Vitis 34 (4), 229–232 (1995)

The vein-banding disease syndrome: A synergistic reaction between grapevine viroids and fanleaf virus

by

J. A. Szychowski[1]), M. V. McKenry[2]), M. A. Walker[3]), J. A. Wolpert[3]), R. Credi[4]) and J. S. Semancik[1])

[1]) Department of Plant Pathology, University of California, Riverside, CA, USA
[2]) Department of Nematology, University of California, Kearney Agricultural Center, Parlier, CA, USA
[3]) Department of Viticulture and Enology, University of California, Davis, CA, USA
[4]) Istituto di Patologia Vegetale, Università degli Studi, Bologna, Italia

S u m m a r y: Viroid-free *Vitis vinifera* cultivars Cabernet Sauvignon and Sauvignon blanc were established in controlled field trials in California to evaluate the relationship between grapevine viroids and fanleaf virus for induction of the vein-banding disease. Vein-banding symptoms were observed only on vines which contained the three principal grapevine viroids, grapevine yellow speckle viroids (GYSVd-1, GYSVd-2), and hop stunt viroid (HSVd-g), as well as grapevine fanleaf virus (GFLV). Sauvignon blanc vines which contained the single viroid, HSVd-g, and GFLV were non-symptomatic indicating an absence of a correlation between HSVd-g and the vein-banding disease. The intensity of vein-banding symptoms was directly correlated with an enhanced titer of GYSVd-1 and GYSVd-2. Vein-banding and yellow speckle symptomatic as well as non-symptomatic vines in Italy contained two viroids, GYSVd-1 and HSVd-g. However, symptomatic vines displayed a higher titer of GYSVd-1 than non-symptomatic materials and vein-banding symptomatic vines were GFLV infected. These data experimentally demonstrate that expression of the vein-banding disease is induced by an unique synergistic reaction between a viroid, GYSVd-1 and a virus, GFLV.

K e y w o r d s: viroids, virus, fanleaf, yellow speckle.

Introduction

A range of viroids in grapevine were initially recognized by Flores *et al.* (1985), Sano *et al.* (1985), and Semancik *et al.* (1987). The ubiquitous occurrence of viroids in *Vitis* varieties and rootstock selections both in California and Europe was noted by Szychowski *et al.* (1991). Hybridization of four grapevine viroids from California sources with specific probes for GYSV and GV1B (Koltunow *et al.* 1989), HSVd-g (Sano *et al.* 1988) and CEVd (M. Bar-Joseph, unpublished) indicated a high degree of homology among viroids from other areas of the world. The parameters of biological screening by differential hosts, electrophoretic properties and molecular hybridization permitted the grouping of the grapevine viroids (Semancik and Szychowski 1992).

Two grapevine viroids, GYSVd-1 and GYSVd-2, previously designated as GV-1 and GV-2 (Semancik *et al.* 1987) or GYSV and GV1B (Koltunow and Rezaian 1988; Koltunow and Rezaian 1989) have been reported to induce symptoms of yellow speckle disease in grapevine (Koltunow *et al.* 1989). Symptoms of yellow speckle are ephemeral, most evident at the end of the summer and consist of a few to many chlorotic spots on leaves. Expression of yellow speckle symptoms prevalent in Australia (Woodham *et al.* 1973) but not common in California apparently require defined climatic conditions (Stellmach and Goheen 1988).

Vein-banding disease has been hypothesized to be either a late season expression associated with fanleaf degeneration caused by grapevine fanleaf virus (GFLV) (Martelli and Savino 1988) or the response to a co-infection of yellow speckle and fanleaf virus (Krake and Woodham 1983). Fanleaf degeneration occurs in most countries that have viticulture production based on vinifera cultivars. In California, fanleaf is prevalent in North Coast, Central Coast and Lodi-Livermore vineyard growing areas (Flaherty *et al.* 1992). Krake and Woodham (1983) reported that leaf symptoms similar to a vein-banding disease source could be reproduced only with a mixed infection of fanleaf and yellow speckle disease. Therefore, it was hypothesized that leaf symptoms associated with vein banding disease were due to a yellow speckle infection, intensified by co-infection with purified fanleaf virus.

Symptoms of vein-banding range from yellow flecking adjacent to the veins to coalesced chlorotic regions radiating from the midrib that outline the veins. Symptoms appear on the vines on a limited number of leaves in mid to late summer. Vein-banding disease can be devastating to grapevines with up to 80 % fruit loss in sensitive varieties (Martelli and Savino 1988). Experimental evidence presented here supports the hypothesis that vein-banding is induced by a synergistic reaction between grapevine viroids and fanleaf virus.

