

AMERICAN VINEYARD FOUNDATION
Final Report
1992/1993

Project Title: Involvement of Viroids in Grapevine Diseases: Impact on Clonal Variation, Vine Performance, and Wine Quality

Principal Investigators: J.S. Semancik
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Objectives:

The ubiquitous nature of viroids in virtually every grapevine throughout the world has introduced the question of the role of viroids in vine performance and wine quality. The possible effects induced by these transmissible biologically active molecules constitutes the primary focus of this project. Specific objectives are:

- (1) Grapevine Viroids as the causal agents and involvement in "**yellow speckle**" and "**vein banding**" diseases.
- (2) Performance evaluation of viroid-free vines at the Oakville Experiment Station.
- (3) Development of additional viroid-free grapevines.
- (4) Transmission and field spread of grapevine viroids.

Summary:

The role of viroids as agents of plant disease is well established. Control experiments have been initiated to evaluate the role of grapevine viroids as the causal agent of the **Yellow Speckle (YS)** disease and in association with grapevine leafroll virus in the expression of **Vein Banding (VB)** symptoms. It has not yet been possible to confirm a simple cause-effect relationship between **YS** symptoms and a single viroid for vines in California as was reported from Australia. Controlled environment experiments are continuing to clarify the relationship between viroids and both **YS** and **VB**. A more complex etiology for viroid induced diseases must also be considered.

Performance of the first viroid-free field trials in the world are continuing into the sixth growing season at Oakville with Cabernet Sauvignon and are providing data on vine growth, yield, fruit maturity and wine quality. Introduction of the additional viroid-free varietal, Sauvignon blanc, grafted onto the first viroid-free rootstock, Teleki 5C, is being introduced at Oakville.

The continued development of additional viroid-free commercial varieties and rootstocks, as well as rootstock germplasm sources is essential to our search for grapevines reactive to viroids. This aspect is directed to the understanding of the role of viroids in scion/rootstock interactions. These studies will provide an understanding of the action of viroids not only in graft union disorders but also the potential application of viroids as molecular elements to control vegetative growth of vines.

Monitoring for the incidence of field transmission to viroid-free vines interplanted in a commercial planting of viroid-containing vines has indicated no field spread. Under the extreme condition of prolonged growth of viroid-free and viroid-infected vines in a common container, only about 15% transmission of selected viroids was detected.

Research Accomplishments:

Objective (1): The relationship of grapevine viroids to Yellow Speckle (YS) and Vein Banding (VB) diseases.

With the cooperation of Dr. G.I. Mink, Washington State University, and Dr. M.V. McKenry, Kearney Agricultural Center, experiments have been established to define the role of viroids in YS and VB. The expression of YS appears to be highly dependent upon climatic conditions. Expression is especially high in Australia. Dr. Mink has devised a controlled environment regime to promote symptom development and is testing materials we have developed.

Vein banding symptom development in California is good and the disease appears to be widespread which is consistent with the occurrence of viroids in vines. To test the hypothesis that grapevine fanleaf virus interacts with viroids to induce vein banding, a trial using miniplots with the following configuration has been established at the Kearney Field Station with the cooperation of Dr. McKenry. Xiphinema index from GFLV infected vines have been inoculated to both Cabernet Sauvignon (CS) and Sauvignon blanc (Sb) in the presence and absence of the major grapevine viroids, GYSV-1, GYSV-2, and HSV-g.

Objective (2): Performance trials of viroid-free vines at the Oakville Experiment Station.

Data from the testing of viroid-free vines obtained by shoot-tip culture has now been collected for the second season as the vines become established. This critical testing is the first to be accomplished in the world and will provide invaluable information as to the role of viroids in vine performance and wine quality. Some preliminary indications of the type of data being collected is provided in the following table:

The low yield of viroid-free material was a result of fewer berries/cluster. Parental vines were slower to mature resulting in a harvest date several days later than the viroid-free and viroid-infected treatments. Additional data from the 1992 harvest will be developed.

Sufficient quantities of shoot-tip cultured viroid-free Teleki 5C have been produced to enable replicating the self rooted Cabernet Sauvignon treatments at the Oakville Viroid Trial using the viroid-free rootstock. In addition, the first white varietal to become available, Sauvignon blanc, will be added in 1993. Other varietals and rootstocks are in various stages of propagation and preparation for addition to the Oakville Viroid Trial.

Objective 3: Development of additional viroid-free materials.

