Grapevine viruses: what they are, what we have done, and where we are heading

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Scope of My Research Program

Service to industry:
- Virus diagnosis
- Disease survey

Genetic diversity & evolution biology

Molecular biology, cell biology, virus-host interactions

Biotechnology (virus as vectors)
Presentation Outline

An overview on grapevine viruses and viral diseases

Results of survey for viruses present in Ontario vineyards

Seasonal dynamics of major viruses in Ontario vineyards

Snapshots of basic research activities in in my laboratory

Summary and thoughts on future directions
Grape and wine industry faces many threats

| Insects, birds, nematodes, winter…… | (Armizo et al., 2016) |
Major grapevine viruses and their diseases

~70 Viruses

25 different diseases

Leafroll Diseases

Closteroviridae

Closterovirus (GLRaV-2)
Ampelovirus (GLRaV-1,-3,-4)
Velarivirus (GLRaV-7)

Fanleaf Degeneration and Decline

Secoviridae

Nepovirus (GFLV)

Rugose Wood Complex

Betaflexiviridae

Vitimivirus (GVA,B,D,F)
Foveavirus (GRSPaV)

New diseases continue to emerge………

Syrah decline: causal agents have not been identified
Red blotch: GRBaV (genus Grablovirus, family Geminiviridae)
Leaf mottle and deformation

(Marteli, 2014)
Grapevine leafroll disease complex
Leafroll

Red berried grapes

White berried grapes

The most widespread and detrimental viral disease complex in grapes

Associated with several viruses from the family *Closteroviridae*

Reducing yield (14 - 40%) and quality of grapes; leading to poor quality wines;

Shortens production span of vineyards

Economic costs: $29,902 to $226,405 per hectare, depending on severity, age of vineyard, virus titer, and environmental conditions (Ricketts et al. 2015)
Phylloxera plague, grafting and viruses

“Phylloxera, a true gourmet, finds out the best vineyards and attaches itself to the best wine” (Wikipedia)

- Grafting has been used since 1870’s to rescue European wine industries from destruction by phylloxera and mildews
- Universal practice in modern viticulture worldwide

Global spread of viruses and viral diseases; Mixed infections with multiple viruses and viral strains
Mealybugs identified in Ontario vineyard
Insect vectors known to spread GLRD

<table>
<thead>
<tr>
<th>Family Pseudococcidae</th>
<th>Common name: Mealybugs</th>
<th>Family Coccidae</th>
<th>Common name: Scales, soft scales</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heliococcus bohemicus</em></td>
<td>Bohemian mealybug</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudococcus maritimus</em></td>
<td>Grape mealybug</td>
<td><em>Coccus hesperidium</em></td>
<td></td>
</tr>
<tr>
<td><em>Ps. viburni</em></td>
<td>Obscure mealybug</td>
<td><em>Coccus longulus</em></td>
<td></td>
</tr>
<tr>
<td><em>Ps. comstocki</em></td>
<td>Comstock mealybug</td>
<td><em>Ceroplastes rusi</em></td>
<td></td>
</tr>
<tr>
<td><em>Ps. longispinus</em></td>
<td>Longtailed mealybug</td>
<td><em>Pulvinaria innumerabilis</em></td>
<td>Cottony maple scale *</td>
</tr>
<tr>
<td><em>Ps. calceolariae</em></td>
<td>Citrophilous mealybug</td>
<td><em>Parasaissetia nigra</em></td>
<td></td>
</tr>
<tr>
<td><em>Planococcus citri</em></td>
<td>Citrus mealybug</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Planococcus ficus</em></td>
<td>Vine mealybug</td>
<td><em>Parthenolecanium corni</em></td>
<td>European fruit lecanium *</td>
</tr>
<tr>
<td><em>Phenacoccus aceris</em></td>
<td>Apple mealybug</td>
<td><em>Pulvinaria vitis</em></td>
<td></td>
</tr>
</tbody>
</table>

* Vectors that have been identified in Ontario (source: W. McFadden-Smith)
Red blotch

*Spissistilus festinus*
**Syrah decline**

**Distribution:** France, Australia, Chile, South Africa, Spain, United States, and now Ontario

**Symptoms:** red canopy, swollen graft unions, stem necrosis, eventual death
The unfamiliar Rugose wood disease complex

One of the most damaging diseases, leading to graft incompatibility, decline and eventual death of vines

Several viruses are associated with Rugose wood disease complex

Comprises four distinct diseases based on indexing on differential indicators

LNSG  KSG  GCB  RSP
Grapevine leaf mottling and deformation: the discovery of GPGV

GLMD (grapevine leaf mottling and deformation) first observed in northern Italy in 2003

Short internodes, leaf deformation, smaller cluster, lower yield.....

