Proposal Title:
Diagnosis of grapevine virus diseases in Michigan vineyards.

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Original goals and objectives for the project:
The objectives of this project were:
1) Identify causal agents of virus-like symptoms and grapevine decline in Michigan
2) Establish in-state DNA-based testing capability for grapevine virus diseases.

Literature Review:
Virus diseases are widespread in vineyards throughout the world and are among the more difficult to
detect and identify. Plant viruses are mostly spread via vegetative propagation, although insect
and nematode vectors are responsible for the spread of some grapevine viruses. Grapevine viruses
can be unrecognized causes of low yields and poor plant growth as well as grapevine decline.
Most virus diseases can only be accurately diagnosed by specialized laboratory testing, either via
serological methods such as enzyme-linked immunosorbent assay (ELISA) or DNA sequencing
(polymerase chain reaction- PCR). These tests are fairly expensive (for example $85-$250 per
test, depending on how many viruses are tested for at the same time). For grapevine viruses, most
testing is done in specialized private laboratories in California, such as Agri-Analyis in Davis and
Eurofins STA in Gilroy, CA. Some common grapevine viruses are grapevine fanleaf virus
(GFLV), grapevine leafroll associated viruses (GLRaV-1 through -9), tomato ringspot virus
(ToRSV), tobacco ringspot virus (TRSV), grapevine fleck virus (GFkV), grapevine virus A
(GVA), grapevine virus B (GVB), Arabis mosaic virus (ArMV), and peach rosette mosaic virus
(PR MV), the latter particularly in Michigan juice grapes.

Recent surveys for grapevine viruses in other states have found that several viruses are
widespread in grapevines there. For instance, Mekuria et al. (2009a, 2009b) found that grapevine
leafroll disease is a significant constraint to sustainable growth of the wine grape industry in
Washington state. In a 3 year survey, they detected grapevine leafroll-associated virus (GLRaV) -
1, -2, -3, -4, -5, and -9 in different wine grape varieties showing grape leafroll symptoms. Mixed
infections of these viruses in different combinations were frequently detected in individual
grapevines. GLRaV-3 was most prevalent. The results also revealed the presence of rupestris
stem pitting-associated virus, grapevine Virus A, grapevine Virus B, grapevine fanleaf virus and
grapevine fleck virus as mixed infections with GLRaVs. A similar survey by Martin et al. (2005)
also found Rupestris stem pitting associated virus (RSPaV) and GLRaV-1, -2, and -3 in
Washington and GLRaV-1, -2, and -3 in Oregon. When vineyards in the Finger Lakes region of
New York were tested, GLRaV-1, -2, and 3 were found in nearly two-thirds of the vineyard
blocks (Fuchs et al. 2009). Researchers also found a variety of viruses in surveys of grapevines in
Missouri, such as ToRSV, ArMV, GFkV, GVA, GLRaV-1 and GLRaV-3 (Milkus et al. 1999; Milkus, 2001). Some French hybrids and American cultivars were 100% infected with GLRaV-3.

No formal virus survey had been conducted in Michigan vineyards however there is preliminary evidence that grapevine viruses are causing problems. Tobacco ringspot virus was detected in table grapes at SWMREC about 10 years ago. Diagnostic testing by AgriAnalysis, Inc. to determine the causes of vine decline and virus-like foliar symptoms during the 2009 growing season led to the discovery of grapevine leafroll-associated virus 3 and rupestris stem pitting virus, in commercial vineyards in Michigan. Leaf reddening and yellowing symptoms are common in Michigan, but the cause was not known. While most common grapevine viruses have not been reported in Michigan before, they are widespread in other grape-growing areas and as such, not expected to be of concern to regulatory agencies. However, there is a need to determine the causes of vine decline and virus-like symptoms in Michigan vineyards so growers can start understanding and managing these problems.

