Vitis **33**, 231–234 (1994)

On the ability-inability of *Scaphoideus titanus* Ball to transmit different grapevine yellows agents*)

by

L. Carraro[1)], N. Loi[1)], C. Kuszala[2)], D. Clair[2)], E. Boudon-Padieu[2)] and E. Refatti[1)]

[1)] Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi di Udine, Italia
[2)] Station de Recherches sur les Mycoplasmes des Plantes, INRA, Dijon, France

S u m m a r y : In a six-year transmission trial using *Scaphoideus titanus* Ball the results were negative when the source of inoculum was symptomatic plants from vineyards of the Friuli-Venezia Giulia region. Transmissions were positive when the insects were fed on Perera symptomatic grapevines of the Valdobbiadene area (Veneto region). The data confirm that different types of grapevine yellows (GY) are present in the grapevine areas where we have been working. ELISA and PCR analyses corroborated that transmissions by *S. titanus* were positive only when the Flavescence dorée *sensu stricto* type of GY is present.

K e y w o r d s : MLO, grapevine yellows, Scaphoideus titanus Ball, transmission, diagnosis.

Introduction

Flavescence dorée (FD), one of the grapevine yellows (GY), was reported for the first time in France (Caudwell 1957), where it did considerable damage in Gascony in the years 1955-1965. Schvester *et al.* (1961) proved that the agent of the disease was transmitted by the leafhopper *Scaphoideus littoralis* Ball (= *S. titanus* Ball). Giannotti *et al.* (1969) and Caudwell *et al.* (1971) demonstrated that FD was caused by a mycoplasma-like organism (MLO).

In 1961, Caudwell reported the presence in the north-eastern grapevine areas of France of a disease called Bois noir (BN), symptomatologically indistinguishable from FD but not transmissible by *S. titanus*. An analogous disease, named Vergilbungskrankheit (VK) was described by Gärtel (1959) in Germany. Similar diseases were frequently noted in other grapevine areas of the world.

In Italy, a disease resembling to FD was discovered in Lombardy, in the Oltrepo' pavese grapevine area, in the late sixties and later, in a rather sporadical form, in vineyards of Piedmont and the Piacenza province (Belli *et al.* 1978). In the early eighties, heavy infections of an epidemic form of an analogous disease were detected in Sicily (Granata 1982), Veneto (Belli *et al.* 1983; Egger and Borgo 1983), Emilia-Romagna (Credi and Babini 1984), Franciacorta in Lombardy (Belli *et al.* 1985), Friuli-Venezia Giulia (Carraro *et al.* 1986), Trentino (Mescalchin *et al.* 1986), Tuscany (Conti 1986), Piedmont (Vidano *et al.* 1987), Apulia (Di Terlizzi *et al.* 1993) and Lazio (Quacquarelli, personal communication). It must be remembered that *S. titanus* was present only in grapevine areas of northern Italy i.e. Piedmont, Lombardy, Veneto and Friuli-Venezia Giulia.

Fortusini *et al.* (1989) reported the transmission of the agent of FD by *S. titanus*, using both wild insects captured in the infected vineyards of the Oltrepo' pavese area and ones experimentally reared on affected plants of the same area.

Starting from 1987, transmission trials using *S. titanus* were carried out at Udine to confirm the aetiology of the disease. This was following the statement that such a natural vector of FD was present in almost all the vineyards of the Friuli-Venezia Giulia (F-VG) region. The present paper reports the results obtained in transmission trials carried out during the six-year-period of the investigation and also outcome of the analyses of the grapevine material used.

Materials and methods

In 1987, at the beginning of summer, nymphs (instar 1 or 2) of *S. titanus* were captured in vineyards of the F-VG region. Some of them were used directly for the transmission trial carried out the same year and the rest were used to establish a colony - on potted healthy grapevines in the screenhouse - to obtain individuals for transmission trials the following year.

T r a n s m i s s i o n t r i a l s f r o m F – V G s o u r c e s o f i n o c u l u m : In the transmission trials repeated between 1987 and 1991, the insects were confined in cloth sleeve cages on canes of affected Chardonnay grapevines (in the field or after transplanting into the screenhouse). Following about 30 d of acquisition, the leafhoppers were transferred to the test plants. The latter included a total of 83 Chardonnay grapevines – woody rooted cuttings, very young cuttings obtained by

*) The research has been supported by the Italian Ministry of Agriculture and Forestry (Extraordinary Program of Research on Grapevine Flavescence Dorée).
Correspondence to: Dr. E. Refatti, Università di Udine, Dipartimento di Biologia Applicata alla Difesa delle Piante, Via delle Scienze, 208, I-33100 Udine, Italy.

micropropagation, one-year-old grafted plants, grown in pots or in the field (protected by an insect proof plastic screen – and Baco 22A rooted cuttings. 10-15 potentially infective insects were transferred to each test plant and left there till their death. Inoculated plants were kept in the screenhouse or in the greenhouse and followed for at least 3 years for symptom observation.