GPGV is now detected worldwide, including Ontario and BC

Grapevine Pinot gris virus and GLMD

GLMD first reported in Northern Italy (2003) on Pinot gris, Pinot blanc

Symptoms: short internodes, leaf mottle, deformation, reduction in berry cluster and yield;

Symptoms are most obvious in spring, subdue in summer

NGS revealed GPGV as putative causal agent funded by a winery in Italy (2012)

Long-distance spread: infected planting materials and grafting

Local transmission: mites (Colomerus vitis)

Alternative hosts: wild plants (Chenopodium album, Silene latifolia)

Distribution: global
Presentation Outline

An overview on grapevine viruses and viral diseases

Results of survey for viruses present in Ontario vineyards

Seasonal dynamics of major viruses in Ontario vineyards

Snapshots of basic research activities in my laboratory

Summary and thoughts on future directions
The grape and wine industry of Ontario

- Grapes rank as the second largest fruit crop in Ontario;
- Ontario grape/wine industry generates an economic impact of $4.4 billion annually

- Growers: > 600
- Annual production: 80,400 tons
- Farm gate value: $100 million
Ontario vineyard acreage among regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Acreage</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niagara</td>
<td>14,800</td>
<td>87%</td>
</tr>
<tr>
<td>Essex Pelee Island</td>
<td>1,400</td>
<td>8%</td>
</tr>
<tr>
<td>Edward County</td>
<td>650</td>
<td>4%</td>
</tr>
<tr>
<td>Other</td>
<td>150</td>
<td>1%</td>
</tr>
</tbody>
</table>

Total acreage = 17,000
Total percentage = 100%

http://www.grapegrowersofontario.com/grape_facts
Sales of wine grapes (in tonnes)

<table>
<thead>
<tr>
<th>Year</th>
<th>HYBRID</th>
<th>VINIFERA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>20,160</td>
<td>51,170</td>
</tr>
<tr>
<td>2013</td>
<td>26,658</td>
<td>30,892</td>
</tr>
<tr>
<td>2014</td>
<td>19,660</td>
<td>22,668</td>
</tr>
<tr>
<td>2015</td>
<td>28,722</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of vinifera:
- 2012: 69%
- 2013: 66%
- 2014: 61%
- 2015: 56%

http://www.grapegrowersofontario.com/grape_facts
Viral disease outbreak in Ontario since 2013
Virus disease outbreak in Ontario since 2013
Development of highly effective RNA isolation technologies for diagnosis

Sigma kit gave the highest yield and quality of total RNAs

Failure in isolating nucleic acids from old and diseased leaves

- The standard Sigma kit

<table>
<thead>
<tr>
<th>Leaf Samples</th>
<th>RNA yield (µg)</th>
<th>A260/280</th>
<th>A260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>49-1</td>
<td>3.1</td>
<td>1.08</td>
<td>0.29</td>
</tr>
<tr>
<td>49-3</td>
<td>1.2</td>
<td>1.49</td>
<td>0.48</td>
</tr>
<tr>
<td>49-4</td>
<td>2.1</td>
<td>1.69</td>
<td>0.64</td>
</tr>
<tr>
<td>49-5</td>
<td>1.0</td>
<td>0.71</td>
<td>0.11</td>
</tr>
<tr>
<td>49-6</td>
<td>0.5</td>
<td>0.90</td>
<td>0.13</td>
</tr>
<tr>
<td>49-7</td>
<td>0.6</td>
<td>1.08</td>
<td>0.21</td>
</tr>
<tr>
<td>49-8</td>
<td>1.8</td>
<td>0.86</td>
<td>0.18</td>
</tr>
<tr>
<td>Average</td>
<td><strong>1.5</strong></td>
<td><strong>1.12</strong></td>
<td><strong>0.30</strong></td>
</tr>
</tbody>
</table>

Sigma kit failed to isolate RNA from old and symptomatic grape leaves late in the season.
RNA isolation from old and symptomatic leaves

