Technical Brief

The Davis Grapevine Virus Collection

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Requests for specific strains of California grapevine viruses, virus and virus-like diseases as well as for specific indicator strains of cultivars are constantly received from various research laboratories over the world for use in research, comparative studies, and teaching. The old Davis virus and virus-like disease collection of own-rooted grapevines established in the 1960s had been degenerating due to phylloxera. A new expanded collection has been established as own-rooted plants and also in graft-inoculated cultivars on rootstocks. Each entry in the new collection has a designated number consisting of descriptive letters and numerals (like GFLV-100). New entries have been and will continue to be accepted. Collections of grapevines are pools of reference and source materials of standard type cultivars of viruses, virus and virus-like diseases. There are three additional collections of grapevines on the UCD campus, each of which serves a different purpose: the Department of Viticulture and Enology collection, the UCD Foundation Plant Materials Services collection of indexed and registered cultivars, and the United States Department of Agriculture - Agricultural Research Services (USDA-ARS) National Clonal Germplasm Repository collection.

KEY WORDS: viruses, viroids, pathogen, type virus collection, leafroll, corky bark, Rupestris stem pitting, grapevine fanleaf virus, tomato ringspot virus, GFLV, LR, RSP, TomRSV, CB

In the past, most grapevine virus detection programs have depended upon biological detection methods using herbaceous and woody indicator plants (9). These biological detection methods provided information on the presence or absence of graft-transmissible disease(s) but not on the specific identity of the agent(s) causing disease(s). In addition, the disease symptoms produced in biological indicators are sometimes similar for dissimilar viruses. Thus, in a clean-stock program based on biological indicators, the emphasis is placed on determining which selections are free of disease agents rather than on the identity of the infectious agent (8,9).For the development of more sensitive and specific detection techniques including serology, electron microscopy, electrophoresis and nucleic acid hybridization, the isolation and identification of individual viruses, and/or disease agents is essential. The utility of serological and nucleic acid probes in detection protocols for the grapevine viruses will depend on the availability of diverse virus isolates, since different types and/or strains of each virus may require individualized probes (3,5,18, 19,22,23,28,29). National and international standards for the use of testing procedures for germplasm movement through quarantine (5,21) depend upon internationally established procedures and a type collection of authenticated pathogen samples. Research on grapevine virus biology is dependent upon these procedures and type isolates. Ideally, virus standard isolates are stored as either purified virus preparations, in greenhouse grown high titer herbaceous hosts, or as freeze-dried plants and/or plant tissue (21). Unfortunately, many of the grapevine viruses and presumed viral diseases cannot be preserved in this way, but rather can be maintained only in living grapevines. Furthermore, although some grapevine diseases have been demonstrated to have a viral etiology and the causal virus itself isolated and characterized, there are diseases caused by graft-transmissible agents (GTA) in grapes that are spread in propagating wood for which a viral etiology is suspected but not yet clearly demonstrated. These diseases, including asteroid mosaic, enation disease, fleck, bushy stunt, Rupestris stem pitting, vein necrosis, and others, can only be maintained in grape tissue as researchers work to identify the causal agent(s). This technical brief describes the grapevine virus collection which has been developed at Davis, California.

The Old Davis Collection

Until recently, the only formal collection of grapevine virus isolates in the U.S.A. was a small demonstration collection of vines planted at the Armstrong farm on the Davis Campus by Dr. W. B. Hewitt (UC Davis Plant Pathology Department, emeritus) and Dr. A. Goheen (USDA-ARS, Davis location, retired) in the early 1960s. These virus isolates have been provided by Hewitt and Goheen to scientists around the world in response to numerous requests for grapevine virus standard isolates and the author continued this service (Table 1). Included in the planting are a number of selections

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infected with virus and/or virus-like agents. In addition, some vines with genetic abnormalities which appear to have virus-like symptoms are included. This collection is composed of own-rooted vines which have become infested with phylloxera in recent years. As a result, some vines have died and others are very weak. Healthy control materials of the diseased selection were not included in the original collection. Some significant disease agents described since the collection was propagated, such as Rupestris stem pitting, are not represented in the collection. For many of the selections, indexing records are incomplete. Other selections are infected with more than one disease agent. The remnants of this collection are endangered because construction is scheduled for this site in the winter of 1992.