Transmission trials from F-VG and Veneto sources of inoculum: In 1990 three different potted grapevines, i.e. one Chardonnay and one Pinot gris from vineyards of the F-VG region and one Perera from a vineyard in Valdobbiadene (Veneto region) were used as sources of inoculum. All grapevines had shown evident GY symptoms the previous year and were already showing initial symptoms on the new growth. *S. titanus* leafhoppers were confined on these potted plants. At the end of the feeding period (35 d) the leafhoppers collected from the 3 grapevines amounted respectively to: 109 for Chardonnay, 211 for Pinot gris and 165 for Perera. The 3 groups of insects were transferred into 3 separate insect proof cages kept outdoors, each containing 2 micropropagated and one grafted Chardonnay and 3 grafted Perera grapevines. After 40 d of feeding the 128 surviving *S. titanus* were mixed together and moved into another cage, containing one healthy micropropagated Chardonnay and 3 *Catharanthus roseus* L. plants. 15 d later the 38 surviving adults were sent for serological tests to the Research Station of INRA at Dijon (Osler *et al.* 1992). The 19 inoculated grapevines were transplanted into the screenhouse.

Transmission trials from Veneto grapevines: In 1992 a transmission trial was repeated from the Perera plant previously used. 3 healthy Chardonnay vines were utilized as test plants. All the inoculated plants were kept in the greenhouse during the next winter and then moved to the screenhouse.

Serological and molecular biology analyses: ELISA and Western-blot were carried out on the 38 surviving *S. titanus* of the 1990 transmission trial, using polyclonal antibodies against FD *sensu stricto*, obtained from INRA Dijon (Osler *et al.* 1992).

ELISA tests using antibodies specific to FD were carried out on leaf tissues from different grapevine samples. The procedures for the extraction of MLO antigens from grapevine and for ELISA were conducted according to Caudwell and Kuszala (1992).

PCR using primers for the amplification of a fragment of the 16S rDNA gene of MLOs (Ahrens and Seemüller 1992) was applied to grapevine DNA extracted from leaf midribs, according to the procedures already described (Daire *et al.* 1992; Daire *et al.* 1993 a).

Grapevine sampling: In the light of the latest data confirming the presence of different types of GY in Italy (Bianco *et al.* 1993; Caudwell 1993; Chen *et al.* 1993; Daire *et al.* 1993 b; Davis *et al.* 1993; Kuszala *et al.* 1993), leaf samples were collected in 1992 and in 1993 for ELISA and PCR analyses. They included samples of Perera, Chardonnay and Pinot gris plants growing in the same vineyards where the grapevines used as source of inoculum in our trial came from. The same analyses were performed on the potted Perera grapevine used as source of inoculum as well as on 2 of the test plants showing symptoms in 1993.

Results

The results obtained in the 6-year transmission trials, using sources of inoculum of different origins, are summarized in Tab. 1.

Transmission trials from grapevines of the F-VG region: None of the 83 Chardonnay test plants reacted positively when *S. titanus* that had fed for acquisition on grapevines of the F-VG re-

Table 1

Results of the six-year transmission trials carried out using *S. titanus* as vector and grapevines from Friuli-Venezia Giulia and from Veneto as sources of inoculum

	Friuli-Venezia Giulia grapevines			Veneto grapevines (*)		
Year	Source of inoculum	Test plant	Symptoms on test plant	Source of inoculum	Test plant	Symptoms on test plant
1987-89	Chardonnay	Chardonnay 74	no	/	/	/
		Baco 22A 10	no			
1990	Chardonnay	Chardonnay 3	no	Perera	Chardonnay 3	no
		Perera 3	no		Perera 3	yes (3/3)
	Pinot gris	Chardonnay 3	no			
		Perera 3	no			
1991	Chardonnay	Chardonnay 3	no	/	/	/
1992	/	/	/	Perera	Chardonnay 3	yes (2/3)

(*) Perera source of inoculum and test plants as well as the symptomatic Chardonnay test plants reacted positively for FD-*sensu stricto* to ELISA and/or PCR analyses.

gion were used for inoculation. Transmission trials on the test plants Perera and Baco 22A were negative as well.

