

# Historical occurrence of Grapevine Red Blotch-Associated Virus

**Maher Al Rwahnih, Adib Rowhani and Deborah Golino**

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University of California, Davis.**

# Novel grapevine viruses discovered by NGS

- 2009 Al Rwahnih et al.: Description of Grapevine Syrah virus 1 (California, USA)
- 2011 Giampetruzzi et al.: Description of Grapevine Pinot gris-associated virus (Italy)
- 2011 Zhang et al.: Description of Grapevine vein clearing virus (Midwest US), the first DNA virus found in *Vitis*.)
- 2012 Al Rwahnih et al.: Description of Grapevine virus F (California, USA)
- 2012 Al Rwahnih et al.: Identification of Grapevine red blotch-associated virus (California, USA). (Same as Grapevine Cabernet franc-associated virus: New York. Krenz et al., 2012)
- 2013 Al Rwahnih et al.: Identification of plant virus satellite. (California, USA)
- 2014 Maliogka and Katis: A putative badnavirus identified in vines affected by Roditis leaf discoloration (Greece)
- 2015 Al Rwahnih et al.: A putative reovirus identified in Cabernet Sauvignon vines (California, USA)

# ***Grapevine red blotch-associated virus* Is Widespread in the United States**

B. Krenz, J. R. Thompson, H. L. McLane, M. Fuchs, and K. L. Perry

## ***Grapevine red blotch-associated virus* is widespread in California and U.S. vineyards.**

M. R. SUDARSHANA (1), A. Gonzalez (1), A. Dave (1), A. Wei (2), R. Smith (3), M. M. Anderson (3), A. M. Walker (3)

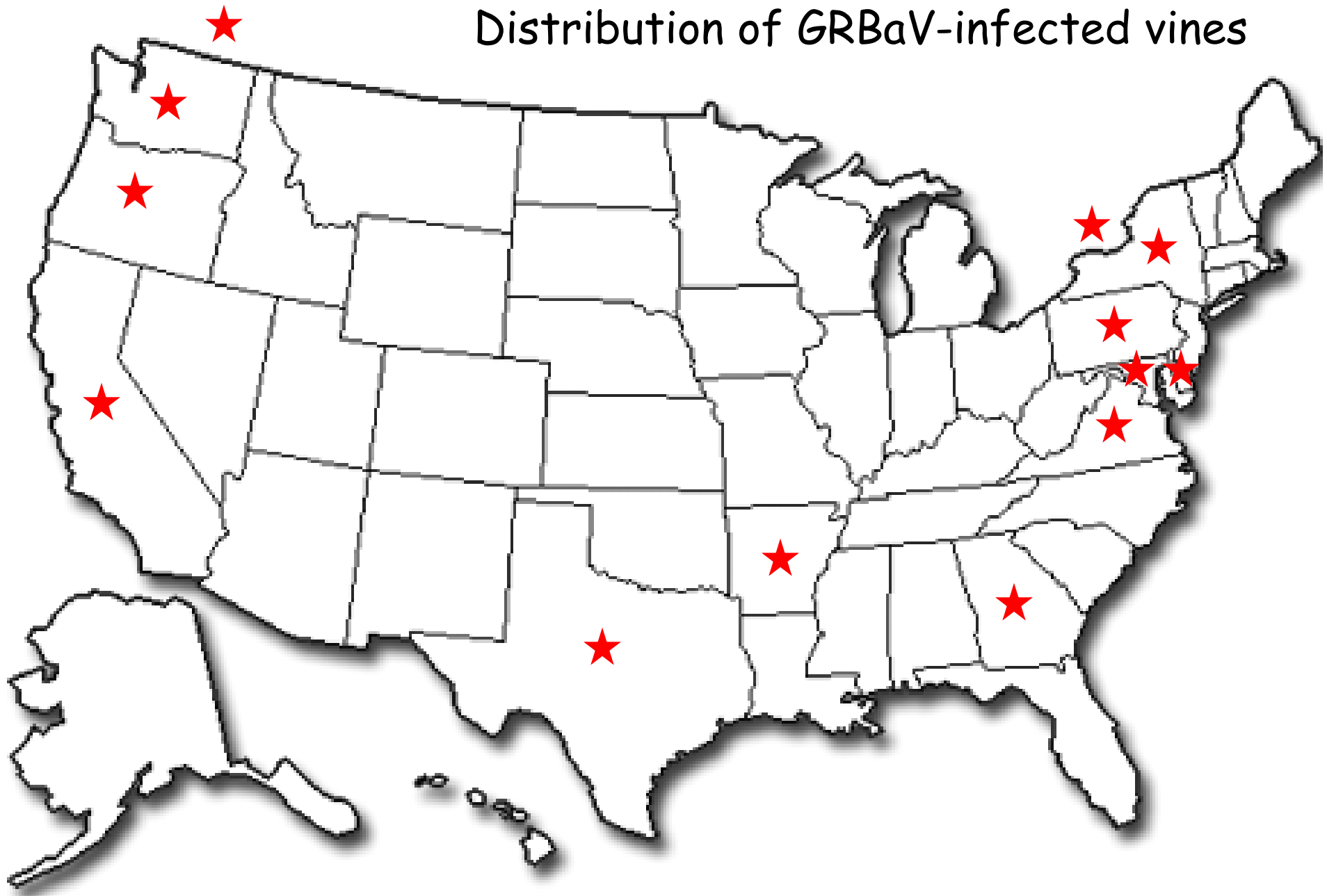
## **Detection and genetic diversity of Grapevine red blotch-associated virus isolates in table grape accessions in the National Clonal Germplasm Repository in California**

