belonging to 16SrV group found in grapevine and it fulfills the definition of Flavescence dorée disease (ANONYMOUS 1991). In particular, it shows that *S. titanus* transmits the FD (group V, subgroup C = 16SrV-C) phytoplasma.

Moreover, the results reported in Tables 1 and 2 show that *S. titanus* may be considered an efficient vector of the FD phytoplasma in Italy. In fact, in most cases (7 out of 11 in July and 10 out of 13 in August), when *S. titanus* resulted to be infected by the FD phytoplasma, typical symptoms were observed in the inoculated plants. In other cases, also, a single infected adult was capable to transmit the disease to grapevine (Table 2, experiment No. 2G) and broadbean (Table 2, exp. No. 2B, No. 8B and No. 9B). Besides *S. titanus* did not acquire phytoplasmas belonging to the subgroup A of 16SrV group. Phytoplasmas in the 16SrV-A subgroup were previously detected in grapevine, often in double infection with other phytoplasmas (BIANCO *et al.* 1996b). However, the presence of the 16SrV-A phytoplasma in that vineyard was very low: also, further investigations are needed in order to evaluate the capability of this phytoplasma to cause symptoms in grapevine and to search for possible alternative plant host(s) of 16SrV-A. Since we never detected such phytoplasma in *S. titanus*, we could also suppose that a different vector may be involved in its spread in vineyard.

On the other hand several *S. titanus* failed to infect plants although they contained the FD phytoplasma: it is conceivable to suppose that in these cases (No. 2, No. 4, No. 7 and No. 8: Table 1; No. 4G, No. 10G and No. 12G: Table 2) the latent period of the transmission cycle was not completed yet, as probably occurred in Table 1 (experiment No. 18); although this grapevine plant did not become infected, the broadbean seedling, inoculated the successive week by the same insect batch, became infected.

On the basis of our results, *S. titanus* seems to be able to acquire the phytoplasma belonging to subgroup B of the group 16SrI (16SrI-B, type strain aster yellows phytoplasma), but unable to transmit it to test plants (*V. vinifera* and *V. faba*). Other authors, on the basis of serological tests, reported the ability of *S. titanus* to acquire phytoplasmas associated with GY in Italy (OSLER *et al.* 1992) and in New York (MAIXNER *et al.* 1993). BERTACCINI *et al.* (1993) detected phytoplasmas probably related to 16SrI-G (now renamed 16SrXII-A) using non ribosomal specific probes. Thus, in our experiments, we have shown the ability of *S. titanus* to acquire phytoplasmas belonging to 16SrI-B subgroup. Although this phytoplasma may naturally infect grapevine (ALMA *et al.* 1996), we have not detected it, so far, in grapevine samples collected in the Vicenza province during this and previous surveys (BIANCO *et al.* 1996b). In the light of the present findings we suggest the possibility that *S. titanus* may occasionally feed on alternative host plants, present in vineyards and infected by 16SrI-B phytoplasma subgroup. Therefore, further investigations should be carried out in order to evaluate its capability to transmit 16SrI-B phytoplasmas to grapevine plants.

Furthermore, in the same vineyard, several symptomatic grapevine plants were found to be infected by a phytoplasma belonging to the stolbur group (16SrXII-A, formerly named 16SrI-G) (DAVIS *et al.* 1997), but we have not detected this phytoplasma in *S. titanus*. Thus, considering earlier reports of the spread of phytoplasmas belonging to stolbur group in Vicenza province (BIANCO *et al.* 1996a), we may suspect the activity, in that area, of other vectors as reported for Vergilbungskrankheit in Germany (MAIXNER *et al.* 1995).

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References

AHRENS U., SEEMÜLLER E. (1992): Detection of DNA of plant pathogenic mycoplasma like organisms by a polymerase chain reaction that amplifies a sequence of the 16SrRNA gene. Phytopathology, **82**: 828–832.

