An Overview of Grapevine Viruses in Washington Vineyards

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Estimated economic impact of leafroll in Merlot over a 20-yr period:

A grower experiencing

• 10% decrease in yield and 0.4° Brix reduction = $3,005 per acre
• 30 percent yield decline and 1.0° Brix decrease = $19,800 per acre
Washington State Wine Commission Survey

- The highest ranked topic with a score of 4.5 out of 5.0:
  - the impact of viticultural practices on wine flavor

- The second ranked topics with a score of 4.0 out of 5.0
  - grapevine viruses
  - fermentation practices

Source: http://www.goodfruit.com/wine-grape-revelations/
July 5, 2016
Grape Virology Program

Aligned with industry’s priorities

Grape and wine industry research priority
Management of viruses that impact vine health and fruit quality
Research on critical topics

- How many viruses are present in WA?
- Impact of viruses.
- Spread, with emphasis on leafroll disease.
Grapevine is a ‘treasure trove’ of viruses

AT LEAST 70 VIRUSES AND VIRUS-LIKE AGENTS REPORTED WORLDWIDE

Current status of grapevine viruses

- Conducted surveys in vineyards
- Optimized sampling strategies
- Tested samples using state-of-the-art diagnostic methods
  - PCR technology
  - Next-generation sequencing
Current status of grapevine viruses

As of 2016

GLRaV-1
GLRaV-2
GLRaV-3
GLRaV-4
GLRaV-4 (strain -5)
GLRaV-4 (strain -9)

GLRaV

Leafroll complex

GRSPaV

Rugose wood complex

GVA
GVB
GVE
GFLV
TRSV

Fanleaf degeneration/decline

GFkV

Fleck

GSyV-1

Syrah decline??

GRBaV

Red blotch
Current status of grapevine viruses

- Leafroll: LR1 (4.7%), LR2 (11.0%), LR3 (50.7%), LR4 (9.6%), LR5 (2.9%), LR9 (1.3%)
- Rugose wood: GVA (9.5%), GFLV (14.2%), GVB (4.1%), GVB (3.5%)

Total # of vines tested = 2083
Current status of virus diseases

**Established**
- Leafroll

**Emerging**
- Red blotch
- Soil-borne
# Grapevine leafroll disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cuttings</th>
<th>Mealybugs</th>
<th>Soft scales</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLRaV-1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>GLRaV-2</td>
<td>Yes</td>
<td><strong>No</strong></td>
<td><strong>No</strong></td>
</tr>
<tr>
<td>GLRaV-3</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>GLRaV-4</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>GLRaV-7</td>
<td>Yes</td>
<td><strong>No</strong></td>
<td><strong>No</strong></td>
</tr>
</tbody>
</table>

Wine grape cultivars respond differently to leafroll disease

Red grape varieties
  e.g. Cabernet Sauvignon

White grape varieties
  e.g. Chardonnay
Red blotch

New grape disease reduces yields, quality

A new disease that threatens the health of grapevines nationwide highlights the need for stronger clean plant campaigns. The disease, first reported in a Napa Valley vineyard in 2006, has been identified in infected vines in the top three U.S. grape growing regions of California, Washington, and New York, as well as a few other East Coast and southern states, and Canada.

Washington State University researchers are tentatively calling the new disease grapevine red leaf disease due to the red to purple color on leaves of red varieties. Symptoms range from red veins and blotches to total reddening of the leaves in some red grape cultivars. Veins on the leaf underside can be pink or red, or the major veins can still be green.

California and New York scientists have dubbed it “grapevine red blotch disease,” but WSU scientists preferred to use the broader term “redleaf” to cover the full spectrum of symptoms, so that growers pay attention to all suspicious red leaves.

The difference in symptoms could be due to own-rooted vines being used in Washington and grafted vines in California, New York, and other places.

Misidentified in vineyards?

The symptoms of redleaf disease in red-fruited cultivars may look similar to those produced by grapevine leafroll disease, but the new disease is distinct in several respects. Like leafroll, redleaf symptoms show up just after veraison and look very similar to leafroll, which could be why growers and university researchers never paid much attention to it in the vineyard. Many just thought it was leafroll virus. But the epidemiological aspects are quite distinct.

It’s like a person having the flu or West Nile virus. Symptoms can be similar, but the nature of the virus and mode of its spread are very different, and the same treatment doesn’t work for both. With leafroll disease, growers have focused on controlling grape mealybug and scale infestations to slow the spread of the disease. But with redleaf, a new vector is suspected, and a different set of management tactics is required.

How does it spread?

