2014 Research Report to the Michigan Grape & Wine Industry Council

Proposal Title:
Impacts of grapevine leafroll virus on Chardonnay vines and the role of potential vectors.

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Original goals and objectives for the project:
The overall goal of this project is to assess the distribution and damage caused by Grapevine leafroll (mostly caused by GLRaV-3) in an infected 'Chardonnay' vineyard in Southwest Michigan and to investigate the presence and role of potential vectors in the spread of the virus. This vineyard is also infected with Tobacco ringspot virus (TRSV) which is vectored by dagger nematodes. The specific objectives of the study are to:

1) Study the effect of GLRaV-3 on yield and fruit composition in 'Chardonnay' grapevines
2) Study the spatial pattern of GLRaV-3 and TRSV in 'Chardonnay' grapevines
3) Identify and quantify mealybugs and other potential vectors in a GLRaV-3 infected vineyard
4) Assess whether potential vector(s) contain GLRaV-3.

Results and Conclusions
Grapevine leafroll disease was widespread in the 'Chardonnay' study vineyard, with 85% of live vines showing symptoms ranging from mild to very severe. In ELISA tests, 99% of vine samples tested positive for the virus (GLRaV-3) regardless of symptom severity. Thus estimating infection based on symptoms may underestimate the actual infection incidence. In addition, 19% of the vines had Tobacco ringspot virus (TRSV) symptoms, but fewer tested positive for the virus. Both viruses were well distributed throughout the vineyard, but there appeared to be fewer vines with grapevine leafroll symptoms where there was high TRSV incidence, although about 10% of the vines showed symptoms of both viruses. Statistical analysis of spatial patterns of virus symptoms and winter injury is ongoing. Fruit yields were very low and variable throughout the field due to winter injury. Yields declined with increasing symptom severity, with moderate, severe, very severe, and very severe + TRSV categories having 2, 70%, 85%, and 91% lower yields than apparently healthy vines, respectively. Mealybugs were found throughout this and surrounding vineyards and were identified as the grape mealybug, *Pseudococcus maritimus*. In another leafroll virus-infected vineyard in Jackson Co., numerous boxelder bugs were found but it is unknown if they can vector plant virus vectors. Samples of both types of insects are currently being tested for the presence of leafroll viruses by PCR

in Prosser, WA. This study shows that leafroll viruses can be damaging to grapevines, especially in combination with TRSV, and that mealybugs are the likely vectors of leafroll viruses in Michigan vineyards. The results also highlight the importance of clean plant material for vineyard establishment. Guidelines for prevention and management of grapevine virus diseases and their vectors will be formulated upon conclusion of the project.

Time line

This project was conducted from January 1 until December 31, 2014 and represents year 1 of a 2-year project.

Work accomplished during period (2014) including methods (by Objective)

1) Study the effect of GLRaV-3 on growth, yield and fruit composition in 'Chardonnay' grapevines

In early fall, we selected and marked a total of 42 vines in the 'Chardonnay' study vineyard in southwest Michigan with differing degrees of leafroll symptoms, including "apparently healthy", "mild", "moderate", "moderately severe" and "severe". We chose seven vines in each category. We also included seven vines that showed severe symptoms of GLRaV-3 as well as Tobacco ringspot virus. Vine leaf samples were tested by ELISA (enzyme-linked immunosorbent assay) for all Grapevine leafroll-associated viruses (1, 2, 3, 4-9) and tobacco ringspot virus (TRSV). All vines, except two of the apparently healthy vines, were found to be infected. Six out of seven vines with leafroll plus TRSV symptoms tested positive for TRSV. The virus infection status of all 42 vines was confirmed by Dr. Naidu at WSU using PCR (polymerase chain reaction) on petioles which were removed from the leaf blade and sent to Prosser, WA. The ELISA was more sensitive than the PCR, but this may have been due to the condition of the petiole samples by the time they got to Prosser. Vines were also tested for additional viruses (GVA, GVB, GVE, and Grapevine red blotch-associated virus); these tests are still in progress. Vines in the entire vineyard were severely winter injured in the winter of 2013/2014 and yields were extremely low. We harvested all the fruit on the selected vines and counted and weighed the clusters; we also counted the number of healthy and rotten berries in each cluster and determined the berry weight of healthy berries. Clusters were frozen for analysis for Brix, TA, pH according to standard laboratory procedures in the Sabbatini lab, which is currently in progress. There was a lot of variability in the number of clusters per vine throughout the vineyard, and some vines whose foliage looked apparently healthy had no fruit at all due to winter injury. Vines with mild leafroll symptoms showed almost a 50% increase in yield (0.9 kg/vine) compared to apparently healthy vines (0.46 kg/vine), but this may have been due to high yield variability and the relatively low sample number (seven vines in each category). Vines with moderate symptoms showed a 2% reduction compared to apparently healthy

Fig. 1. Severe symptoms of Grapevine leafroll virus infection in 'Chardonnay' vines.

vines, while those with severe symptoms had a 70% reduction, with very severe symptoms an 85% reduction, and with very severe symptoms + TRSV a 91% reduction in yield. When comparing these results to 2013 yield data, it appears that virus infection suppressed yield more after a severe winter than a relatively mild winter. Economic losses in the study vineyard will be estimated to assist the grower in deciding whether to take the vineyard out of production in 2015 and to develop guidelines for virus management.