Davis is a well known viticultural center and, because grape virology research has a long history in the Davis Plant Pathology Department, requests are received from around the world for the "type strain" of grapevine virus and virus-like diseases. Materials in the current virus collection are not well characterized and cannot satisfy the exacting standards required for type strains.

A standard collection of well characterized grapevine virus isolates was needed. In addition to the need for research isolates, a teaching collection was needed to provide examples of disease symptoms in vines for students, researchers, farm advisors, and California Department of Food and Agriculture (CDFA) biologists. Examples of grapevines with genetic abnormalities, such as variegation and internode shortening (bushiness), would be useful for differentiation between these abnormalities and symptoms caused by GTA. If properly organized, the same collection could serve all these purposes. As a result, the decision was made to propagate a new Davis grapevine virus collection.

The New Davis Collection

The new Davis grapevine virus and virus-like disease collection includes a block of own-rooted virus source vines (VSV) and a trellised clonal virus collection (CVC). The CVC contains three commercial varieties and a rootstock, all chip-bud inoculated with the same set of selected virus isolates.

Selections have come from a number of sources. In addition to the old virus collection, many potentially valuable virus isolates exist in the other Davis grape collections. Davis has three large grape collections: one in the Viticulture and Enology Department, one at Foundation Plant Material Services, and one at the USDA-ARS National Clonal Germplasm Repository. The virus indexing records from Dr. Austin Goheen's program contain data on many of the plants in these three collections. In addition, collaborators have provided field-selected materials of potential interest.

In this way, vines infected with diverse isolates of grapevine viruses and/or GTA have been identified, propagated, and planted in block B-6 of the UC Davis Plant Pathology Armstrong farm. Tests show this site to be free of vector nematodes. The VSV collection represents accessions of virus-infected grapes, propagated in the original *Vitis* varietal host background in which they were discovered. Entries have been selected for a number of different reasons: historical selections of Hewitt and Goheen, source vines of the isolates in the CVC described in this brief, virus isolates used for antisera production (28), vines which are infected with isolates antigenically related to various grapevine virus types (18), vines with interesting indexing histories (11), healthy vines of the same clonal identity as diseased accessions, presumed genetic aberrations with virus-like symptoms, *etc.*

Thirty-two pairs of like clones which should differ only in their viral status have been identified and are established in the VSV collection. These pairs originated from individual vines cured of disease by heat treatment in Dr. Goheen's grapevine clean stock program when, largely due to circumstances, some of the original virus-infected source plants survived in one of the three Davis grapevine collections.

The infection status of each accession in the new collection has been determined when possible using the historic Davis indexing records. Many of these records contain data on a single index result, and in some cases, data is not available for specific indicators because of changes made in particular indicators as the program has evolved (11). Only limited serological testing and nucleic acid analysis has been performed on most of the selections. Some of these selections have served as the source of some recent research projects which have provided additional information about various isolates (3,12,22,23,28). As further biological and/or other forms may be performed on the selections, records will be updated.

The CVC is composed of certified commercial varieties which have been inoculated with selected virus isolates. Three *Vitis vinifera* L. grape varieties widely planted in California were selected to produce bench grafted plants: Thompson Seedless Davis (FPMS selection 02A), Cabernet Sauvignon (Davis selection FPMS 05), and Chardonnay (Davis FPMS selection 04). All are grafted to *Vitis rupestris* St. George FPMS selection 15 which has been chosen for its freedom from GTA (it is self-indexing for many of the most important ones) and the vigor it will provide, producing plentiful wood for grapevine virus research. Additional vines of St. George FPMS 15 alone were chip-budded with each isolate. A core of 24 virus isolates has been grafted to all three varieties and the rootstock (See Table 1). That core includes isolates from the old collection as well as a number of new isolates. The original source vine of the 24 isolates has been propagated in the VSV. After chip budding with the virus isolates mentioned above, they were planted adjacent to the VSV collection in the summer of 1989 with the exception of the St. George which was planted in summer 1990. Healthy indicator vines for field indexing varieties are included as a guard row: Cabernet Franc, St. George, LN 33-1, and Baco 22A. The vineyard is trained to a bilateral cordon system with one cordon wire and two wires on 36-inch

Table 1. The grapevine virus isolate numbers for each of the 24 isolates in the Davis virus collection. Each isolate has been propagated in the original source vine and transmitted to Cabernet Sauvignon, Thompson Seedless, Chardonnay, and St. George vines.