WSU bench graft tests showed that redleaf disease is graft-transmissible. Symptoms on the scion (virus-free Cabernet Franc) were similar to those observed in the source vines used as the rootstock. Based on the
Leafroll symptoms

- Green veins
- Interveinal reddening
- Downward rolling of leaf margins

Red blotch symptoms

- Red veins
- Red blotches
- Interveinal reddening
Is it leafroll or red blotch?
cv. Cabernet franc
Is it leafroll or red blotch?

Difficulty with symptom-based diagnosis

cv. Cabernet Sauvignon

cv. Syrah

cv. Petite Sirah

Red blotch

Leafroll

Leaf roll
Is it leafroll or red blotch?

cv. Sauvignon Blonc

cv. Chardonnay

Difficulty with symptom-based diagnosis

cv. Semillon
Survey conducted in 2014 & 2015 seasons in five AVAs.

Red-berried cultivars: samples from grapevines exhibiting symptoms of GLD or GRBD and suspected for GLD- or GRBD-like symptoms.

White-berried cultivars: random samples from grapevines due to the absence of visual symptoms of GLD and GRBD.

Leafroll (GLD) and red blotch (GRBD) in WA vineyards

Adiputra et al., unpublished data
Leafroll and red blotch in WA vineyards

~1,500 samples during 2014 and 2015 seasons:
14 Red-fruited + 5 White-fruited
Tested by RT-PCR (GLRaV-3) and PCR (GRBaV)

Adiputra et al., unpublished data
Fanleaf degeneration/decline

Grapevine fanleaf virus
Tobacco ring spot virus

cv. Grenache

cv. Merlot

cv. Cab franc

cv. Merlot

cv. Grenache
Grapevine fanleaf virus – *Xiphinema index* absent
Tobacco ring spot virus – *Xiphinema americanum* present
Current status of virus diseases

- Leafroll (GLRaV-3)
  - predominant & widely distributed
- Red blotch (GRBaV)
  - less predominant compared to GLRaV-3
- Soil-borne viruses (TRSV)
- Co-infections can occur

  - Symptoms are similar, though not identical, in many red-berried cultivars
  - Like leafroll, no apparent symptoms of red blotch in white-berried cultivars
  - Symptom-based diagnosis is not reliable and diagnostic assays should be used for reliable ID
Issues regarding sampling and virus testing
<table>
<thead>
<tr>
<th>Disease</th>
<th>Early season (Spring)</th>
<th>Late season (Fall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanleaf</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Leafroll</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Red blotch</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
When to collect samples?

Leafroll  Pre-Véraison  Red blotch

Post-Véraison
When to collect samples?

GLRaV-3 titer (by ELISA)

No correlation between presence of virus and time of symptom expression.
When to collect samples?

Symptoms on foliage during the season

Canes during winter/dormancy period show no symptoms
When to collect samples?

Collect leaf samples with petioles during the season.

Collect cane samples 3-4 nodes length in Winter.
What type of test to use?

<table>
<thead>
<tr>
<th>Samples</th>
</tr>
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<tbody>
<tr>
<td>(+)</td>
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<tr>
<td>(-)</td>
</tr>
</tbody>
</table>

Molecular assays (PCR)

Serological assays (ELISA)
Limitations of ELISA

Variability among viruses (e.g. GLRaV-2 strains)

ELISA

OD (A405)

RT-PCR

e.g. Red blotch virus can be tested only by PCR

332 bp
What type of samples?

<table>
<thead>
<tr>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr</td>
</tr>
<tr>
<td>-ve</td>
</tr>
<tr>
<td>+ve</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
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<td>6</td>
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<td>7</td>
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<tr>
<td>8</td>
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<td>9</td>
</tr>
</tbody>
</table>

Leaf

Cane

Detection by PCR methods
Take-home message

- Viruses distributed systemically in different parts – can use leaves, canes, and roots of an infected grapevine for testing.

- Viruses can be detected throughout the season using leaf (petioles) and cane samples.

- Viruses can be detected using dormant canes in winter.
Impact of grapevine viruses

Leafroll
Red blotch
Grapevine leafroll disease
Impact on fruit yield

Infected Healthy

cv. Cabernet Sauvignon

Healthy Infected

cv. Merlot

Infected Healthy

cv. Cabernet Sauvignon

Healthy Infected

cv. Merlot
Leafroll : Impact on yield (cv. Merlot)

Average loss/year ~25%

Total weight of clusters per vine (Kg)

Year

2008 2009 2010 2011 2012

No leafroll Leafroll

2008: -17.17%
2009: -27.82%
2010: -15.82%
2011: -20.57%
2012: -11.89%

No leafroll Leafroll

Average loss/year ~25%
cv. Cabernet Sauvignon     cv. Pinot noir

Infected Healthy     Infected

Leafroll disease
Impact on fruit quality

cv. Cabernet franc               cv. Chardonnay

Infected Healthy

Infected Healthy
# Leafroll: Impact on fruit quality

Total soluble solids (°Brix) at harvest (cv. Merlot)