**Results and Conclusions**

The survey yielded several grapevine viruses that were previously unrecognized in Michigan, specifically GLRaV-1 and -2, and grapevine fleck virus. Roughly 1/3 of the samples were positive using ELISA. The most common viruses were GLRaV-3 and tobacco ringspot virus. GLRaV-3 causes a chronic infection and will lead to a reduction in yield, brix, and color, but will not kill the plant. In contract, TRSV is lethal and can spread by nematodes. Syrah vines may indeed suffer from Syrah decline but this could not be verified by ELISA (only PCR). These results emphasize the need for clean planting material and also show that not all virus-like symptoms are caused by viruses. Leaf reddening is also often caused by crown gall, physical injury or other some other disorder. Fungicide or herbicide injury may also sometimes mimic virus diseases.

**Time Line**

**2010:** Samples (155) were taken in late summer of 2010 and tested for 12 different viruses with ELISA. Tissue from each sample was saved and frozen for testing for phytoplasma and rupestris stem pitting virus during the winter and spring of 2011.

**2011:** Frozen samples from the summer 2010 survey will be subjected to PCR to tests for phytoplasma, rupestris stem pitting virus, and Syrah virus 1 (which cannot be detected by ELISA). We will also use PCR to test samples from the 2010 and 2011 surveys. Additional grapevines will be tested by ELISA in summer of 2011 (the number of samples depending on available leveraged funds).

**Work accomplished during period including methods (by Objective)**

1) **Identify causal agents of virus-like symptoms and grapevine decline in Michigan**

Eighteen Michigan farms in 6 different Michigan counties were visited in the summer of 2010 and 155 leaf and petiole samples were taken from a total of 45 vineyards. Samples were taken from vines exhibiting virus-like symptoms or general vine decline. All samples were tested by ELISA for the following 12 viruses: GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4-9, GFkV, GVA, GVB, ArMV, GFLV, TRSV, ToRSV, and PRMV (Table 1). We will also use PCR to test samples from the 2010 and 2011 surveys. Of the 155 samples tested, 341% (52 samples) tested
ELISA positive for either GLRV-1, GLRV-2, GLRV-3, GFkV, ToRSV, TRSV, or PRMV. All samples were ELISA negative for GLRV4-9, ArMV, GFLV, GVA and GVB. There were 10 samples that tested positive for more than one virus (Table 2). These samples came from 6 different vineyards.

There were 26 known cultivars represented in the survey with 4 vineyards of an unknown cultivar. Ten varieties had samples that were ELISA positive for at least one virus (Table 3). A total of 16 varieties tested negative for all viruses. Therefore symptoms could be due to other causes, such as crown gall infection, vine stress or mechanical injury, nutrient deficiency, phytoplasmas, viruses for which no ELISA kits are available, Syrah decline, or other problems. The varieties with no positives were: Barbera 2665-8 (1/1), Cabernet Sauvignon (1/1), Chardonnel (1/5), Dornfelder (1/1), Lagrein (1/3), Merlot (1/1), Orange Muscat (1/2), Pinot Noir (6/27), Rkatsiteli (1/2), Roussanne (1/1), Sin Sault (1/1), Syrah (2/7), Teroldego (1/2), Thomcord (1/3), Vignoles (1/1), and Zweigelt (1/2). Numbers in parenthesis represent (# vineyard/# samples) tested.