Disease detected by indexing	Isolate number	Original source vine*	References to research using isolate
Asteriod Mosaic	AstMo 100	St. George VC R1 V1, 2	15
Asteroid Mosaic	AstMo 101	Merlot VC R2 V1, 2	15
Corky Bark	CB 100	Semillon VC R3 V7, 8	22,23
Corky Bark	CB 117	Refosco VC R3 V24, 25	work in progress
Corky Bark	CB 118	Ruby Cabernet FPMS-1	work in progress
Corky Bark	CB 120	St. George VC R3 V10, 11	work in progress
Fanleaf	GFLV 100	St. George VC R1 V4, 5	2
Fanleaf	GFLV 101	St. George VC R1 V6,7	2
Fanleaf	GFLV 102	St. George VC R1 V9, 10	2
Fanleaf	GFLV 108	Cabernet Sauvignon FS	27
Fleck	Fleck 101	St. George VC R3 V1, 2	16
Leafroll	GLRaV 101	Italia FV B3 V9	18
Leafroll	GLRaV 102	GR #817 B11 V27	18
Leafroll	GLRaV 106	Thompson Seedless FPMS-1A	18
Leafroll	GLRaV 109	Pinot Noir FS	18,28
Leafroll	GLRaV 110	Thompson Seedless VC R2 V21, 22	work in progress
Rupestris Stem Pitting	RSP 100	Gewürtztraminer FV C2 V5	3,26
Rupestris Stem Pitting	RSP 102	Pinot noir FV G6 V3, 4	3
Rupestris Stem Pitting	RSP 105	Thompson Seedless FPMS-4A	3
Rupestris Stem Pitting	RSP 108	Lanot 244 C0284-14B1	work in progress
Rupestris Stem Pitting	RSP 109	Pinot noir C1186-09A1	work in progress
Rupestris Stem Pitting	RSP 110	Weiser Burgunder C-0782-40B2	work in progress
Rupestris Stem Pitting	RSP 111	Faberreb 0628C-1047-06A1	work in progress
Yellow Speckle**	YS 100	Mission VCR2 V10	

* C = Canadian = Sanachton Station Accession Number; FPMS = Foundation Plant Material Service Selection Number; FS = Field Selection; FV = FPMS Foundation Vineyard; GR = Germplasm Repository; VC = Hewitt Virus Collection.
**See text.

cross arms.

The plants were originally chip-budded while in containers with two grafts in the spring of 1989, one from a bud and one from an internode. After the vineyard was planted, data was taken throughout the summer on the effectiveness of the inoculation by a visual check on chip-bud growth. In the fall, the growing shoot of the buds was cut out, and the chips were observed for healing. If the cambium was still green, it was assumed that the virus had been successfully transferred from the chip to the healthy vine. If none of the previous grafts healed properly, the vine was reinoculated from the same source vine in spring and/or fall of 1990 by field side-grafting. The vineyard was pruned back to two-bud spurs during the dormant season. Replacement vines were planted as needed in spring 1990.

The source plants of each of the 24 virus isolates is being re-indexed to provide additional information on the virus isolates. Additional indexing will be done by colleagues in New York, Canada, and Israel to insure the identity of the most important virus isolates in the collection. Screening of each disease isolate with available grapevine virus antisera collections and for dsRNA content is underway.