Maher Al Rwahnih<sup>a</sup>, Adib Rowhani<sup>a</sup>, Deborah A. Golino<sup>a</sup>, Christina M. Islas<sup>a</sup>, John E. Preece<sup>b</sup> & Mysore R. Sudarshana<sup>c</sup>

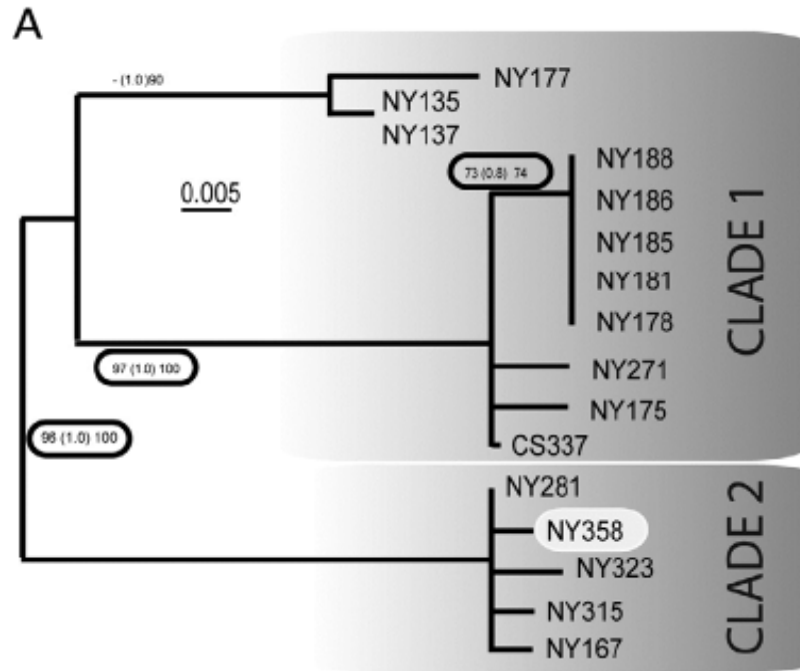
## **Grapevine Red Blotch-Associated Virus, an Emerging Threat to the Grapevine Industry**