The procedures to obtain viroid-free vines by shoot-tip culture have provided a number of vinifera cultivars for the testing of the effect of viroids on vine performance and wine quality. With the introduction of new rootstocks, graft union disorders as well as the appearance of unusual vine characteristics have appeared. Similar responses to the presence of viroids have been observed in other crops. Therefore, we have increased our development of a broader range of germplasm to expose materials reactive to the presence of viroids. The emphasis has been on additional rootstock materials which can be tested with the several vinifera cultivars already in the process of propagation. The following materials are in the early stages of testing for the presence of viroids in vines developed from shoot-tips prior to readaptation to field conditions and increased production.

ROOTSTOCKS ENTERED INTO SHOOT-TIP CULTURE PROGRAM FOR VIROID-FREE VINES

A) Commercial

Richter 110
 Couderc 3309
 O39-16
 Paulsen 1103
 Ruggeri 140

B) Experimental

Kober 125AA
 Paulsen 775

C) Germplasm

Vitis berlandieri
 V. champini
 V. rotundifolia
 V. rufotomentosa

Objective 4: Transmission and field spread of grapevine viroids.

We have continued to monitor for field transmission of viroids by analysis of viroid-free Cabernet Sauvignon vines which have been interplanted to a commercial vineyard. Vines established in the vineyard have been determined to contain the two viroids, GYSVd-1 and HSVd-g. The

viroid-free vines receive normal cultural treatment to best approximate a typical commercial vineyard situation. Dormant wood has been collected annually over the past four growing seasons. Analysis for viroids by sPAGE of tissue forced from this wood has indicated no spread of the viroids occurred in the first three years. Preliminary results of the latest analysis indicates that some field transmission may have occurred. It will be necessary to confirm this indication with analysis of additional material from the commercial planting.

Outside Presentation of Research:

- Semancik, J.S. and J.A. Szychowski. 1992. Relationships among the viroids derived from grapevines. J. Gen. Virology 73: 1465-1469. (reprint included)
- Semancik, J.S., J.A. Szychowski, and J.A. Wolpert. 1992. Viroids in grapevines, a threat or opportunity? Practical Winery & Vineyard 13: 39-43. (reprint included)
- Szychowski, J.A., J.A. Wolpert, G.I. Mink, R. Credi, and J.S. Semancik. 1992. Viroids associated with yellow speckle and vein banding diseases of grapevine and the incidence of viroid transmission. Am. Society of Enology and Viticulture (abstr.). (oral presentation)
- Wolpert, J., J. Szychowski, A. Bledsoe, N. Duran-Vila, and J. Semancik. 1992. Effect of viroid content on growth, yield, fruit maturity and wine quality of own-rooted Cabernet Sauvignon grapevines. Am. Society of Enology and Viticulture (abstr.). (oral presentation)

Presentations scheduled to be made at the ICVG Meetings in September 1993:
(copies of the extended abstracts prepared are included here)

- 1) Semancik, J.S. Current status of research on grapevine viroids.
- 2) Semancik, J.S., Szychowski, J.A., Credi, R., Mink, G.I., McKenry, M. and Wolpert, J.A. The role of grapevine viroids in yellow speckle and vein banding diseases.
- 3) Wolpert, J.A., Szychowski, J.A., Duran-Vila, N., and Semancik, J.S. Performance of viroid-free cabernet sauvignon vines.

AMERICAN VINEYARD FOUNDATION

Project Title: INVOLVEMENT OF VIROIDS IN GRAPEVINE DISEASES:
IMPACT ON CLONAL VARIATION, VINE PERFORMANCE, AND WINE QUALITY

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Executive Summary Statement of Research Findings

VIROIDS are small, transmissible RNA molecules restricted to plants which are associated with disease. Viroids are extremely resistant to thermotherapy but viroid-free plants can be obtained by culture of very small shoot tips. The viroids we have detected in California and European grapevines present an unusual relationship for any crop with virtually every vine containing viroids.

The basic goal of this project is to determine the significance of viroids to the growth and productivity of grapevines and wine quality. The widespread occurrence of viroids in grapevines throughout the world clearly indicates that all vine characteristics as well as wine quality, have been observed through a viroid background.

The considerable investment made to the Viroid Trial at Oakville is returning information essential to our understanding of the impact of viroids on vine performance and wine quality. The viroid-free vines we have established at Oakville are the first control materials planted to a viroid test in the world and are critical to monitoring the effect of viroids on vines. Additional commercial, experimental, and germplasm cultivars have been entered into the viroid-free vine program to identify specific materials sensitive to the effects of grapevine viroids.