- Our modified RNA isolation system works well with both young and old leaves throughout the season

<table>
<thead>
<tr>
<th>Leaf Samples</th>
<th>RNA yield (µg)</th>
<th>A260/280</th>
<th>A260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Modified</td>
<td>Standard</td>
</tr>
<tr>
<td>49-1</td>
<td>3.1</td>
<td>8.8</td>
<td>1.08</td>
</tr>
<tr>
<td>49-3</td>
<td>1.2</td>
<td>8.5</td>
<td>1.49</td>
</tr>
<tr>
<td>49-4</td>
<td>2.1</td>
<td>8.6</td>
<td>1.69</td>
</tr>
<tr>
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<td>1.0</td>
<td>5.7</td>
<td>0.71</td>
</tr>
<tr>
<td>49-6</td>
<td>0.5</td>
<td>5.9</td>
<td>0.90</td>
</tr>
<tr>
<td>49-7</td>
<td>0.6</td>
<td>13.1</td>
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</tr>
<tr>
<td>49-8</td>
<td>1.8</td>
<td>8.1</td>
<td>0.86</td>
</tr>
<tr>
<td>Average</td>
<td>1.5</td>
<td>8.4</td>
<td>1.12</td>
</tr>
</tbody>
</table>

The modified Sigma kit is effective in isolating high quality RNA from various tissues over the season, including old and symptomatic grape leaves

The detection limits of PCR and RT-PCR after optimization

![Detection limits of PCR and RT-PCR after optimization](image-url)
Multiplex RT-PCR: a single test detects multiple viruses

Primers: conserved or degenerate primers targeting all known variants of each virus
Province-wide survey for viruses

• **Purpose:** to assess the prevalence and distribution of major grape viruses in Ontario

• **Sampling strategies:**

  Places sampled:

  Three production regions (Niagara Peninsula, Lake Erie North Shore and Prince Edward County)

  Numbers of samples:

  **2016:** 700 composite samples (*Vitis vinifera* wine grapes)

  **2017:** 800 composite samples (table, juice, hybrid wine grapes, and a few wild grapes)
Province-wide survey for viruses

• Sampling strategies

Sampling time:

Late summer and early fall: August and September

Sampling method:

15 samples per vineyard block, two leaves per vine

Composite sample: 10 leaves from five vines within a panel
17 viruses were targeted

Grapevine leafroll-associated viruses (GLRaV-1, 2, 3, 4, 7)
Grapevine red blotch-associated virus (GRBaV)
Grapevine rupestris stem pitting-associated virus (GRSPaV)
Grapevine virus A (GVA)
Grapevine virus B (GVB)
Grapevine pinot gris virus (GPGV)
Grapevine fanleaf virus (GFLV)
Arabis mosaic virus (ArMV)
Tomato ringspot virus (TomRSV)

Minor viruses:

Grape fleck virus (GFkV)
Grapevine rupestris vein feathering virus (GRVFV)
Grapevine asteroid mosaic-associated virus (GAMaV)
Grapevine Syrah virus-1 (GSyV-1)
### Prevalence of major viruses: vinifera wine grapes

<table>
<thead>
<tr>
<th>Viruses tested</th>
<th>Total no. of samples tested</th>
<th>No. of samples tested positive</th>
<th>Percentage of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRSPaV</td>
<td>563</td>
<td>473</td>
<td>84.0</td>
</tr>
<tr>
<td>GLRaV-3</td>
<td>657</td>
<td>315</td>
<td>47.9</td>
</tr>
<tr>
<td>GFkV</td>
<td>657</td>
<td>143</td>
<td>21.8</td>
</tr>
<tr>
<td>GPGV</td>
<td>657</td>
<td>142</td>
<td>21.6</td>
</tr>
<tr>
<td>GRBV</td>
<td>657</td>
<td>120</td>
<td>18.3</td>
</tr>
<tr>
<td>GVA</td>
<td>657</td>
<td>41</td>
<td>6.2</td>
</tr>
<tr>
<td>GAMaV</td>
<td>563</td>
<td>34</td>
<td>6.0</td>
</tr>
<tr>
<td>GRVFV</td>
<td>563</td>
<td>33</td>
<td>5.9</td>
</tr>
<tr>
<td>GLRaV-2</td>
<td>657</td>
<td>29</td>
<td>4.4</td>
</tr>
<tr>
<td>GVB</td>
<td>657</td>
<td>20</td>
<td>3.0</td>
</tr>
<tr>
<td>GLRaV-1</td>
<td>657</td>
<td>14</td>
<td>2.1</td>
</tr>
<tr>
<td>GSyV-1</td>
<td>563</td>
<td>9</td>
<td>1.6</td>
</tr>
<tr>
<td>TomRSV</td>
<td>563</td>
<td>9</td>
<td>1.6</td>
</tr>
<tr>
<td>ArMV</td>
<td>563</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Prevalence of major viruses in vineyard blocks of vinifera wine grapes