A grapevine virus isolate numbering system has been instituted for the Davis grapevine virus collection. A unique number has been assigned to each accession which includes a set of letters representing the disease type or virus (AstMo = asteroid mosaic; CB = corky bark disease; GFLV = grapevine fanleaf virus; GLRaV = grapevine leafroll associated virus; RSP = Rupestris stem pitting; TomRSV = tomato ringspot virus, *etc.*). Graft-transmissible agents which have not yet been proven to be viral are included in this system as are vines with the virus-like symptoms of putative chimeras and other genetic abnormalities. Since the mutations in the vines with virus-like symptoms are not graft-transmissible, those accessions can not involve more than one *Vitis* varietal background. Because the accession number refers to the pathogen rather than the grapevine, it remains consistent even when the varietal background of the host changes; *i.e.*, GFLV isolate 100 exists in the VSV collection and has been grafted to all the varieties in the CVC, where it is still called GFLV isolate 100. Accession numbers will not be reused if additional data obtained on an isolate should demonstrate that the original identification of the disease agent was incorrect; the old accession number will be eliminated with a notation in the records that a new

number has been assigned representing the corrected diagnosis. If a vine should prove to be infected with more than one virus, the isolate number of the first diagnosed disease agent will be continued and a new isolate number assigned to the additional virus isolates. Thus, if a vine containing a leafroll isolate should also be infected with Rupestris stem pitting, a separate isolate number would be applied to each agent.

Discussion and Future Research Plans

There are a number of virus, or presumed virus, diseases of grapevines which have not been included in the Davis grapevine virus collections. Some of these diseases are caused by exotic pathogens such as the European nepoviruses. These pathogens could not be included in the collection without endangering the health of domestic vineyards. Others, such as yellow mottle (alfalfa mosaic virus) and yellow dwarf (tomato spotted wilt virus) are caused by plant viruses which are available from other sources (1) and are not believed to be economically important in grapes.

In addition, there are grapevine virus diseases which may exist in Davis grapevine collections or in other U.S. collections which have not yet been included in the new collection because the test which detects them is not yet a part of the standard grapevine virus indexing used in the United States. Most U.S. importation, quarantine, and certification programs rely on a standard woody index on the indicators *V. rupestris* cv. St. George, the hybrid LN-33 and *V. vinifera* Cabernet Franc (9). Martelli (21) has defined several diseases based on the use of additional indicators: for example, Kober stem pitting detected on the rootstock Kober 5BB; grapevine line pattern virus detected on *V. vinifera* Jubileum 75; and vein necrosis detected on the rootstock Richter 110R. Efforts are underway to identify isolates of these diseases for addition to the Davis collection. The importance of these diseases, like RSP, have not yet been estimated by high stringency replicated field trials.

It is not yet possible to insure that any of the virus isolates in the collection represent single infections of a given virus, free of secondary infections by other virus or virus-like agents. Many of the isolates in the collection have not been indexed on a complete set of indicators nor screened with all available tests. Some multiple infections can not be determined using current detection techniques. Corky bark disease, as defined by Goheen, will produce pitting and grooving above and below the inoculation point on the indicator St. George (9); if Rupestris stem pitting disease is also present, the mild pitting it produces below the inoculation point would be obscured by the pitting and grooving produced by the corky bark agent. Thus, a selection infected by both corky bark and Rupestris stem pitting diseases would emerge from the indexing system identified as corky bark infected with no indication that Rupestris stem pitting disease was also present. The development of an antisera or a DNA probe to the Rupestris stem pitting agent would be a first step in identifying multiple infections of this type. As information is gained about each virus isolate in the collection and technology advances for distinguishing between causal agents, multiple viral infections are likely to be found in the selections in the collection. Although some grapevine diseases have been demonstrated to be caused by well described viruses like grapevine fanleaf virus (fanleaf degeneration) (17) and tomato ringspot virus (yellow vein) (13), other diseases believed to be caused by viruses have not been definitely associated with a single causal agent (rupestris stem pitting disease) (3). Growing evidence suggests that more than one causal agent maybe involved in producing similar symptoms on some indicators [*e.g.*, grapevine leafroll disease (18,29)]. As a result, many of the accessions in this collection represent vines found to be infected with a particular disease by biological indexing but which may eventually prove to infected with more diverse causal agents than currently identified.

Given the rapid advances in viral diagnostics, some diseased selections have undoubtedly been misdiagnosed. For example, the yellow speckle isolate in the collection (see Table 1) was included based on symptoms observed in the selection in the 1960s. At that time, it was suspected that a virus caused the disease. Since then, it has been determined that yellow speckle disease in grapes has a viroid etiology, with two viroids capable of causing the symptoms associated with the disease (19). Recent tests on YS isolate 100 suggest that this selection is infected with the hop stunt viroid, rather than either yellow speckle viroid (A. Hadidi, personal communication).