Experiments designed to define the role of the grapevine viroids in YELLOW SPECKLE and VEIN BANDING diseases have been initiated. These studies are critical to the identification of the specific viroids present in vines and the scope of the threat of these diseases.

CURRENT STATUS OF RESEARCH ON GRAPEVINE VIROIDS

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Viroids were first identified through biological expression as the causal agents of plant disease. However, the physical-chemical aspects of grapevine viroid detection, isolation and characterization has advanced more rapidly than the understanding of the intrinsic biological activity of viroids and the consequences on vine performance. This fact has been due, in part, to the lack of alternate herbaceous hosts and the initial lack of viroid-free control vines.

The organizational scheme for grapevine viroids first proposed and accepted in 1990 at the 10th Meeting of the ICVG (1) has gained general acceptance in the viroid literature (2) as presented in Table 1.

Table 1. TOWARD A CONSENSUS NOMENCLATURE FOR THE GRAPEVINE VIROIDS

Group	Synonymous Designations	Nucleotide Number	Disease	Grapevine Viroid
1	GVd-s, CEVd-g	369	(NR)	CEVd-g
2	AGVd GVd-c	369 (NR)	(NR) (NR)	AGVd GVd-c
3	GVd-f, GVd-1, GYSVd GVd-2, GVd-1B	367 363	YELLOW SPECKLE YELLOW SPECKLE	GYSVd-1 GYSVd-2
4	HSVd-g (Riesling), GVd-3 HSVd-g (Japan), GVd-3	298 297	(NR) (NR)	HSVd-g

(NR) = no report.

The relationships originally proposed from physical properties and molecular hybridization reactions with viroid-specific probe, have been reinforced by additional comparative studies (3) employing the polymerase chain reaction (PCR) analysis (Table 2).

Table 2. COMMON NUCLEOTIDE SEQUENCES OF GRAPEVINE VIROIDS IDENTIFIED BY POLYMERASE CHAIN REACTION (PCR) ANALYSES AND VIROID SPECIFIC PROBES

Group	Grapevine Viroid	Viroid Residues	PCR Product* Residues	Cloned Viroid Probes*		
				GYSVd-1	HSVd-g	AGVd
1	CEVd-g	369	---	--	--	--
2	AGVd	369	375	--	--	+
3	GYSVd-1	367	223	+	--	--
	GYSVd-2	363	363	+	--	--
4	HSVd-g	297	297	--	+	--

* Data reported by Rezaian et al., 1992, Intervirology 34:38-43.

Although the viroids as a group have elicited much interest as unique small, RNA molecules with unusual structural features, the biological activity of viroids to alter the "normal" plant growth and development remains the most dramatic and definitive property of the viroid molecule. It has become evident that viroid replication is not inexorably tied to viroid pathogenesis and the production of disease symptoms.

The grapevine viroids (GVd) occupy an unusual niche among the viroids in that:

- 1) the GVd's are spread worldwide in a virtual ubiquitous manner (4),
- 2) only yellow speckle (YS) has been confirmed as a viroid induced disease of vines which is expressed principally in Australia (5).

Nevertheless, this nominal disease threat attributed to viroids does not obviate the fact that, **every vine response including viticultural characters, disease expression, indexing reaction, and even wine quality is observed through a viroid background.** Acknowledgment of this statement supports the importance of the clarification of the biological potential of grapevine viroids. This remains as the primary challenge on the scene of grapevine viroid research today.

Analysis of 24 cDNA clones of GYSVd-1 indicates a high frequency of sequence variations which occur principally in the pathogenic (P) domain (6). A comparison of clones of GYSVd-2, which also induces yellow speckle symptoms, did not display a similar sequence variability centered in the P domain. A review of the properties of GYSVd-1 and GYSVd-2 (Table 3) and particularly the common ability to induce YS symptoms between two viroids sharing only 73% sequence homology challenges the implied relationship as "strains". By convention it is generally accepted that related strains share a 90% sequence homology.