<table>
<thead>
<tr>
<th>Viruses tested</th>
<th>No. of blocks tested</th>
<th>No. of positive blocks</th>
<th>Percentage of positive blocks</th>
<th>No. of blocks with all sample positive</th>
<th>Percentage of blocks with all sample positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRBV</td>
<td>137</td>
<td>36</td>
<td>26.3</td>
<td>8</td>
<td>22.2</td>
</tr>
<tr>
<td>GLRaV-1</td>
<td>137</td>
<td>12</td>
<td>8.8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>GLRaV-2</td>
<td>137</td>
<td>16</td>
<td>11.7</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>GLRaV-3</td>
<td>137</td>
<td>94</td>
<td>68.6</td>
<td>36</td>
<td>38.3</td>
</tr>
<tr>
<td>GRSPaV</td>
<td>127</td>
<td>117</td>
<td>92.1</td>
<td>109</td>
<td>93.2</td>
</tr>
<tr>
<td>GVA</td>
<td>137</td>
<td>20</td>
<td>14.6</td>
<td>3</td>
<td>15.0</td>
</tr>
<tr>
<td>GVB</td>
<td>137</td>
<td>9</td>
<td>6.6</td>
<td>4</td>
<td>44.4</td>
</tr>
<tr>
<td>GPGV</td>
<td>137</td>
<td>62</td>
<td>45.3</td>
<td>5</td>
<td>8.1</td>
</tr>
<tr>
<td>GFkV</td>
<td>137</td>
<td>60</td>
<td>43.8</td>
<td>18</td>
<td>30.0</td>
</tr>
</tbody>
</table>

* The percentage is calculated by the number of blocks, in which all the samples are positive for a given virus, divided by the number of positive blocks for a given virus.
Mixed infections of vinifera grapes with multiple viruses

<table>
<thead>
<tr>
<th>No. of viruses in mixed infections</th>
<th>No. of samples with mixed infections</th>
<th>Percentage of samples with multiple viruses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
<td>4.4</td>
</tr>
<tr>
<td>1</td>
<td>136</td>
<td>23.7</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>31.9</td>
</tr>
<tr>
<td>3</td>
<td>142</td>
<td>24.7</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

-95.6% of samples tested positive for at least one virus; -72% of samples are infected with two or more viruses
### Prevalence of major viruses in major vinifera cultivars

#### Red cultivars

<table>
<thead>
<tr>
<th>Viruses tested</th>
<th>Cab Franc</th>
<th>Cab Sauvignon</th>
<th>Pinot noir</th>
<th>Merlot</th>
<th>Syrah</th>
<th>Chardonnay</th>
<th>Riesling</th>
<th>Pinot gris</th>
<th>Sauvignon blanc</th>
<th>Gewürz-traminer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRBV</td>
<td>25.8</td>
<td>22.2</td>
<td>7.6</td>
<td>5.6</td>
<td>64.7</td>
<td>21.2</td>
<td>2.0</td>
<td>20.6</td>
<td>26.3</td>
<td>0.0</td>
</tr>
<tr>
<td>GLRaV-1</td>
<td>1.3</td>
<td>0.0</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
<td>4.0</td>
<td>0.0</td>
<td>5.3</td>
<td>6.3</td>
</tr>
<tr>
<td>GLRaV-2</td>
<td>5.2</td>
<td>25.0</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td>7.6</td>
<td>4.0</td>
<td>0.0</td>
<td>10.5</td>
<td>0.0</td>
</tr>
<tr>
<td>GLRaV-3</td>
<td>49.7</td>
<td>22.2</td>
<td>33.7</td>
<td>13.9</td>
<td>86.3</td>
<td>56.1</td>
<td>53.0</td>
<td>58.8</td>
<td>31.6</td>
<td>68.8</td>
</tr>
<tr>
<td>GVA</td>
<td>1.9</td>
<td>2.8</td>
<td>3.3</td>
<td>0.0</td>
<td>0.0</td>
<td>16.7</td>
<td>15.0</td>
<td>0.0</td>
<td>5.3</td>
<td>18.8</td>
</tr>
<tr>
<td>GVB</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.5</td>
<td>8.0</td>
<td>0.0</td>
<td>5.3</td>
<td>0.0</td>
</tr>
<tr>
<td>GPGV</td>
<td>35.5</td>
<td>5.6</td>
<td>8.7</td>
<td>16.7</td>
<td>35.3</td>
<td>15.2</td>
<td>13.0</td>
<td>14.7</td>
<td>47.4</td>
<td>6.3</td>
</tr>
</tbody>
</table>