Viroids have been demonstrated to be virtually ubiquitous in grapevines (24). Although yellow speckle disease is known to have a viroid etiology (19), the effect of other viroids in grapes is unknown. Even yellow speckle seems to be highly variable in expression of symptoms. Viroids are mechanically transmissible by grafting and are likely to be transmitted in chip buds, rootstocks, or scion wood. Therefore, the viroid status of selections in the Davis grapevine virus collection is likely to be a composite profile (25). It might include viroids present in the original selection which provided the virus isolate, any viroids that selection might have acquired during its horticultural past from understocks, and, in the case of the 24 isolates in the CVC established in commercial varieties, those viroids present in the scion and rootstock materials to which the selection was grafted.

The ideal collection of grapevine virus isolates would contain single infections of characterized virus isolates present in the absence of viroids or other infectious agents. Because this ideal is not yet within practical reach, the Davis grapevine virus collection was propagated without regard to the viroid status of the materials. Although viroid-free grapes have been created (7), techniques must still be developed to transmit each of the grapevine viruses of interest to these viroid-free grapevines. Further work in this area could answer a

number of questions about the synergisms between grapevine viruses and viroids in addition to providing viroid free isolates of the grapevine viruses. Until the time that viroid-free, virus-infected selections might be available, the complex viroid background of the current Davis grapevine virus collection must be noted and should be recorded by researchers using the materials.

It is also likely that many of the isolates are additionally infected with *Agrobacterium tumefaciens*. *A. tumefaciens* biovar 3 occurs in systemic latent infections in grapes (6). Although it might be possible to exclude the pathogen from grapes in the virus collection by either shoot tip culture or heat therapy, the possible effects on the virus profile of the materials makes these treatments undesirable at this stage in collection development for the production of virus-infected wood.

The clonal virus collection will eventually provide identical selections in some commercial cultivars differing in viral status. This wood can be used as a source of propagating materials for trials on the effects of the grapevine viruses as well as a source of the virus isolates themselves. Although it is clearly established that some grapevine viruses like GFLV cause severe, economic disease in grapevines (12), the impact of other diseases such as Rupestris stem pitting need to be determined by replicated field studies. In addition, studies are needed to quantify the effects of diseases such as leafroll upon vine performance and wine quality. The author has heard accounts of growers using grapevine leafroll diseases in the field as a devigorating agent; the results of this practice on both vine health and wine quality needs to be better studied.

Research on virus detection will also be facilitated by the availability of each isolate in the CVC in five varietal backgrounds (the original source of the isolate, Thompson Seedless, Chardonnay, Cabernet Sauvignon, and Rupestris St. George) since it will be possible to test new probes against the same isolate of the disease agent in these different host varieties (See Table 1).

Assigning isolate numbers to each virus accession should allow the unique identity of the infecting agent to be preserved despite a multiplicity of *Vitis* varietal sources. For example, the first antisera to grapevine corky bark associated virus was produced with virus purified from a Semillon vine in the old Hewitt virus collection (22,23). That isolate of corky bark virus has been assigned the virus isolate number CB 100.

In addition to vines in the VSV collection propagated from the original infected Semillon, we now have Thompson Seedless, Cabernet Sauvignon, Chardonnay, and Rupestris St. George vines in the VCV collection which have been graft inoculated with CB 100. The CB 100 antisera has been used successfully to confirm the presence of the agent in the inoculated VCV collection. Wood from the collection, infected with CB 100, can be used to produce bench-grafted vines to test the effect of this corky bark associated virus.

At this writing, much remains to be accomplished before this collection can serve the many purposes for which it is intended. It is already being used for teaching on the Davis campus. Students and visitors are able to observe grapevine virus disease symptoms and compare them to non-infectious abnormalities such as spontaneous mutations. Requests have been received from scientists throughout the world for collection selections. Several research projects are underway using materials from one or another part of the collection. As long as support for the collection can be maintained, the collection will be available to scientists for grapevine virus research; this research should, in turn, provide new information about the accessions in the collection as well as contributing to knowledge which will help control the grapevine virus diseases. The current collection represents a necessary starting point serving as the foundation for a better characterized collection of grapevine virus isolates.

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