### Table 1. ELISA positive grapevine samples listed by county.

<table>
<thead>
<tr>
<th>County</th>
<th># Individual vinyards tested</th>
<th>Total # samples</th>
<th># Positive</th>
<th>% positive</th>
<th>Positive samples by virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allegan</td>
<td>4</td>
<td>23</td>
<td>7</td>
<td>26.1</td>
<td>GLRaV-1 0 0 2 0 5 0 0</td>
</tr>
<tr>
<td>Berrien</td>
<td>18</td>
<td>50</td>
<td>30</td>
<td>60.0</td>
<td>GLRaV-2 1 3 12 3 4 2 5</td>
</tr>
<tr>
<td>Jackson</td>
<td>5</td>
<td>19</td>
<td>8</td>
<td>31.5</td>
<td>GLRaV-3 0 3 5 0 0 0 0</td>
</tr>
<tr>
<td>Leelanau</td>
<td>13</td>
<td>35</td>
<td>1</td>
<td>2.8</td>
<td>GFkV 0 0 0 0 1 0 0</td>
</tr>
<tr>
<td>Old Mission</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>TRSV 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Van Buren</td>
<td>4</td>
<td>25</td>
<td>6</td>
<td>24.0</td>
<td>ToRSV 0 0 2 1 2 1 0</td>
</tr>
<tr>
<td>Total/ave</td>
<td>45</td>
<td>155</td>
<td>52</td>
<td>34%</td>
<td>PRMV 1 6 21 4 12 3 5</td>
</tr>
</tbody>
</table>

### Table 2. Grapevine samples with mixed viral infections according to ELISA results

<table>
<thead>
<tr>
<th># of Samples</th>
<th># of Vineyards</th>
<th>Grape variety</th>
<th>ELISA positives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GLRaV-2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Chambourcin</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Pinot Noir</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Riesling and Chardonnay</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Chancellor</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Unknown</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3. ELISA positive samples listed by cultivar

<table>
<thead>
<tr>
<th>Variety</th>
<th># of locations</th>
<th># of samples</th>
<th>% positive</th>
<th>ELISA positives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GLRaV-1</td>
</tr>
<tr>
<td>Cabernet Franc</td>
<td>5</td>
<td>10</td>
<td>60.0</td>
<td>6</td>
</tr>
<tr>
<td>Chambourcin</td>
<td>1</td>
<td>6</td>
<td>33.3</td>
<td>2</td>
</tr>
<tr>
<td>Chancellor</td>
<td>2</td>
<td>13</td>
<td>30.8</td>
<td>3</td>
</tr>
<tr>
<td>Chardonnay</td>
<td>3</td>
<td>12</td>
<td>50.0</td>
<td>3</td>
</tr>
<tr>
<td>Concord</td>
<td>2</td>
<td>7</td>
<td>28.6</td>
<td>2</td>
</tr>
<tr>
<td>Delaware</td>
<td>1</td>
<td>1</td>
<td>100.0</td>
<td>1</td>
</tr>
<tr>
<td>Marquis</td>
<td>1</td>
<td>10</td>
<td>20.0</td>
<td>2</td>
</tr>
<tr>
<td>Noiret</td>
<td>1</td>
<td>8</td>
<td>25.0</td>
<td>1</td>
</tr>
</tbody>
</table>
2) Establish in-state DNA-based testing capability for grapevine virus diseases.

Team members Annemiek Schilder and graduate student Srdjan Acimovic traveled to Agriculture and Agri-food Canada in Harrow, Ontario to receive training from Dr. Roberto Michelutti and Dr. Yaima Arocha Rosete (experts on phytoplasma research and detection) on specific PCR techniques for the detection of phytoplasmas. We will be applying these techniques on samples from the 2010 and 2011 grape virus surveys that tested ELISA negative. This objective will be completed with the 2011 funds. Depending on matching funds from the Viticulture Consortium-East, specific PCR primer sequences and protocols for detection of grapevine viruses will be obtained from Dr. Adib Rowhani at Foundation Plant Services in Davis, CA. These will be evaluated on grapevine samples. DNA extraction and sequencing protocols will be optimized. We will test Syrah vines for Syrah virus 1 to confirm Syrah decline.

Communication Activities, Accomplishments and Impacts

The results of this research were shared with grape growers at the following meetings: Great Lakes Expo, Dec 2010 and the Northwest Orchard and Vineyard Show, Jan 2011.

Research publications resulting from this project

A publication of the survey results is anticipated after the second year of data collection.

Funding partnerships

Funding for this project from MGWIC was used to try to leverage additional funding in 2010 from GREEEN ($19,151) but was not granted. In 2011, we are using MGWIC renewal funds to leverage funds from Viticulture Consortium-East ($28,666 pending).

References