#### White cultivars

Three major viruses (GLRaV-3, GRBV and GPGV) are widely present in multiple vinifera cultivars, with less prevalence in Merlot.
Summary: virus survey

1,500 samples representing wine grapes (both vinifera and hybrids), juice, table and wild grapes were sampled from three regions across the province.

14 viruses were detected, including all major viruses that are targeted in certification programs worldwide (except GFLV).

GLRaV-3, GRBaV, GPGV, and GRSPaV are the most prevalent, detected in all types of grapes.

The vast majority of samples are infected with multiple viruses, as expected.
Presentation Outline

An overview on grapevine viruses and viral diseases

State of survey for viruses present in Ontario vineyards

Seasonal dynamics of major viruses in Ontario vineyards

Snapshots of basic research activities in my laboratory

Summary and my thoughts on future directions
Seasonal dynamics and spatial distribution of major grape viruses

**Objective:** to understand the seasonal and tissue distribution of major viruses in cool climate vineyards. Providing guideline on best time window and tissue type for virus testing.

Vines infected with each virus were identified based on our initial survey, and labeled.

Progression of symptoms recorded monthly

Different tissues were collected monthly from May to October

Nucleic acids and proteins were isolated using our in-house protocol

Titer of each virus was assessed via PCR, qPCR and western blot
Sample collection

Tissues (leaf, inflorescence, fruit and phloem) were collected monthly, from May to October.
Seasonal progression of disease symptoms of GLRaV-3 in Cabernet Franc
Grape fruits of healthy versus infected Cabernet franc (September)
Distribution of four viruses in different tissues collected over the season

Chardonnay, ID 49-10, mixed infected with four viruses
Temporal dynamics of GLRaV-3 in leaves

RT-qPCR

Fold change

- control  May  June  July  August  September  October

Western blot
Distribution of GLRaV-3 among different tissues (June vs. September, Chardonnay)

June

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>0</td>
</tr>
<tr>
<td>Fruit</td>
<td>60</td>
</tr>
<tr>
<td>Petiole</td>
<td>40</td>
</tr>
<tr>
<td>Mid rib</td>
<td>0</td>
</tr>
</tbody>
</table>

September

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>0</td>
</tr>
<tr>
<td>Fruit</td>
<td>0</td>
</tr>
<tr>
<td>Petiole</td>
<td>80</td>
</tr>
<tr>
<td>Old leaf</td>
<td>60</td>
</tr>
<tr>
<td>Young leaf</td>
<td>100</td>
</tr>
<tr>
<td>Cambium</td>
<td>120</td>
</tr>
</tbody>
</table>
Seasonal and tissue distribution of GRBaV

qPCR

Fold change

May  June  July  August  September  October

Fold change

qPCR (September samples)

PCR

May  June  July  August  September  October  Young leaf  Old leaf  Fruit  Cambium  Seed  Petiole  - control  + control
Summary: seasonal dynamics and tissue distribution

Best time window and tissue source for detection:

Using fruits: June
Using leaf: August – September
Cambium: Late season and dormancy

Recommendation for routine and high-throughput testing:

Source of virus: Leaf tissue
Template prep: Our improved nucleic acid isolation protocol
Test methods: PCR or qPCR (GRBaV);
RT-PCR or RT-qPCR for other viruses.
Composite samples;
Multiplex tests
A bit on metagenomics

• Next Generation Sequencing (NGS) has been the most powerful tool for virus discovery (leading role of grape virologists)