Materials and methods

With the availability of shoot-tip cultured viroid-free grapevines (Duran-Vila *et al.* 1988), a field plot was es-

Correspondence to: Dr. J. S. Semancik, University of California, Department of Plant Pathology, Riverside, CA 92521, USA. Fax: (909) 787-4294.

tablished to investigate possible interactions between the causal agents of yellow speckle and fanleaf. Four microplots were established in June 1992 under field conditions at the University of California Kearney Agricultural Center in Parlier, California. The plots were ca. 40 m^2 each with plastic lined sides to a depth of ca. 1.5 m. Prior to planting the plots were fumigated with methyl bromide. Plant materials included own-rooted, shoot-tip cultured viroid-free Cabernet Sauvignon (Tab. 1 A, C) and clonal propagations graft inoculated with tissue from a source vine containing GYSVd-1, GYSVd-2, and HSVd-g (Tab. 1 B, D) which had been infected with a partially purified viroid extract.

Two months after planting, vines in two plots either without (Tab. 1 A) or with (Tab. 1 D) viroids were inoculated with soil from a known nematode infested fanleaf vineyard by incorporating one liter of soil per vine around the base of each plant. The average number of nematodes inoculated per vine were: 5 *Criconemella xenoplax,* 1052 *Xiphinema index* and 211 *X. americanum.*

Two plots of similar design incorporated Sauvignon blanc vines containing either GYSVd-1, GYSVd-2 and HSVd-g or HSVd-g only. Both plots were inoculated with the nematode infested soil as described.

Vines were pruned to two buds during dormancy each year and disease symptoms were monitored throughout the growing season. Apical tissues were collected in June and August 1994 from each vine and combined within every microplot. Symptomatic vein-banding leaves were also collected during the August sampling.

Vein-banding and yellow speckle (Tab. 2) symptomatic and non-symptomatic tissues from Italy were collected either in June or late summer. Four separate collections were made from these vines over three growing seasons. One collection was performed in June and three in September. Apical leaves were macerated in liquid nitrogen, lyophilized, sealed in plastic bags and kept at room temperature.

Table 1

Vein-banding disease symptoms on Cabernet Sauvignon induced by a synergistic reaction between viroids and fanleaf virus

Properties	Microplot A	B	C	D
Grapevine viroids *	-	+	-	+
X. index	+	-	-	+
GFLV (ELISA)	1.165	0.031	0.023	0.638
Vein-banding symptoms	NS **	NS	NS	SEVERE

* GYSVd-1, GYSVd-2 & HSVd-g determined by sPAGE.
** Non-symptomatic.

Tissue extraction and detection of viroids were performed essentially as previously reported (RIVERA-BUSTAMANTE *et al.* 1986, SZYCHOWSKI *et al.* 1988). To promote rehydration of the lyophilized material, 1 g of tissue was homogenized with 10 ml sodium sulfite buffer, 2 ml H_2O, and 18 ml phenol (pH 7.5) and kept at 4 °C for 30 min prior to centrifugation. Detection of GFLV was accomplished by enzyme-linked immunosorbent assay (ELISA) as reported by ROWHANI *et al.* (1992).

Results

Characteristic vein-banding symptoms (Fig. 1, right) became visible on the mature leaves of vines which were dual infected with grapevine viroids and GFLV (Tab. 1 D) during the third leafing season. Leaves on vines in the other three plots (Tab. 1 A, B, C) lacking one or both disease agents remained non-symptomatic (Fig. 1, left). Presence of fanleaf virus was detected by ELISA, 1.165 and 0.638 (Tab. 1 A, D, respectively) only in the vines previously inoculated with *X. index*. Vein-banding symptoms were only expressed in Sauvignon blanc vines which contained

Table 2

Yellow speckle (YS) symptoms and viroid titer associated with seasonal growth; variety Pagadebit

No.	YS *	June YS	June TITER **	September YS	September TITER
1	POS	NEG	++++	POS	++++
2	NEG	(no data)	(no data)	NEG	+
3	NEG	NEG	+	NEG	+
4	NEG	NEG	+	NEG	+
5	POS	NEG	+	POS	++++
6	NEG	NEG	+	NEG	+
7	POS	NEG	++++	POS	++++
8	NEG	NEG	+	NEG	+
9	POS	NEG	++++	POS	++++
10	NEG	NEG	+	NEG	+

* Symptoms expressed in previous September.
** Notations of (+++) and (+) signify relative concentration of GYSVd-1 detected in sPAGE.

the three grapevine viroids while vines which contained HSVd-g alone were non-symptomatic (Fig. 2, right and left, respectively).

