Update on Grapevine Red Blotch Virus

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GRBaV Presentation Outline

• Virology
• Red Blotch Is Widely Distributed in the U.S.
• Correlation of Disease Severity with Virus Level
• Virus distribution in a vine
• Virus level variation among vines
• Red Blotch is graft-transmissible
• Status of CDFA-certified Grapevine Stock
• USDA Assistance Program
• Sampling and Testing Strategies
GRBaV Understanding to Date

- There are two genetic clades of GRBaV with up to 8% sequence heterogeneity; no as of yet recognized biological differences among isolates.
- GRBaV has been detected in both cultivated and wild vines, not in other hosts.
- GRBaV is an atypical virus. **No serological assays** are available despite intense effort by several research groups. No twin particles has been observed.
- GRBaV is **graft transmissible**, is NOT mechanically transmissible.
- **Symptom severity** is strongly correlated to virus titer level.
- Insector vector – Three-corned alfalfa treehopper was shown to transmit GRBaV under greenhouse conditions.
What is a Red Blotch Virus?

Geminiviruses: a tale of a plasmid becoming a virus
By Mart Krupovic*, Janne J Ravantti and Dennis H Bamford

*BMC Evolutionary Biology 2009, 9:112
Leafroll 3 Virus by Comparison

It is hard to tell Red Blotch from Leafroll Infection.

PN 777/3309, Medford, Oregon
Grapevine red blotch-associated virus is widespread in the United States.

Marc Fuchs et al, Phytopathology. 2014 May 7.
LR3 and RBaV in CA Vineyards

**Figure 2** Proportion of all samples tested at Agri-Analysis contaminated with GRBaV or LR3. Note: Fall through the end
LR3 and RBaV in CA Vineyards

Yearly Average Incidence Rate of GRBaV and GLRaV-3 in All Samples Tested at Agri-Analysis (Davis, CA) between October 2012 and March 2016
Other Key Viruses in CA Vineyards

Monthly Average Incidence Rate of GRBaV and GLRaV-3 in All Samples Tested at Agri-Analysis (Davis, CA) between April 2015 and March 2016
CS07xO3916 – Normal Fall Foilage
CS07xO3916 – Speckled Red Leaf Foilage
CS07xO3916 – Complete Red Leaf Foilage
Symptom Severity Depends on GRBaV Virus Level
GRBaV is Distributed Throughout the Vine
YES, GRBaV is found in berries

- Seed or pip
- Exocarp – extending inwards to the vascular bundles, on one definition
- Mesocarp – the bulk of the pericarp
- Endocarp – separating the pericarp from the seed
- Epidermis or skin
- Peripheral vascular bundles

Agri-Analysis
Your plant and seed testing resource
GRBaV Level in Vines Can Vary by Several Orders of Magnitude

![Bar chart showing GRBaV levels in different vines, with one vine (#19) having significantly lower levels compared to others.](chart.png)
GRBaV is Graft-Transmissible
**RBoV is Graft Transmissible**

**Figure 1** Virus levels in sampled tissues illustrated in photo above.
After a 5-month test under greenhouse conditions, three of the 15 plants exposed to three-cornered alfalfa tree hoppers (ATH) became positive for GRBaV.

Work of Dr. Brian Bahder, Dr. Mysore "Sudhi" Sudarshana and Dr. Frank Zalom

SOURCE: UCANR
GRBaV Vector Questions

1. Is ATH the only vector to transmit GRBaV or there are other insects involved?

2. What is the distribution and prevalence rate of ATH in California vineyards and other major wine regions of the U.S.?

3. Is ATH responsible for the spread of RB in California vineyards? or lack thereof in other states and other countries?

4. Does ATH transmit other viruses besides red blotch?

5. What should growers do to manage ATH in their vineyards?
LR3 & RBaV in Certified Nursery Stock

- GRBaV is found in CDFA-certified scion blocks.
- GRBaV is also found in CDFA-certified rootstock blocks but at a lower frequency 101-14, 420A, 3916,
- GRBaV is frequently found in non-CDFA-certified scion field selections and clones.
- There is a strong correlation between the presence of Red Blotch symptoms and the GRBaV virus. This suggests that removal of symptomatic vines may help control the disease.
"Red Blotch virus is present in some certified nursery stock."

"better understanding of how clean grape nursery stock can become infected with virus and better detection techniques are required to eliminate damaging viruses from our certified stock."

Dr. Deborah Golino, Director, Foundation Plant Services, UCDAVI

Source: Wine Business Monthly Article by James Stamp and Alan Wei, August, 2014
Table 4. Testing of RS and scion IB materials. November 2012-May 2014

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*-1, *-2: different increase block sources
Factors Affecting Quality of Certified Nursery Stock

• GRBaV is not part of certification until fall of 2016
• Infected mother vines (rootstock and scion wood)
• Spread by Insect Vectors (Alfalfa treehopper, mealy bugs, etc.)

• Insufficient sampling rate and sampling frequency of certified blocks
• Virus variability and testing methods
The 2014 Farm Bill authorized Tree Assistance Program (TAP) to provide financial assistance to eligible growers to rehabilitate eligible trees, bushes, and vines lost by natural disasters.

- Red Blotch was approved in Select Counties in California and Oregon for loss in excess of 15 percent mortality.

- TAP is administered by the Farm Service Agency (FSA) of the U.S. Department of Agriculture (USDA).

- Jennifer Blume, County Executive Director, San Luis Obispo County FSA Office, 65 S. Main St Ste 106, Templeton, CA 93465 (805)434-0398 Ext 2
USDA RED BLOTCH ASSISTANCE
Testing Requirement

• A claim of loss due to GRBaV must be verified through polymerase chain reaction (PCR) testing performed at a commercial lab.

• A minimum of ten (10) symptomatic vines must be tested from each block claimed to be infected. This sampling, coupled with visual indicators identifiable to the FSA representative, will be used to determine the extent of damage. Visual indicators:

  • Red Varieties: reddening of underside secondary/tertiary veins, red blotches on leaves;

  • White Varieties: very pale yellow blotches and irregular chlorosis.
Three Types of GRBaV TESTING SERVICES At AGRI-ANALYSIS

- **Presence and Absence** of GRBaV Testing by Polymerase Chain Reaction (PCR);

- **Quantitative Testing of GRBaV Titer** by qPCR. Our research had shown that Virus titer can vary by several orders of magnitude from a slightly symptomatic vine to a heavily symptomatic one.

- **GRBaV Clade Type Identification**. GRBaV is known to exist in two clade types, differing by up to 8% in nucleotide composition.
Sampling & Testing Strategies

• Certified Materials are often found to be infected with Red Blotch and LR3 and shall be presumed as such until proven clean.

• Test every mother vine

• Test statistically significant number of final grafts

• Use composite sampling to reduce testing cost
Simple Math on Sampling Five Mother Vines

- **5 mother vines** → 1,000 scion cuttings
- $200 testing for ten virus panel
- $200/1,000 = 0.2 per cutting
- 0.2/3 = 6.7%!
Question everything
ACKNOWLEDGEMENT

Agri-Analysis Team:

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Dr. Xiaodong Wang
Martin Gil
Andy Zinkl
Dr. Sara Rhokit
Alessandro Viani
Dr. Alan Wei
Comparison of AA and BIOReba ELISA

- Bioreba Reagent
- Agri-Analysis New Polyclonal

Data points for samples 9775-4, 9775-6, 9775-9, 9854-27, 9858-17, and 9730-1.