  • *Grapevine vein clearing virus* (Zhang et al. 2011)
  • *Grapevine Pinot gris virus* (Giampetruzzi et al. 2012)
  • *Grapevine virus F* (Al Rwahnih et al. 2012)
  • *Grapevine Syrah virus-1* (Al Rwahnih et al. 2012)
  • Grapevine Roditis leaf discoloration-associated virus (Maliogka et al. 2015)

• Application of NGS for virus detection, discovery and solving the etiology of new diseases

• Isolation of total RNAs from multiple vines with disease symptoms
• Several sequencing platforms (Illumina HiSeq 2500, NextSeq500 & PE150)
• CLC Genomic Workbench
Solving the mystery of decline in grapevine

Vineyard planted 1983
Showing leafroll
Severe reduction in yield and brix
Berries were abandoned

Visitation to vineyard
Nov 7, 2014

Tested with PCR and NGS

Vineyard block pulled out 2015
The power of next gen sequencing

<table>
<thead>
<tr>
<th></th>
<th>Vine no. 1</th>
<th>Vine no. 2  (Red canopy)</th>
<th>Vine no. 3  (No red canopy)</th>
<th>Riesling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. reads</td>
<td>57,719,419</td>
<td>56,885,806</td>
<td>68,538,997</td>
<td>73,385,886</td>
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<tr>
<td>Total sequence</td>
<td>2,943,690,369</td>
<td>2,901,176,106</td>
<td>3,495,488,847</td>
<td>3,742,680,186</td>
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<tr>
<td>GRSPaV</td>
<td>19,310</td>
<td>12,795</td>
<td>14,432</td>
<td>14,273</td>
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<tr>
<td>GLRaV-1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>82,891</td>
</tr>
<tr>
<td>GLRaV-3</td>
<td>1,799</td>
<td>1,649</td>
<td>3,033</td>
<td>5,035,598</td>
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<tr>
<td>GRBaV</td>
<td>174,274</td>
<td>3,285</td>
<td>24,557</td>
<td>--</td>
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<tr>
<td>GVA</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>93,437</td>
</tr>
<tr>
<td>GVB</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>681,446</td>
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<tr>
<td>GVF</td>
<td>--</td>
<td>--</td>
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<td>1,040</td>
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<tr>
<td>GPGV</td>
<td>53,991</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>GYSVd-1</td>
<td>3,805</td>
<td>3,968</td>
<td>6,087</td>
<td>5,659</td>
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<tr>
<td>GYSVd-2</td>
<td>424</td>
<td>724</td>
<td>1,013</td>
<td>1,007</td>
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<tr>
<td>HSVd</td>
<td>9,616</td>
<td>7,019</td>
<td>10,819</td>
<td>14,733</td>
</tr>
</tbody>
</table>
Solving the mystery of decline in Riesling

Six viruses and three viroids detected.
Two new and distinct strains of GLRaV-3 completely sequenced

H. Xiao et al. 2018
Presentation Outline

An overview on grapevine viruses and viral diseases

State of survey for viruses present in Ontario vineyards

Seasonal dynamics of major viruses in Ontario vineyards

Snapshots of basic research activities in my laboratory

Summary and my thoughts on future directions
Genome structure of select grape viruses

GLRaV-3 (genome size: ~18,500 nts; genus Ampelovirus, family Closteroviridae)

GPGV (genome size: 7,259 nts; Genus Trichovirus, family Betaflexiviridae)

GRSPaV (genome size: 8,725 nts; Genus Foveavirus, family Betaflexiviridae)
Basic research activities

**Ultimate Goal:** to advance grape virology and to make it one of the best model systems in virology

NGS for discovery of novel viruses, etiology (Syrah decline) and viral effects on fruit yield and quality

Genetic diversity and evolution biology of major grape viruses

Establishment of infectious cDNA viral clones and tissue culture/agro-based infectivity systems

Localization and structure of viral replication machinery

Transcriptomics to study the impact of viruses on gene expression, vine growth, yield and quality of grapes and wine

Elucidating function of novel genes and non-coding genetic elements in GLRaVs (AlkB domain, long non-coding sequences, etc.)
Genetic diversity and population structure

Meng et al. 2006. JGV 87, 1725

0.01 substitutions/site

GRSPaV-1 (Vein necrosis)

GRSPaV-SG1 (Vein necrosis)

GRSPaV-SY (Syrah decline?)