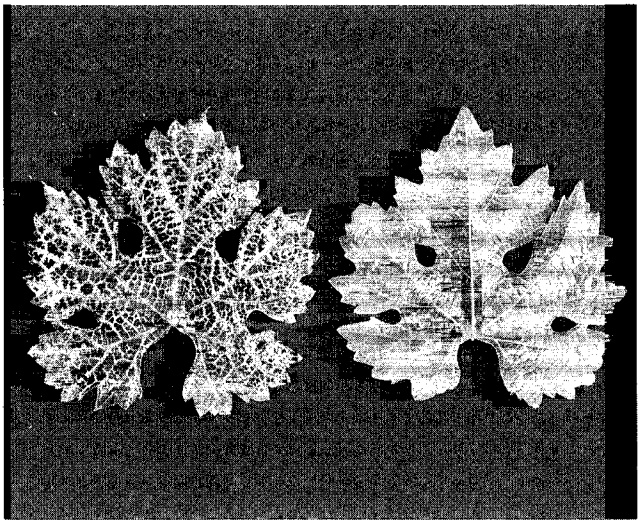


Fig. 1: Non-symptomatic (left) and vein-banding symptomatic (right) Cabernet Sauvignon leaves which occurred during third leafing of the vines.

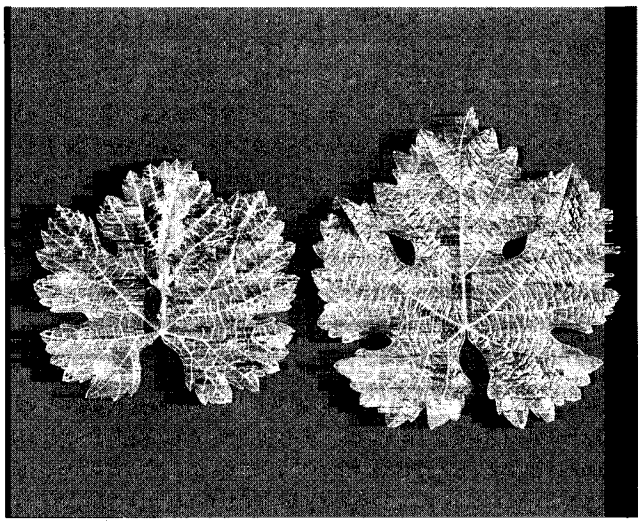
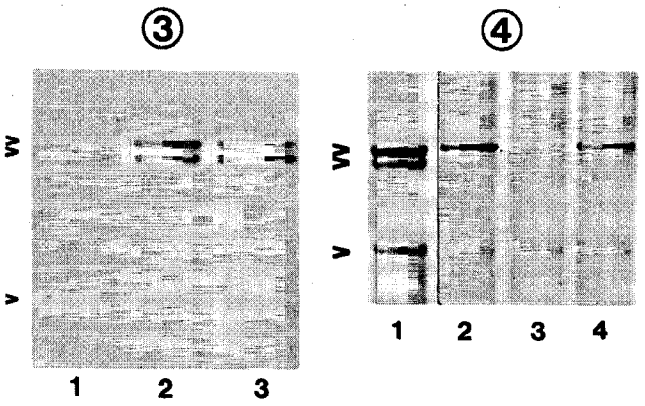


Fig. 2: Non-symptomatic (left) and vein-banding symptomatic (right) Sauvignon blanc leaves which occurred during third leafing of the vines.

Sequential PAGE confirmed the presence of GYSVd-1, GYSVd-2, and HSVd-g only in the inoculated Cabernet Sauvignon vines (Tab. 1 B, D). Titer of both GYSVd-1 and GYSVd-2 was increased in vein-banding symptomatic tissues. Furthermore, the two viroid-RNAs were concen-trated in chlorotic regions of symptomatic leaves (Fig. 3, lane 2) when compared to green areas of the same leaf (Fig. 3, lane 1). A similar increase in the titer of GYSVd-1 was also detected in naturally-infected vein-banding symptomatic vines observed in Italy. This vein-banding tissue source contained only two viroids, GYSVd-1 and HSVd-g (Fig. 4, lane 2). The presence of GFLV in vein-banding tissues was also verified.