Mysore R. Sudarshana, Keith L. Perry, and Marc F. Fuchs

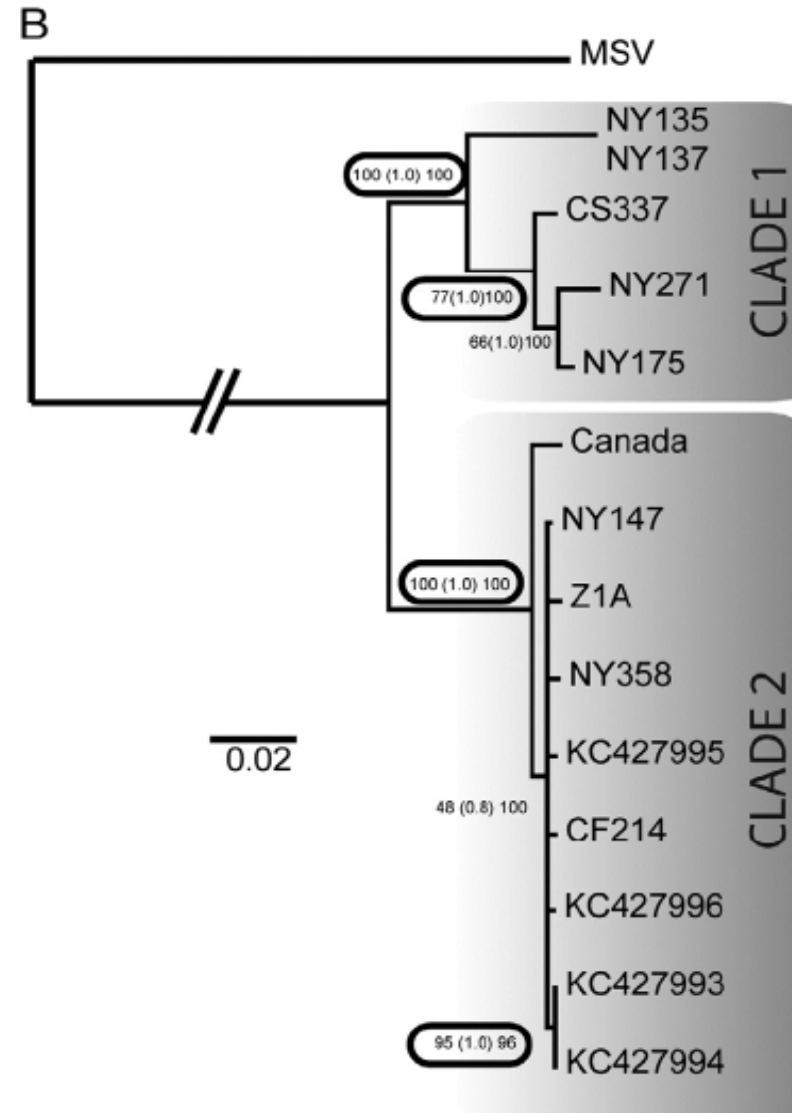
# Distribution of GRBaV-infected vines



# Molecular variability



Replicase gene



complete genome.

Is Red Blotch a novel disease?  
Is it caused by an invasive  
introduction?

# UC Davis Center for Plant Diversity/Herbarium

- The Herbarium archives contain over 300,000 dried specimens.
- Each specimen is labeled and mounted on archival paper.
- Specimen data includes species, cultivar, place and date of origin, and the collector's name.







# Example of a specimen records



Consortium of California Herbaria

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UC Davis

## Accession Detail Results

[UCD is the home institution for this record](#)

Please cite data retrieved from this page: Data provided by the participants of the Consortium of California Herbaria ([ucjeps.berkeley.edu/consortium/](http://ucjeps.berkeley.edu/consortium/); Fri Oct 31 15:16:31 2014).