ALBANESE G., DAVIS R.E., GRANATA G., DALLY E.L., SANTUCCIO T., TESSITORI M. (1995): DNA-based analyses to detect and identify phytoplasmas in yellows-diseased grapevines in Sicily. Petria, **6**: 65–76.

ALMA A., DAVIS R.E., VIBIO M., DANIELLI A., BOSCO D., ARZONE A., 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.

ANONYMOUS (1991): Round table discussion. In: Proc. 10th Meet. ICVG, Volos, Greece, 1990: 14–19.

BELLI G., FORTUSINI A., OSLER R., AMICI A. (1973): Presenza di una malattia del tipo "flavescence dorée" in vigneti dell'oltrepo' pavese. Riv. Patol. Vegetale, suppl. al vol. **9**: 50–56.

BELLI G., FORTUSINI A., RUI D., PIZZOLI L., TORRESIN G. (1983): Gravi danni da flavescenza dorata in vigneti di Pinot nel Veneto. L'Inform. Agrario, **XXXIX**: 24231–24433.

BELLI G., FORTUSINI A., RUI D. (1985): Recent spread of flavescence dorée and its vector in vineyards of Northern Italy. Phytopathol. Mediter., **24**:189–191.

BERTACCINI A., ARZONE A., ALMA A., BOSCO D., VIBIO M. (1993): Detection of mycoplasmalike organisms in *Scaphoideus titanus* Ball reared on flavescence dorée infected grapevine by hybridizations using DNA probes. Phytopathol. Mediter., **32**: 20–24.

BERTACCINI A., VIBIO M., STEFANI E. (1995): Detection and molecular characterization of phytoplasmas infecting grapevine in Liguria (Italy). Phytopathol. Mediter., **34**: 137–141.

BIANCO P.A., DAVIS R.E., PRINCE J.P., LEE I.M., GUNDERSEN D.E., FORTUSINI A., BELLI G. (1993): Double and single infections by aster yellows and elm yellows MLOs in grapevines with symptoms characteristic of Flavescence dorée. Riv. Patol. Veget., Ser. V, **3**: 69–82.

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

BIANCO P.A., CASATI P., DAVIS R.E., SCATTINI G. (1996b): Two different phytoplasmas belonging to group 16SrV may occur in grapevines affected by Flavescence dorée disease. IOM Letters, **4**: 192–193.

CARRARO L., LOI N., KUSZALA C., CLAIR D., BOUDON-PADIEU E., REFATTI E. (1994): On the ability-inability of *Scaphoideus titanus* Ball to transmit different grapevine yellows agents. Vitis, **33**: 231–234.

CAUDWELL A. (1957): Deux années d'études sur la flavescence dorée, nouvelle maladie grave de la vigne. Ann. Amél. Plant., **4**: 359–393.

CAUDWELL A. (1993): Advances in grapevine yellows research since 1990. Extended abstr. 11th Meet. ICVG, Montreux, Switzerland, 6–9 Sept. 1993: 79–83.

CAUDWELL A., GIANNOTTI J., KUSZALA C., LARRUE J. (1971a): Étude du rôle de particules de type "mycoplasme" dans l'étiologie de la flavescence dorée de la vigne. Examen cytologique des plantes malades et des cicadelles infectieuses. Ann. Phytopathol., **3**: 107–123.

CAUDWELL A., LARRUE J., KUSZALA C., BACHELIER J.C., (1971b): Pluralité des jaunisses de la vigne. Ann. Phytopathol., **3**: 95–105.

DAIRE X., CLAIR D., LARRUE J., BOUDON-PADIEU E., ALMA A., ARZONE A., CARRARO L., OSLER R., REFATTI E., GRANATA G., CREDI R., TANNE E., PEARSON R., CAUDWELL A. (1993): Occurrence of diverse MLOs in tissues of grapevine affected by grapevine yellows in different countries. Vitis, **32**: 247–248.