<table>
<thead>
<tr>
<th>Year</th>
<th>Healthy</th>
<th>Leafroll</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>24.83 ±0.09</td>
<td>23.30 ±0.10</td>
<td>-6.16% ***</td>
</tr>
<tr>
<td>2010</td>
<td>25.03 ±0.32</td>
<td>23.10 ±0.29</td>
<td>-7.71% *</td>
</tr>
<tr>
<td>2011</td>
<td>23.50 ±0.06</td>
<td>22.53 ±0.15</td>
<td>-4.13% *</td>
</tr>
<tr>
<td>2012</td>
<td>25.96 ±0.05</td>
<td>24.58 ±0.00</td>
<td>-5.31% ***</td>
</tr>
</tbody>
</table>

*, p = 0.05-0.01; **, p = 0.01-0.002; ***, p < 0.002
Impacts of Grapevine Leafroll Disease on Fruit Yield and Grape and Wine Chemistry in a Wine Grape (Vitis vinifera L.) Cultivar

Olufemi J. Alabi\textsuperscript{1,\textsuperscript{*}}, L. Federico Casassa\textsuperscript{2,\textsuperscript{\textbullet}}, Linga R. Gutha\textsuperscript{1,\textsuperscript{\textbullet}}, Richard C. Larsen\textsuperscript{2}, Thomas Henick-Kling\textsuperscript{2}, James F. Harbertson\textsuperscript{2}, Rayapati A. Naidu\textsuperscript{1,\textsuperscript{*}}

1 Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, Washington, United States of America, 2 Viticulture and Enology Program, Washington State University, Wine Science Center, 2710 Crimson Way, Richland, Washington, United States of America
“The impacts of leafroll on yield and fruit and wine quality traits were variable between the seasons, with greater impacts observed during a cooler season, suggesting the influence of Genotype (G)-by-Environment (E) interactions on overall impacts of the disease”
Grapevine Red Blotch Disease

Impacts on fruit yield

cv. Merlot
Impacts of red blotch

Total soluble solids (°Brix)

<table>
<thead>
<tr>
<th>Year</th>
<th>Healthy</th>
<th>Infected</th>
<th>[%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>24.18</td>
<td>21.00</td>
<td>-13.15%</td>
</tr>
<tr>
<td>2014</td>
<td>24.36</td>
<td>21.46</td>
<td>-11.90%</td>
</tr>
<tr>
<td>2015</td>
<td>25.32</td>
<td>22.78</td>
<td>-10.03%</td>
</tr>
</tbody>
</table>
Summary points

• Both leafroll and red blotch affect fruit yield and quality in the cv. Merlot examined.

• Impacts on berry sugars and anthocyanins more pronounced than grape juice pH and TA.
Spread of grapevine viruses

All viruses can be spread via planting materials

No chemicals to cure infection and make a sick plant healthy

Prevention better than cure
- use ‘clean’ plants
- control vectors
Virus spread via top-grafting
The ‘Mantra’ of ‘start clean’

Use virus-tested ‘clean’ plants as the first line of defense
Source of planting stock matters

Nurseries

Clean Plant Center Northwest

Healthy vineyards

Neighbors/Friends/Your own blocks

Outside the state

http://cpcnw.wsu.edu
Source of planting stock matters

Blocks planted with virus-tested ‘clean’ cuttings
Spread via compromised planting stock

5.5 acre block
Symptomatic vines – 11.8%
GLRaV-3 +ve

Cabernet Sauvignon
Spread via compromised planting stock

cv. Cabernet Sauvignon 2015 planting

11.8% in 2015

Roguing

1.0% in 2016
Spread via compromised planting stock stock
cv. Syrah 2015 planting

3.5% in 2015

Roguing

0.05% in 2016
Test source vines before planting new vineyards

Cuttings with no virus

Healthy vine

Cuttings with virus

Infected vine
The ‘Mantra’ of ‘stay clean’

- Do ‘clean’ plantings remain healthy? (stay free from viral infections?)

- What is the risk of leafroll spread from neighboring old blocks?

Monitored new plantings for several seasons (time and space)
Monitor spatial distribution and temporal spread of grapevine leafroll disease

Geographically separate locations, but adjacent to old blocks totally infected with leafroll
Monitor spatial distribution and temporal spread of grapevine leafroll disease

- Monitor blocks for leafroll symptoms during each season.
- Record symptomatic vines in a XY-matrix using the row number and vine position as co-ordinates.
- Confirm symptomatic vines for GLRaV-3.
- Compare the data obtained in previous seasons.
- Spatial and temporal distribution maps for each season using cumulative data of new infections from that season and the preceding seasons.
Results
cv. Cabernet Sauvignon

- Increased number of symptomatic vines each season
- A gradient of infected vines – indication of initial spread from heavily infected old blocks
- Clustering of symptomatic vines with time – indication of secondary spread
Results

cv. Syrah

- Increased number of symptomatic vines each season.
- A gradient of infected vines – indication of initial spread from heavily infected old blocks.
- Clustering of symptomatic vines with time – indication of secondary spread.