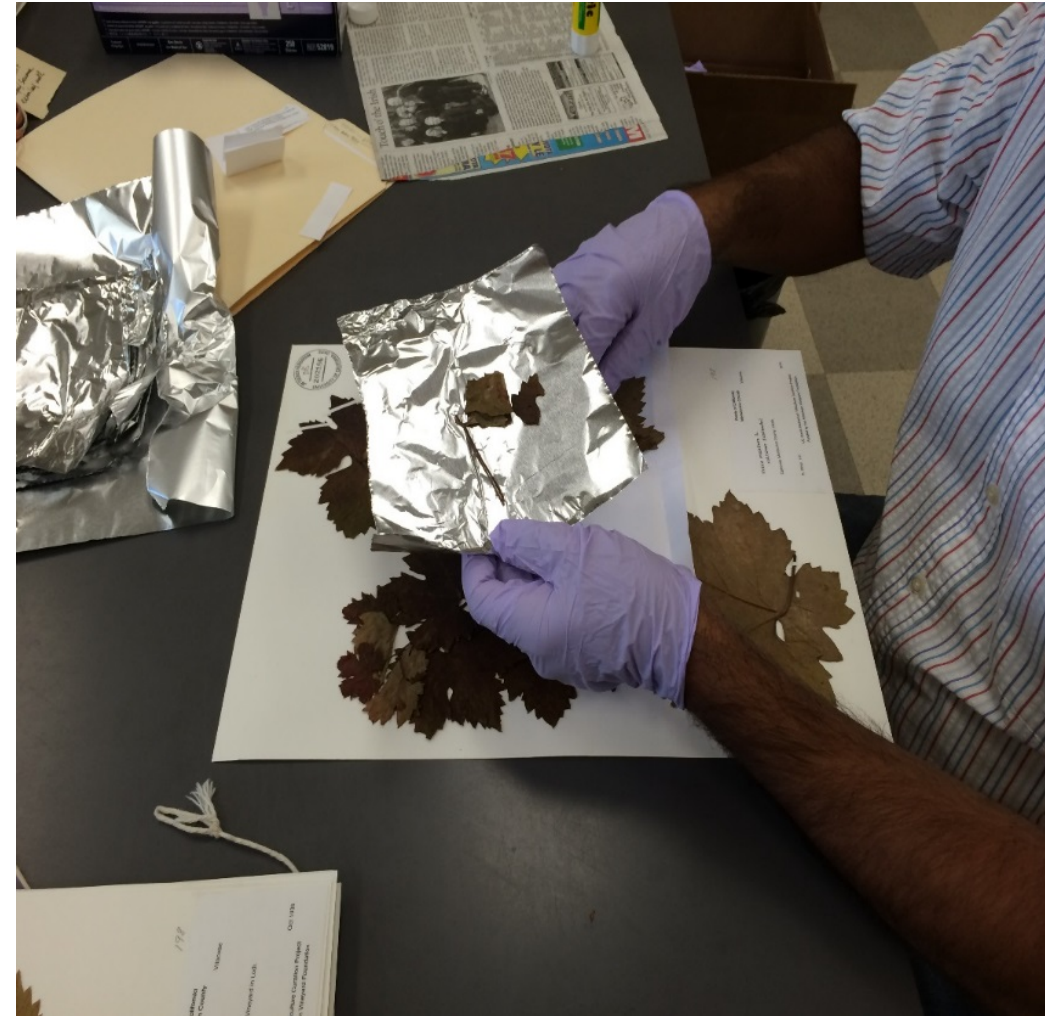
Records are made available under the [CCH Data Use Terms](#).

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Specimen number	UCD17955
Determination	<i>Vitis vinifera</i> <a href="#">More information: Jepson Online Interchange</a>
Collector, number, date	H. Olmo, s.n., 08 11 1940
County	Fresno
Locality	Wahtoke Vineyard in Sanger
Coordinates	36.7042 -119.5553 <a href="#">BerkeleyMapper</a> [or without layers, <a href="#">here</a> ]
Datum	WGS84; ER = 3.612 km
Coordinate source	Geolocate (copied from UCD17950)
Annotations and/or curatorial actions	<i>Vitis vinifera</i> L. cv. Murocain 2010-10-06 Original determination

# Sample collection

- Fifty six grapevine specimens were collected (Approximately 0.5 g of leaf/petiole tissue).
- 23 *Vitis vinifera* and 33 American hybrid samples
- specimens were harvested and pressed between 1937-1950.
- Pieces of foil containing each sample were placed in individual Ziplock bags to prevent cross contamination.
- The 56 sample bags were transported to a lab in which research on grapevine had NOT previously been conducted.



# List of samples: Vitis cultivars from Napa or Sonoma County

Sample #	Herbarium ID#	Variety/cultivar	Location (County)	Year of collection	Collector	Tissue
1	DAV202170	Vitis vinifera L. cultivar Early Burgundy	Sonoma	1940	H. Olmo	Leaves
2	DAV202866	Vitis vinifera L. cultivar aff. Napa Gamay	Napa	1939-1940	H. Olmo	Leaves
3	DAV202172	Vitis vinifera L. cultivar Petite Bouschet	Sonoma	1937	H. Olmo	Leaves
4	DAV202202	Vitis vinifera L. cultivar Rosetta	Sonoma	1938?	H. Olmo	Leaves and petioles
5	DAV202196	Vitis vinifera L. cultivar Zinfandel	Napa	1935	H. Olmo	Leaves and petioles
6	DAV202174	Vitis vinifera L. cultivar Black Malvoisie	Sonoma	1935	H. Olmo	Leaves and petioles
7	DAV202201	Vitis vinifera L. cultivar Burger	Napa	1935	H. Olmo	Leaves and petioles
8	DAV202171	Vitis vinifera L. cultivar Carignane	Sonoma	1937	H. Olmo	Leaves and petioles
9	DAV202870	Vitis vinifera L. cultivar aff. Merlot	Napa	1939-1940	H. Olmo	Leaves and petioles;
10	DAV202872	Vitis vinifera L. cultivar aff. Gamay	Sonoma	1939-1940	H. Olmo	Leaves and petioles
11	DAV202227	Vitis vinifera L. cultivar Grey Riesling	Sonoma	1938	H. Olmo	Leaves and petioles
12	DAV202860	Vitis vinifera L. cultivar Early Burgundy	Napa	1939-1940	H. Olmo	Leaves and petioles