A similar increase in titer of GYSVd-1 was also observed in yellow speckle (YS) disease expressing tissues from field grown vines in Italy (Tab. 2). Only two grapevine viroids, GYSVd-1 and HSVd-g were found in this material by sPAGE. The yellow speckle symptomatic tissues revealed an increased GYSVd-1 titer (Fig. 4, lane 4) compared to non-symptomatic tissues (Fig. 4, lane 3).

These results were consistent for all collections made in late summer. A viroid titer differential was also observed in the June collection well in advance of any disease symptoms. Titer increase was principally detected in vines found to express symptoms later in the same growing season.



Figs. 3 (left) and 4 (right): Polyacrylamide (5 %) gel containing 8 M urea after sequential PAGE and silver staining. **3**: Grapevine viroid standard (lane 3) containing GYSVd-1, GYSVd-2, and HSVd-g, position indicated from top to bottom respectively by arrowheads. Nucleic acid preparations from green (lane 1) and chlorotic regions (lane 2) of a vein-banding symptomatic leaf of Sauvignon blanc. **4**: Viroid standard containing GYSVd-1, GYSVd-2 and HSVd-g, position indicated from top to bottom respectively by arrowheads, from the variety Cardinal (lane 1). Nucleic acid preparations from vein-banding symptomatic (lane 2), non-symptomatic (lane 3), and yellow speckle symptomatic tissue (lane 4) from the Italian cultivar Pagadebit.

Discussion

Vein-banding disease has been an ill-defined syndrome postulated to be either a late season expression of infection by GFLV or a consequence of infection by both GFLV and the yellow speckle agent. Experimental data reported here demonstrates that the vein-banding disease syndrome is induced by the unique synergistic reaction between a viroid, GYSVd-1 and a virus, GFLV.

The three principal grapevine viroids, GYSVd-1, GYSVd-2, and HSVd-g, were inoculated into viroid-free materials as a broad spectrum survey and to ensure that any viroid-induced effect would be observed. However, vein-banding symptoms were also observed in the absence of GYSVd-2. The question nevertheless remains, whether GYSVd-2 alone can also participate in a synergistic reaction with GFLV to produce vein-banding symptoms. The absence of vein-banding symptoms on Sauvignon blanc vines which contained both HSVd-g and GFLV indicates that no direct correlation exists between HSVd-g and vein-banding disease.

Vein-banding symptoms which first developed only during the third leafing season might be affected by the

temporal variability reported for symptom expression of GFLV coupled with the uncertainties of field inoculation of GFLV by the *X. index* vector. Vines which contained the two viroids reported to be the causal agents of yellow speckle disease, GYSVd-1 and GYSVd-2 (KOLTUNOW *et al.* 1989), have not displayed yellow speckle symptoms to date under field conditions in California.

The enhanced viroid titer observed in the vein-banding symptomatic material may suggest a linkage between some critical viroid concentration and symptom expression. A similar relationship between viroid titer and symptom expression is also observed in host responses resulting from infection by variants of avocado sunblotch viroid (SEMANCIK and SZYCHOWSKI 1994).

All materials received from Italy contained one of the causal agents of yellow speckle, GYSVd-1, however, only some vines expressed symptoms. An increased viroid titer was generally observed in tissues collected both in June and September from vines which ultimately displayed late season symptoms of yellow speckle or vein-banding. In only one vine (Tab. 2, No. 5), ultimately positive for yellow speckle, was viroid titer lower in the June sampling than in September. Viroid replication and accumulation may, therefore, precede symptom expression and as such might be used to predict eventual disease onset.

Experimental evidence presented here demonstrates that vein-banding disease syndrome is caused by a synergistic reaction between viroids and grapevine fanleaf virus. This unusual interaction between viroids and viruses extends the biological potential of viroids. Previous studies have shown that virtually all grapevines contain viroids and 85 % of vines carry at least one of the reported causal agents of yellow speckle (SZYCHOWSKI *et al.* 1991). Present studies demonstrate that at least one of the agents of yellow speckle disease, GYSVd-1, is also involved in vein-banding disease syndrome. Given the pervasive presence of the viroid background, the previous proposed association of vein-banding disease with grapevine fanleaf degeneration sites is now understandable. Studies reported here caution that viroids may affect vine properties even in the absence of a direct causal relationship and may be factors in the unknown disorders occurring in the Napa Valley of California (WEBER and WOLPERT 1993) as a result of extensive replanting.

Acknowledgements

The authors acknowledge JACK CLARK for photographic assistance, ELIZABETH DE JAGER for technical assistance, and the American Vineyard Foundation for partial support of these studies.

Literature cited

DURAN-VILA, N.; JUAREZ, J.; ARREGUI, J. M.; 1988: Production of viroid-free grapevines by shoot tip culture. Amer. J. Enol. Viticult. **39**, 217-220.