Transmission trials from Veneto grapevines: In 1991 one of the 3 Perera test plants inoculated in 1990 by *S. titanus* fed on the Perera affected plant showed GY symptoms. The 2 other Perera plants showed mild symptoms in 1992 and very evident GY symptoms in 1993. One out of 3 Chardonnay grapevines inoculated in 1992 from the affected Perera plant started to show GY symptoms during the next winter in the greenhouse and another plant showed clear symptoms of the disease in 1993.

Table 2

ELISA A 405 nm values of a part of the grapevine samples examined

Sample	A 405 nm values	Positivity
Symptomatic Chardonnay (1992 test plant)	0.163	+
Symptomatic Perera (1990 test plant)	1.200	+
FD-infected Alicante Bouschet from France	0.208	+
Healthy Alicante Bouschet	0.007	–
BN-infected Chardonnay from Friuli-Venezia Giulia (*)	0.007	–
BN-infected Chardonnay from Friuli-Venezia Giulia (*)	0.012	–

(*) The samples resulted BN-infected at the PCR analysis.

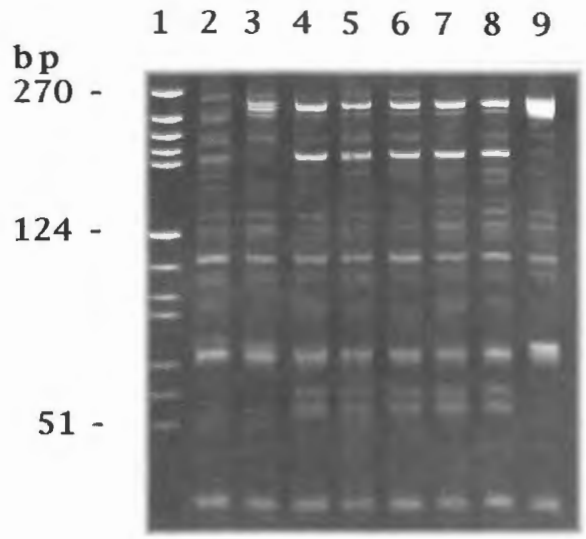

Figure: Polyacrylamide gel electrophoresis of *Alu* I restricted MLO 16S rDNA fragments PCR-amplified from GY-affected grapevines. Weak bands were obtained together with absence of MLO-DNA like profiles. Lane 1: molecular weight marker; lane 2: healthy grapevine cv. Chardonnay; lane 3: grapevine cv. Chardonnay symptomatic test plant of the transmission trial carried out in 1992; lanes 4-8: BN-infected Chardonnay plants; lane 9: FD-infected Alicante Bouschet from France.

Results of the ELISA and PCR analyses: As reported by Osler *et al.* (1992), 11 out of 38 surviving *S. titanus* adults used for the 1990 transmission trials, when individually submitted to ELISA tests, reacted positively to the antibodies against FD *sensu stricto*. The data were confirmed by the Western-blot patterns.

The Perera samples collected in the Valdobbiadene area, the Perera grapevine used as source of inoculum and one of the inoculated Perera and Chardonnay test plants that showed GY symptoms in 1993, reacted positively to ELISA using specific antibodies against FD (Kuszala *et al.* 1993). In contrast, the results of the ELISA analyses on the grapevine samples collected in the vineyards of F-VG region were all negative. In Tab. 2 part of the results obtained at the ELISA tests using specific antibodies against FD are reported.

Similar results were obtained using PCR. In the Figure results are reported obtained in one of the test. Lanes 3 and 9 show the typical profile of FD *sensu stricto*. Lanes from 4 to 7 correspond to the BN profiles.

All symptomatic Chardonnay and Perera vines examined, including the inoculated Chardonnay, were found to be affected by FD *sensu stricto*. The results of analyses were negative for FD in the symptomatic Chardonnay and Pinot gris grapevines of the F-VG region, but demonstrated the presence of a different MLO type similar to the one infecting BN-diseased grapevine in Burgundy (Daire *et al.* 1993 a, b).