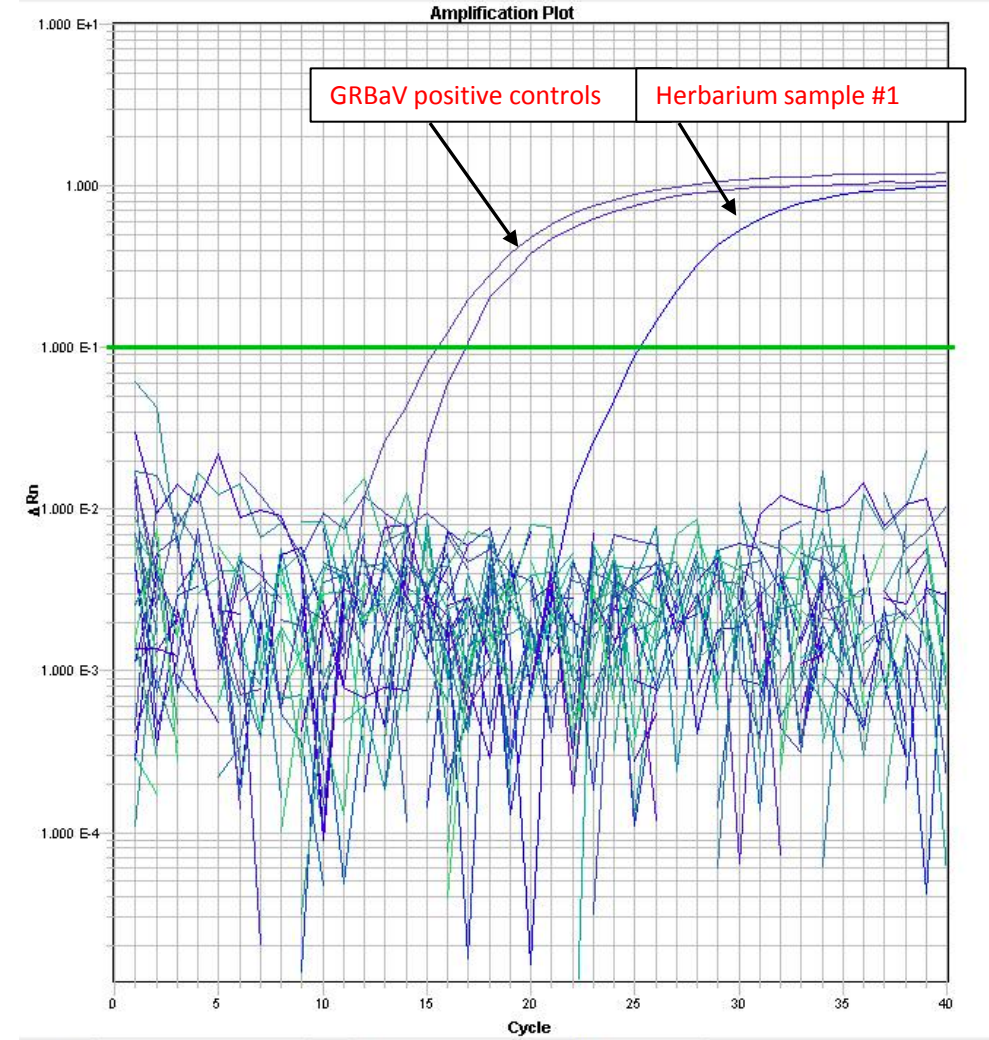
# Precautions to prevent cross-contamination

- Extractions were conducted in an isolated lab (The Michelmore Lab)
- Samples processed with ALL new materials and reagents.



# GRBaV PCR Results

- All herbarium samples were tested for GRBaV using both conventional and Quantitative PCR assays.
- Only one sample was found to be positive for GRBaV (sample #1).