DAVIS R.E., DALLY E.L., BERTACCINI A., LEE I.M., CREDI R., OSLER R., SAVINO V., CARRARO L., DI TERLIZZI B., BARBA M. (1993): Restriction fragment length polymorphism analyses and dot hybridizations distinguish mycoplasmalike organisms associated with flavescence dorée and southern European grapevine yellows disease in Italy. Phytopathology, **83**: 772–776.

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

FORTUSINI A., SARACCHI M., BELLI G. (1989): Trasmissione sperimentale della Flavescenza dorata della vite mediante *Scaphoideus titanus* Ball in Italia. Vignevini, **9**: 43–46.

LEE I.M., HAMMOND R.W., DAVIS R.E., GUNDERSEN D.E. (1993): Universal amplification and analysis of pathogen 16SrDNA for classification and identification of mycoplasmalike organisms. Phytopathology, **83**: 834–842.

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

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. Int. J. Syst. Bacteriol., **42**: 226–233.

MAIXNER M., 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.

MAIXNER M., AHRENS U., SEEMÜLLER E. (1995): Detection of the German grapevine yellows (Vergilbungs-

krankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Eur. J. Plant Pathol., **101**: 241–250.

NAMBA S., KATO S., IWANAMI S., OYAIZU H., SHIOZAWA H., TSUCHIZAKI T. (1993): Detection and differentiation of plant-pathogenic mycoplasmalike organisms using polymerase chain reaction. Phytopathology, **83**: 786–791.

OSLER R., BOUDON-PADIEU E., CARRARO L., CAUDWELL A., REFATTI E. (1992): First results on the trials in progress to identify the vector of the agent of a Grapevine yellows in Italy. Phytopathol. Mediter., **31**: 175–181.

PRINCE J.P., DAVIS R.E., WOLF T.K., LEE I.M., 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.

SCHAFF D., LEE I.M., DAVIS R.E. (1992): Sensitive detection and identification of Mycoplasma-like Organisms in plants by polymerase chain reactions. Biochem., Biophys. Res. Commun., **186**: 1503–1509.

VEGA F.E., DAVIS R.E., BARBOSA P., DALLY E.L., PURCELL A.H., LEE I.M. (1993): Detection of a plant pathogen in a nonvector insect species by the polymerase chain reaction. Phythopathology, **83**: 621–624.

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Souhrn

BIANCO P. A., ALMA A., CASATI P., SCATTINI G., ARZONE A. (2001): **Přenos fytoplazem skupiny 16SrV křístem** *Scaphoideus titanus* Ball **v severní Itálii**. Plant Protect. Sci., **37**: 49–56.

Onemocnění Flavescence doreé (FD) bylo definováno jako choroba působená fytoplazmami, které patří do skupiny 16SrV (žloutenka jilmu) a jsou přenášeny hmyzem *Scaphoideus titanus* Ball. Studovali jsme přenos agens FR křísem *S. titanus* ve vinohradu v oblasti Veneto, abychom zjistili, které fytoplazmy mohou být v této oblasti přenášeny. Fytoplazma za skupiny 16SrV-C byla detekována a identifikována v dospělcích *S. titanus* odchycených na poli v rostlinách révy vinné (*Vitis vinifera* L. cv. Gamay) a bobu (*Vicia faba* L. cv. Arlà), na nichž křísti sáli. Experimentální přenos fytoplazmy náležející do skupiny 16SrI-B křístem *S. titanus* nebyl prokázán.

Klíčová slova: Flavescence doreé; žloutenka révy vinné; *Scaphoideus titanus*; přenos; PCR

Corresponding author:

Dr. PIERO ATTILIO BIANCO, Centro CNR per il Miglioramento Sanitario delle Colture Agrarie, c/o Istituto di Patologia Vegetale, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy, tel.: + 39 2 23 69 11 42, fax: + 39 2 70 63 12 87, e-mail: piero.bianco@unimi.it