FLORES, R.; DURAN-VILA, N.; PALLAS, V.; SEMANCIK, J. S.; 1985: Detection of viroid and viroid-like RNAs from grapevines. J. Gen. Virol. **66**, 2095-2102.

FLAHERTY, D. L.; CHRISTENSEN, L. P.; LANINI, W. T.; MAROIS, J. J.; PHILLIPS, P. A.; WILSON, L. T.; (Ed.) 1992: Grape Pest Management, 108 and 285. Division of Agriculture and Natural Resources, University of California, Oakland, California.

KOLTUNOW, A. M.; KRAKE, L. R.; JOHNSON, S. D.; REZAIAN, M. A.; 1989: Two related viroids cause grapevine yellow speckle disease independently. J. Gen. Virol. **70**, 3411-3419.

- -; REZAIAN, M. A.; 1988: Grapevine yellow speckle viroid: structural features of a new viroid group. Nucleic Acids Res. **16**, 849-864.

- -; - -; 1989: Grapevine viroid 1B, a new member of the apple scar skin viroid group contains the left terminal region of tomato planta macho viroid. Virology **170**, 575-578.

KRAKE, L. R.; WOODHAM, R. C.; 1983: Grapevine yellow speckle agent implicated in the aetiology of vein banding disease. Vitis **22**, 40-50.

MARTELLI, G. P.; SAVINO, V.; 1988: Fanleaf degeneration. In: R. C. PEARSON and A. C. GOHEEN (Eds.): Compendium of Grape Diseases, 48-49. APS Press, St. Paul, Minnesota.

RIVERA-BUSTAMANTE, R.; GIN, R.; SEMANCIK, J. S.; 1986: Enhanced resolution of circular and linear molecular forms of viroid and viroid-like RNA by electrophoresis in a discontinuous-pH system. Anal. Biochem. **156**, 91-95.

ROWHANI, A.; WALKER, M. A.; ROKNI, S.; 1992: Sampling strategies for the detection of grapevine fanleaf virus and the grapevine strain of tomato ringspot virus. Vitis **31**, 35-44.

SANO, T.; KUDO, H.; SUGIMOTO, T.; SHIKATA, E.; 1988: Synthetic oligonucleotide hybridization probes to diagnose hop stunt viroid strains and citrus exocortis viroid. J. Virol. Methods **19**, 109-120.

- -; UYEDA, I.; SHIKATA, E.; MESHI, T.; OHNO, T.; OKADA, Y.; 1985: A viroid-like RNA isolated from grapevine has high sequence homology with hop stunt viroid. J. Gen. Virol. **66**, 333-338.

SEMANCIK, J. S.; RIVERA-BUSTAMANTE, R.; GOHEEN, A. C.; 1987: Widespread occurrence of viroid-like RNAs in grapevines. Amer. J. Enol. Viticult. **38**, 35-40.

- -; SZYCHOWSKI, J. A.; 1992: Relationships among the viroid derived from grapevines. J. Gen. Virol. **73**, 1465-1469.

- -; - -; 1994: Avocado sunblotch disease: a persistent viroid infection in which variants are associated with differential symptoms. J. Gen. Virol. **75**, 1543-1549.

STELLMACH, G.; GOHEEN, A. C.; 1988: Other virus and viruslike diseases. In: R. C. PEARSON and A. C. GOHEEN (Eds.): Compendium of Grape Diseases, 53-54. APS Press, St. Paul, Minnesota.

SZYCHOWSKI, J. A.; DOAZAN, J. P.; LECLAIR, P.; GARNIER, M; CREDI, R.; MINAFRA, A.; DURAN-VILA, N.; WOLPERT, J. A.; SEMANCIK, J. S.; 1991: Relationship and patterns of distribution among grapevine viroids from California and Europe. Vitis **30**, 25-36.

- -; GOHEEN, A. C.; SEMANCIK, J. S.; 1988: Mechanical transmission and rootstock reservoirs as factors in the widespread distribution of viroids in grapevines. Amer. J. Enol. Viticult. **39**, 213-216.

WEBER, E.; WOLPERT, J. A.; 1993: Unknown disorder appears in Napa. Grape Grower **25** (10), 4.

WOODHAM, R. C.; TAYLOR, R. H.; KRAKE, L. R.; 1973: Grapevine virus and virus-like diseases in southern Australia. Riv. Patol. Veg. Ser. IV, **9** (Suppl.), 17-22.

Received July 3, 1995