TABLE 3. COMPARISON OF GRAPEVINE VIROIDS INDUCING YELLOW SPECKLE SYMPTOMS

	GYSVd-1	GYSVd-2
1) Synonyms:	GV-f; GV-1; GYSV	GV-2; GV-1b
2) Residues:	367	363
3) Homology for GYSVd-1:	100%	73%
4) Symptom Expression:		
a) grapevines:	yellow speckle	yellow speckle
b) other host:	none	none
5) Occurrence in vines:		
a) frequency all vines:	85%	13%
b) predominant vines:	wine	table

This review prompts a closer examination on the subject of the "variable" and "erratic" expression of yellow speckle symptoms which has frequently been repeated in the YS literature. "One of the most remarkable features of GYS is the extreme variability in symptom expression" which is noted "...following an exceptionally hot summer" (7). It has been reported that 38% of the cultivars are YS positive by self expression and 24% more by indexing, yet approximately 85% of the vines from California and European sources carry one of the two viroids implicated in the yellow speckle disease.

Contributing to this perspective is the statement that "...the leaf symptom associated with vein banding disease are due to a yellow speckle infection, INTENSIFIED by co-infection with fanleaf virus" (8).

What then exactly is the YELLOW SPECKLE disease? And what is the relationship of yellow speckle to the VEIN BANDING syndrome?

- a) Has the YS syndrome been accurately described as a simple disease expression induced by two different viroids?
- b) Does a complex situation exist with multiple disease expressions described under the single generic term "yellow speckle"?
- c) Does GYSVd-1 comprise a population of size related viroids only some of which are competent to induce YS or VB symptoms?
- d) Is YS an acceptable symptom expression induced by GYSVd or a "stress condition" aggravated by the presence of viroids? Is the prime factor in the expression of YS the viroid or climate?
- e) Are "yellow speckle" and "vein banding" (VB) distinct diseases or degrees of severity?

In any discussion of YS and VB as expressions of physiological modification heightened by the presence of viroids, the consideration can be introduced that viroids might not constitute consummate pathogens but offer a potential for controlling some aspect of vine growth and development (9). Since the viroid is not integrated into the host genome and does not contribute genetic information which is translated into protein, the viroid apparently acts by influencing the expression of the normal host genome. In essence, this process may involve exploitation of the inherent potential for variation that resides in the vine. The fact that viroids are currently being used to dwarf citrus in commercial plantings suggests that this procedure may provide a practical tool for "customizing" vine growth.

THE ROLE OF GRAPEVINE VIROIDS IN YELLOW SPECKLE AND VEIN BANDING DISEASES

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The role of viroids as agents of plant disease is well established. The ubiquitous distribution of viroids in vines throughout the world, however, raises questions as to the role of viroids as primary causal agents or as factors in disease expression. Although the two viroids GYSVd-1 and GYSVd-2 have been so designated for inducing yellow speckle (YS) symptoms on grapevines in Australia, it has not yet been possible to confirm a simple cause-effect relationship between YS symptoms and a single viroid for vines in California. Experiments have been initiated to evaluate the role of grapevine viroids as the causal agent of yellow speckle disease and the expression of vein banding (VB) symptoms in association with grapevine leafroll virus.

With these circumstances and a review of the properties of yellow speckle and vein banding diseases, a more complex etiology for viroid induced grapevine diseases might be entertained. The extreme variability in YS and VB symptom expression plus the erratic occurrence of the diseases, which usually appear following an exceptionally hot period, might indicate primarily a stress condition which is only aggravated by the presence of the viroid. Is then the climate or viroids the prime factor inducing yellow speckle symptoms? A controlled environment regime to promote YS symptom development has been used to test the following materials.

CULTIVAR	VIROID CONTENT	YELLOW SPECKLE
Cabernet Sauvignon	viroid-free	unknown
" "	GYSV-1, GYSV-2, HSV-g	"
" "	GYSV-1	"
" "	GYSV-2	"
Sauvignon blanc	viroid-free	"
" "	GYSV-1, GYSV-2, HSV-g	"
Cabernet franc	GYSV-1	"
O39-16	GYSV-2	"
Zinfandel	GYSV-1 and HSV-g	"
Mission	GYSV-1 and HSV-g	++++

Synergism among pathogenic agents is not an uncommon occurrence. It is important to recognize that because of the virtual ubiquitous occurrence of viroids in grapevines, all vine responses including disease expressions are viewed against a viroid background and potentially modulated by them. The origin of the vein banding symptom has evoked some difference of opinion and has been recognized as either a response to fanleaf alone or as the result of a mixed infection of yellow speckle with the presence of fanleaf.

To test the hypothesis that grapevine fanleaf virus interacts with viroids to induce vein banding, a field trial using miniplots has been established. Xiphinema index from GFLV infected vines have been inoculated to both Cabernet Sauvignon and Sauvignon blanc in the presence and absence of the major grapevine viroids, GYSV-1, GYSV-2, and HSV-g.