Discussion

We succeded in experimentally transmitting the MLO agent of FD *sensu stricto*, both to Perera and to Chardonnay cultivars using *S. titanus*. On the other hand, all the transmission trials of the agent of the GY form(s) present in F-VG were negative. This was in spite of the fact that the leafhopper species is present in the region and the grapevine yellows disease(s) has also a rather active natural vector (Osler *et al.* 1993). It should be noted that the presence/absence or abundance of *S. titanus* populations was not related to the presence and frequency of the affected grapevines in the vineyards of the F-VG region (Refatti *et al.* 1988). This means that the vector(s) of GY form(s) appear(s) to be a species of leafhopper different from *S. titanus*.

The data acquired could help to explain the results of the ELISA analyses carried out on the *S. titanus* surviving the transmission trials carried out in 1990 (Osler *et al.* 1992), in which 29 % of the leafhoppers reacted positively to the FD *sensu stricto* antibodies. The data appear to be related to the fact that only one of the 3 sources of inoculum used was affected by this form of GY. Both our data and that of Fortusini *et al.* (1989) (obtained by using Chardonnay plants affected by FD as the source of inoculum) confirm the fact that *S. titanus* can quite easily transmit FD-MLO in Italy, too. The Perera cultivar can be considered a good indicator plant for FD. The data obtained in our 6-year transmission trials confirm the results

reported by DAIRE *et al.* 1993 b and by KUSZALA *et al.* 1993, i.e. in the grapevine areas of northeastern Italy there are different GY-type diseases: FD *sensu stricto* in Valdobbiadene (Veneto region) and BN in the F-VG region. It should be noted that in *C. roseus* plants inoculated by dodder in 1987 using a symptomatic grapevine cv. Chardonnay of the F-VG region as source of inoculum an MLO belonging to the X-disease group was detected by PRINCE *et al.* (1993) and by DAIRE *et al.* (1993 a). By using the same *C. roseus* plants CHEN *et al.* (1993) were able to obtain oligonucleotide primers that were successfully used for GY diagnosis on grapevines from F-VG region and New York State. The same authors discussed the possibility that periwinkles and grapevines were infected from closely related but not identical MLOs.

Literature cited

AHRENS, U.; SEEMÜLLER, E.; 1992: Detection of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology **82**, 828-832.

BELLI, G.; FORTUSINI, A.; OSLER, R.; 1978: Present knowledge of disease of the type "Flavescence dorée" in vineyards of northern Italy. Proc. 6th Meeting ICVG, Cordoba 1976, 7-13.

- - ; - - ; RUI, D.; 1985: Recent spread of "Flavescence dorée" and its vector in vineyards of northern Italy. Phytopathol. Medit. **24**, 189-191.

- - ; - - ; OSLER, R.; AMICI, A.; 1973: Presenza di una malattia del tipo "Flavescence dorée" in vigneti dell'Oltrepo' pavese. Riv. Patol. Veget. Ser. IV **9**, 51-56.

- - ; - - ; RUI, D.; PIZZOLI, L.; TORRESIN, G.; 1983: Gravi danni da Flavescenza dorata in vigneti di Pinot nel Veneto. Inform. Agrar. **35**, 24431-24433.

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. S V **3**, 69-82.

CARRARO, L.; OSLER, R.; LOI, N.; REFATTI, E.; GIROLAMI, V.; 1986: Diffusione nella regione Friuli-Venezia Giulia di una grave malattia della vite assimilabile alla Flavescenza dorata. Un vigneto chiamato Friuli **5**, 4-9.

CAUDWELL, A.; 1957: Deux années d'études sur la Flavescence dorée, nouvelle maladie grave de la vigne. Ann. Amélior. Plantes **4**, 359-393.

- - ; 1961: Etude sur la maladie du Bois noir de la vigne: ses rapports avec Flavescence dorée. Ann. Epiphyties **12**, 241-262.

- - ; 1993: Advances in grapevine yellows researches since 1990. Proc. 11th Meeting ICVG, Montreux 1993, 79-93.

- - ; KUSZALA, C.; 1992: Mise au point d'un test ELISA sur les tissus de vignes atteintes de Flavescence dorée. Res. Microbiol. **143**, 791-806.

- - ; GIANNOTTI, S.; KUSZALA, C.; LARRUE, J.; 1971: Etude du role de particules de type "mycoplasme" dans l'etiologie de la Flavescence dorée de la vigne. Examen cytologique des plantes malades et des cicadelles infectieuses. Ann. Phytopathol. **3**, 107-123.