GRSPaV-BS (RSP)
Model on origin & evolution

Ancestor of GRSPaV

GRSPaV-1 (Vitis riparia)  GRSPaV-SG1 (Vitis rupestris)  GRSPaV-BS (Vitis vinifera?)  GRSPaV-SY (Vitis vinifera?)  GRSPaV-PN (Vitis vinifera?)

Mixtures of Viral Variants (In commercial grape varieties)

Transmission via pollen  Transmission via grafting

Construction of infectious viral clones: gateway to fundamental research

- pRSP28-2\(_{(\text{Cam})}\) (top)
- pRSP-GFP2\(_{(\text{Cam})}\) (bottom)

Subgenomic promoter from a distant strain
Demonstration of infectivity of viral cDNA clones in experimental host plants

- pRSP-GFP2\textsubscript{(Cam)}
- PVX-GFP
- pRSP-CP:GFP\textsubscript{(Cam)}
The ultimate goal: launching infection of viral cDNA clones in the real host, grapevine

Clean, virus-free plants

Shoot development

Rooting stage

Vacuum infiltration

Recovery

Growth
GFP-tagged viral cDNA clone is infectious in grapevines via agro-based infection

-GFP was detected in roots of grapevine cv. Thompson 30 dpi
-RT-PCR detects GRSPaV from grapevine plantlets showing GFP
-One of ten plants showed systemic infection 6 months after inoculation

Structure and localization of viral replication complexes

- pRSP-GFP2
- pREP-GFP

Images at 2 dpi, 3 dpi, 6 dpi, and 8 dpi.
Presentation Outline

An overview on grapevine viruses and viral diseases

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Seasonal dynamics of major viruses in Ontario vineyards

Snapshots of basic research activities in my laboratory

Summary and my thoughts on future directions
Looking ahead: what needs to be done?

Generation and certification of clean plants and its implementation

Ecology and natural reservoirs of grapevine viruses:

- Survey for viruses in wild grapes
- Comprehensive survey for viruses in non-vinifera grapes (hybrid wine grapes, juice grapes, table grapes, rootstocks)

Identification of vectors for GLRaVs, GRBaV, and GPGV in Ontario

Studies of transmission biology of major viruses by vectors

Novel control strategies against major viral diseases using viral vectors and RNA silencing
Overall Summary

- Developed the most effective method for isolating nucleic acid from grapes.
- Performed large-scale province-wide surveys for all major viruses and determined distribution of viruses in wine, juice and table grapes.
- Detected 14 viruses in Ontario vineyards, with GLRaV-3, GRBaV, GPGV and GRSPaV being the most prevalent.
- Applied and continue to use NGS technology and the bioinformatic pipeline for virus detection, discovery, and virus-host interactions.
- Established all experimental systems essential for diverse fundamental research on several major grape viruses.
- We are pursuing many fundamental studies pertaining to GLRaV-3, GPGV, and GRSPaV.
Collaborators and funding agencies

Funding:

Ontario Grape & Wine Research Inc.

NSERC Discovery Grant Program;

NSERC Engage grant program;

OMAFRA-UoG Partnerships Program

-Production Systems
-Emergency Management
-Gryphon’s LAAIR program

Dr. Wendy McFadden-Smith (OMAFRA)
Angelo Pavan and Gabe Demarco (Cave Spring Cellars)
Rob Power (Creekside winery)
Emily Aubie and Katie Dickeson
Many grape growers and wineries
Dr. Andy Reynolds (Brock)
Dr. Lorne Stobbs (AAFC-Vineland)
Bernard Kim (Norgen Biotek)
The Grape Virology Crew at Guelph
Niagara Peninsula is the largest and most important region for grapes and tender fruit production in Ontario.

<table>
<thead>
<tr>
<th>Fruit crops</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grapes</td>
<td>94%</td>
</tr>
<tr>
<td>Peaches, nectarines, apricots</td>
<td>90%</td>
</tr>
<tr>
<td>Plums</td>
<td>80%</td>
</tr>
<tr>
<td>Sweet cherries</td>
<td>75%</td>
</tr>
<tr>
<td>Sour cherries</td>
<td>60%</td>
</tr>
</tbody>
</table>
Seasonal progression of disease symptoms of GLRaV-3 in Chardonnay

June

July

August

September

October

Healthy
Grape fruits from healthy versus infected Chardonnay (September)
Viral diseases can be difficult to diagnose if based solely on symptoms.