A. Map of the block

B. Spread of GLD in time and space


2.20 3.97 5.74 11.10 13.40 18.10 25.46
Results

cv. Petite Sirah

- Increased number of symptomatic vines each season.
- A gradient of infected vines – indication of initial spread from heavily infected old blocks.
- Clustering of symptomatic vines with time – indication of secondary spread.
Multi-year field studies on the spread of GLD in three wine grape cultivars have provided convincing evidence that:

- Young vineyards planted with ‘clean’ virus-tested planting stock can become infected with GLD.
- Rate of spread may depend on site-specific influences (viz. proximity to infected blocks, weather-driven factors, vector species composition, virus strains, etc.).
Early intervention by roguing

A. Map of the block

B. Spread of GLD in time and space

**Cabernet Sauvignon**

<table>
<thead>
<tr>
<th>Year</th>
<th>Infection level (%)</th>
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<tbody>
<tr>
<td>2007</td>
<td>0.15</td>
</tr>
<tr>
<td>2008</td>
<td>0.39</td>
</tr>
<tr>
<td>2009</td>
<td>0.66</td>
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<tr>
<td>2010</td>
<td>1.16</td>
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<tr>
<td>2011</td>
<td>3.13</td>
</tr>
<tr>
<td>2012</td>
<td>4.9</td>
</tr>
<tr>
<td>2013</td>
<td>5.6</td>
</tr>
<tr>
<td>2014</td>
<td>8.72</td>
</tr>
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**Syrah**

<table>
<thead>
<tr>
<th>Year</th>
<th>Infection level (%)</th>
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</thead>
<tbody>
<tr>
<td>2004</td>
<td>2.20</td>
</tr>
<tr>
<td>2008</td>
<td>3.97</td>
</tr>
<tr>
<td>2009</td>
<td>5.74</td>
</tr>
<tr>
<td>2011</td>
<td>11.10</td>
</tr>
<tr>
<td>2012</td>
<td>13.40</td>
</tr>
<tr>
<td>2013</td>
<td>18.10</td>
</tr>
<tr>
<td>2014</td>
<td>25.46</td>
</tr>
</tbody>
</table>

**Petite Sirah**

<table>
<thead>
<tr>
<th>Year</th>
<th>Infection level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>4.41</td>
</tr>
<tr>
<td>2012</td>
<td>15.15</td>
</tr>
<tr>
<td>2013</td>
<td>24.30</td>
</tr>
<tr>
<td>2014</td>
<td>42.14</td>
</tr>
</tbody>
</table>
Blame the bugs?

- Grape mealybugs (*Pseudococcus maritimus*) as vectors
- Soft scale insects (*Parthenolecanium corni*) as vectors

Roguing: Remove infected vines

Remove symptomatic (infected) vine
Remove at least one (or two) non-symptomatic vines on either side
Roguing as a post-planting strategy

Roguing (within 3-4 years post-planting) and replanting for reducing leafroll spread in new plantings
Overall Conclusions

• Virus diseases continue to be a major concern
  - leafroll viruses
  - red blotch virus
  - soil-borne viruses
  - what else is out there?

• Visual symptoms are not reliable
  - Accurate diagnosis is vital
Overall Conclusions

- Spread via cuttings used for new plantings
- Spread by insect vectors (e.g. mealybugs, scale insects) from old blocks
- Why leafroll 3 is more widespread and prevalent?
- Vector control strategies – when to spray & what to spray?
Technical contributors

Dr. Basu Bagewadi
Dr. Prashant Swamy
Dr. Sridhar Jarugula
Ms. Bhanu Donda
Ms. Nagateja Natra
Mr. Jati Adiputra
Mr. Leslie Walker
Ms. Lakshmi Movva
Many post-docs and graduate students worked earlier
Thanks to:

- Wine Research Advisory Committee of the Washington State Wine Commission
- Washington Association of Wine Grape Growers
- WSU-CAHNRS Agricultural Research Center
- Washington Grape and Wine Research Program
- WSU Viticulture & Enology Program
- CAHNRS Agricultural Extension
- WSU New Faculty Seed Grant Program
- Nursery Research & Grapevine Research Funds
- Specialty Crop Block Grant Program
- Specialty Crop Research Initiative
- Northwest Center for Small Fruits Research
- Viticulture Consortium-West
- Washington State Commission on Pesticide Registration
- Altria - Chateau Ste. Michelle Wine Estates
- Washington Wine Industry Foundation