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 grapevine yellows disease agent. Phytopathology **83**, 915-922.

CONTI, M.; 1986: Micoplasmi ed altri procarioti unicellulari, agenti fitopatogeni di crescente interesse. Ann. Acc. Agricolt. Torino **129**, 1-17.

CREDI, R.; BABINI, A. R.; 1984: Casi epidemici di giallume della vite in Emilia-Romagna. Vignevini **11** (3), 35-39.

DAIRE, X.; BOUDON-PADIEU, E.; BERVILLE, A.; SCHNEIDER, B.; CAUDWELL, A.; 1992: Cloned DNA probes for detection of grapevine Flavescence dorée mycoplasma-like organism (MLO). Ann. Appl. Biol. **121**, 95-103.

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

- - ; - - ; - - ; ALMA, A.; ARZONE, A.; CARRARO, L.; OSLER, R.; REFATTI, E.; GRANATA, G.; CREDI, R.; TANNE, E.; PEARSON, R.; CAUDWELL, A.; 1993 b: 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 lenght polymorfism analyses and dot hybridizations distinguish mycoplasmalike organisms (MLOs) associated with Flavescence dorée and Southern European Grapevine Yellows disease in Italy. Phytopathology **83**, 772-776.

DI TERLIZZI, B.; ALMA, A.; CASTELLANO, M. A.; SAVINO, V.; 1993: Further studies on yellows-like disorders in Apulia. Proc. 11th Meeting ICVG, Montreux 1993, 95-96.

EGGER, E.; BORGO, M.; 1983: Diffusione di una malattia virus-simile su "Chardonnay" ed altre cultivar nel Veneto. Inform. Agrar. **35**, 25547-25556.

FORTUSINI, A.; SARACCHI, M.; BELLI, G.; 1989: Trasmissione sperimentale della Flavescenza dorata della vite mediante *Scaphoideus titanus* BALL in Italia. Vignevini **16** (9), 43-46.

GÄRTEL, W.; 1959: Die Flavescence dorée oder Maladie du Baco 22 A. Weinberg Keller **6**, 295-311.

GIANNOTTI, S.; CAUDWELL, A.; VAGO, C.; DUTHOIT, J. L.; 1969: Isolament et purification de microorganismes de type mycoplasme a partir de vigne atteintes de Flavescence dorée. C. R. Acad. Agric. **268**, 843-847.

GRANATA, G.; 1982: Deperimenti e giallume in piante di vite. Inform. Fitopatol. **32**, 18-20.

KUSZALA, C.; CAZELLES, O.; BOULUD, J.; CREDI, R.; GRANATA, G.; KRIEL, G.; MAGAREY, P.; MAGNIEN, C.; PEARSON, R.; REFATTI, E.; TANNE, E.; CAUDWELL, A.; 1993: Contribution à l'étude des jaunisses de la vigne dans le monde. Prospection par test ELISA spécifique du mycoplasma-like organism (MLO) de la Flavescence dorée. Agronomie **13**, 929-933.

MESCALCHIN, E.; MICHELOTTI, F.; VINDIMIAN, M. E.; 1986: Riscontrata in alcuni vigneti del Basso Sarca Flavescenza dorata della vite. Terra Trentina **32**, 36-38.

OSLER, R.; BOUDON-PADIEU, E.; CARRARO, L.; CAUDWELL, A.; REFATTI, E.; 1992: First results of the trials in progress to identify the vector of the agent of a grapevine yellows in Italy. Phytopath. Medit. **31**, 175-181.

- - ; CARRARO, L.; LOI, N.; REFATTI, E.; 1993: Symptom expression and disease occurrence of a yellows disease of grapevine in northeastern Italy. Plant Dis. **77**, 496-498.

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.

REFATTI, E.; CARRARO, L.; PAVAN, F.; OSLER, R.; GIROLAMI, V.; 1988: La flavescenza dorata della vite. Notiz. ERSA **1**, 1-16.

SCHVESTER, D.; CARLE, P.; MOUTOUS, G.; 1961: Sur la transmission de la Flavescence dorée des vignes par une cicadelle. C. R. Acad. Agric. **47**, 1021-1024.

VIDANO, C.; ARZONE, A.; ALMA, A.; ARNO', C.; 1987: Auchenorrinchi e diffusione della Flavescenza dorata della vite in Italia. Atti Convegno Flavescenza dorata della Vite, Vicenza/Verona 1987, 57-68.

Received May 24, 1994