# Sample #1

Cultivar: Early Burgundy

Location: Sonoma

Collector: H. Olmo

Year of collection: 1940



## What is Next: Genomic analysis

Obtain the full genome sequence of GRBaV herbarium isolate and compare it with the recently sequenced GRBaV isolates.

# Confirmation by NGS

- 0.1g of tissue was collected from the original specimen
- Total nucleic acid was extracted in an isolated lab and sent to the sequencing facility at the University of Southern California. (That lab has never worked with grapevine material)
- Next Generation Sequencing generated about 88 million Illumina reads
- More than 92,000 reads mapped specifically against the GRBaV genome

The image is a screenshot of the USC Epigenome Center website. The header features the USC Epigenome Center logo and navigation links: HOME, ABOUT THE CENTER, PEOPLE, SERVICES, RESEARCH, and RESOURCES. The main content area is titled "Next Generation Sequencing" and includes a "The Team" section with photos and names of Selene Tyndale and Helen Truong. Below this is a "New Equipment!!" section with photos of a NextSeq 500 and a MiSeq sequencer, each with a caption indicating their approximate read rates. A "Resources" sidebar on the left lists services like Biospecimens, Next Generation Sequencing, and Infinium DNA Methylation450. A "Services" sidebar on the right lists options like Returning Users, Getting Started with NGS, and Data Recovery and Analysis. At the bottom, there is a paragraph about the Molecular Genomics Next-Gen Sequencing core and a link for pricing information.

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## Next Generation Sequencing

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**Resources**

- Links of Interest
- Datasets
- Protocols

**The Team**

Selene Tyndale, Research Lab Technician III

Helen Truong, Research Lab Technician II

**New Equipment!!**

NextSeq 500  
Approx. 400 M Reads/run

MiSeq  
Approx. 15-25 M reads/run

The Molecular Genomics Next-Gen Sequencing core has acquired the latest sequencing instruments from Illumina; the MiSeq and the NextSeq 500. The main advantage for our user base is faster turnaround times. Runs that take two weeks on the HiSeq can now be done within 24 hours. These instruments also provide greatly enhanced capacities for certain types of projects such as targeted resequencing, microbiome studies, and metagenomics. Contact us to find out how you can use these new technologies and capacities to advance your lab's projects.

Pricing and more information [here!](#)

**Operations Overview**

**Next Generation Sequencing**

The Next Gen core features several Illumina instruments, including the pictured HiSeq 2000, to meet your data production needs.

**Next Generation Sequencing**

Charles Nicolet, Ph.D., Director of Sequencing Technology  
(323) 442-7988

**Infinium DNA Methylation450**

Daniel Weisenberger, Ph.D., Laboratory Manager/Coordinator  
(323) 442-7786

**Genotyping / Microarrays**

David Van Den Berg, Ph.D., Production Director  
(323) 442-4341  
(323) 442-4466



# Blast Results

- The full genome sequence of this herbarium isolate (Accession No. KP221559) shared **92-99%** nucleotide identity with other GenBank isolates.
- The virus was a member of a mixed infection with GLRaV-2 and GVB.



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<http://dx.doi.org/10.1094/PDIS-12-14-1252-PDN>

## DISEASE NOTES

# First Report of Grapevine red blotch-associated virus in Archival Grapevine Material From Sonoma County, California

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## FPS Research Shows Red Blotch in Calif. Since 1940?

**FPS Meeting Provides Update on CDFA Grapevine Certification Program Revision Plan**

*Posted by [Ted Rieger](#) on November 21, 2014*

Based on recent tests performed with dead grapevine leaf material on file in the University of California Davis (UCD) Plant Herbarium, UCD Foundation Plant Services (FPS) director Deborah Golino stated, “Red blotch has been present in the Sonoma County winegrape growing area for at least 74 years.” Golino announced the finding at the annual FPS meeting held November 20 in Davis to update nursery industry representatives on FPS activities.

The study used currently available laboratory testing technology for Grapevine Red Blotch-associated Virus (GLRaV) on 56 different grapevine specimens placed in the UCD herbarium from 1937-1950. Only one specimen tested positive--a sample of the cultivar Early Burgundy collected by UCD viticulture professor emeritus Dr. Harold Olmo from a Sonoma County vineyard in 1940.

Thank you