With these tests in place a number of considerations can be discussed for the definition of grapevine viroids and the role of viroids in expression of "disease" symptoms.

(1) Is yellow speckle a single disease caused by GYSVd-1 or GYSVd-2 which is present in over 85% of all grapevines or an amalgam of different diseases, the distinct symptoms of which have not yet been adequately defined?

(2) Is the vein banding symptom induced by yellow speckle viroid in the presence of fanleaf disease or by a yet undefined component of the GYSVd-1 population, i.e., a putative grapevine vein banding viroid or GVBVd?

Since every viroid population is comprised of variants it is difficult to establish the presence of physically distinct subpopulations which may be responsible for the different symptom expression. Nevertheless, analysis of viroids derived from tissue expressing YS and VB symptoms was undertaken to investigate the presence of population heterogeneity which might be linked to disease expression.

A difference in the titer of the "GYSVd-1 like" viroid was noted between nonsymptomatic and YS or VB expressing tissues. A GYSVd-2 like viroid was never found in association with YS expressing tissues. No molecular distinctions could be detected by sPAGE, however, heterogeneity was displayed in apparent full-length cDNA products from PCR amplification with GYSVd-1 specific primers.

PERFORMANCE OF VIROID-FREE CABERNET SAUVIGNON VINES

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California grapevine varieties and rootstocks contain one or more of the three principal grapevine viroids (GYSVd-1, GYSVd-2, and HSVd-g). The effects of viroids on vine performance and wine quality has been impeded by the lack of viroid-free true to type varieties. Performance of the first viroid-free field trials in the world are continuing into the sixth growing season at the Oakville Experiment Station in the Napa Valley, California. In this study, own-rooted Cabernet Sauvignon vines with three different viroid profiles were tested for vine growth, yield, fruit maturity and wine quality. Vines were planted in 1988 on a spacing of 2.4 m X 3.6 m (vine X row) and trained to a bi-lateral cordon, spur-pruned system.

Parental material, certified tested from the Foundation Plant Materials Service, U.C. Davis containing naturally-occurring GYSVd-1 and HSVd-g were used to obtain viroid-free vines by shoot-tip culture (STC). Vines propagated from this viroid-free source were then inoculated with GYSVd-1, GYSVd-2, and HSVd-g.

TABLE 1. EFFECT OF VIROID CONTENT ON GROWTH AND YIELD OF OWN-ROOTED CABERNET SAUVIGNON GRAPEVINES, OAKVILLE, NAPA VALLEY, CA., 1992

TREATMENT	PRUNINGS (kg/vine)	SHOOT NUMBERS	SHOOT WT. (g)	YIELD (kg/VINE)	YIELD/ PRUNINGS
1) STC (Viroid-free)	3.72 a	33.4 a	112 a	9.97 a	2.73 b
2) STC + GYSVd-1 + GYSVd-2 + HSVd-g	3.16 ab	34.0 a	93 a	9.95 a	3.16 ab

Mean separation by Duncan's multiple range, p<0.05.

TABLE 2. EFFECT OF VIROID CONTENT ON YIELD COMPONENTS OF OWN-ROOTED CABERNET SAUVIGNON GRAPEVINES, OAKVILLE, NAPA VALLEY, CA., 1992

TREATMENT	CLUSTERS/ VINE	CLUSTER WT (g)	BERRIES/ CLUSTER	BERRY WT (g)
1) STC (Viroid-free)	64.4 a	155 ab	133 ab	1.16 a
2) STC + GYSVd-1 + GYSVd-2 + HSVd-g	67.4 a	147 b	122 b	1.21 a

TABLE 3. EFFECT OF VIROID CONTENT ON JUICE MATURITY INDICES OF OWN-ROOTED CABERNET SAUVIGNON GRAPEVINES, OAKVILLE, NAPA VALLEY, CA., 1992

TREATMENT	°BRIX	TITRATABLE ACID (g/l ⁻¹)	pH
1) STC (Viroid-free)	22.7 a	8.8 a	3.10 ab
2) STC + GYSVd-1 + GYSVd-2 + HSVd-g	22.7 a	8.4 a	3.15 a

In both 1991 and 1992, measurements were made of vine yield components, juice maturity indices, and dormant season cane pruning weights. In the 1992 harvest, the crop weights were not significantly different. No disease symptoms of yellow speckle or vein banding have been observed to date. Test wines were made from the harvest of each treatment and are being subjected to chemical and sensory evaluation as well as a vertical comparison to the test wine of 1991.