2) Study the spatial pattern of GLRaV-3 and TRSV in 'Chardonnay' grapevines

We inspected all vines in a 10-row section of the vineyard and recorded their level of symptom expression of grapevine leafroll as well as tobacco ringspot symptoms. The level of winter injury was also recorded. Of the 859 vine spaces, 15% had dead or missing vines and 9.8% were replants, indicating that over the past several years, 25% of all vines had died. The majority probably died in the winter of 2013-2014. Virus symptoms were widespread in the vineyard, with 76% of live vines showing grapevine leafroll symptoms, 10% showing TRSV symptoms and 9% showing both leafroll and TRSV symptoms. A total of 90 leaf samples were taken in a grid pattern over the 10 rows and tested by ELISA: 99% of the samples tested positive for GLRaV-3, regardless of symptom severity, including the majority of apparently healthy vines (including many replants), indicating that actual

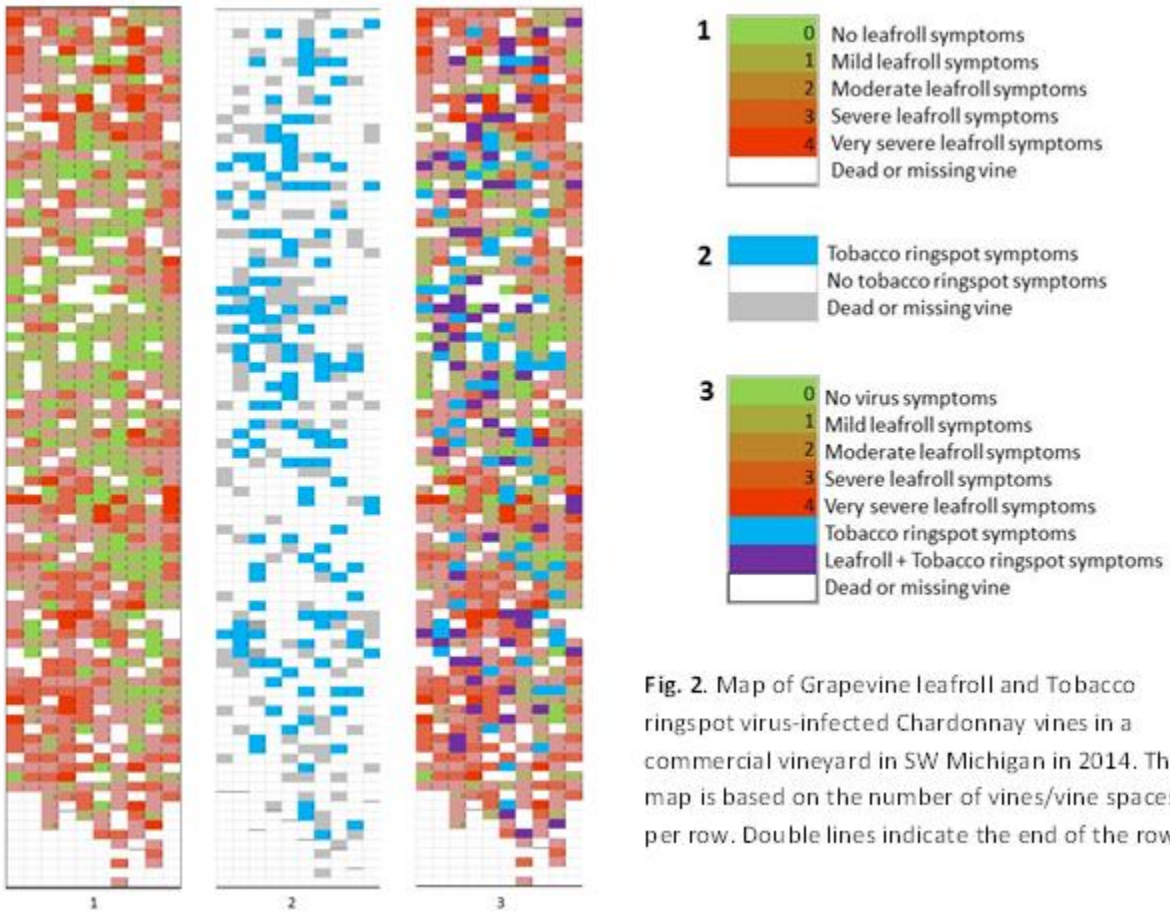

Fig. 2. Map of Grapevine leafroll and Tobacco ringspot virus-infected Chardonnay vines in a commercial vineyard in SW Michigan in 2014. The map is based on the number of vines/vine spaces per row. Double lines indicate the end of the row.

infection was more widespread than symptoms. In addition, 2% of the samples also had GLRaV-4, 1% had GLRaV-1 and 1% had GLRaV-2 virus strains. Whereas 19% of the vines were symptomatic for TRSV, only 9% of the samples tested positive for TRSV, suggesting that the stratified sampling procedure either did not adequately capture the distribution of TRSV-infected vines and/or that the ELISA was not sensitive enough to detect all infections. Dr. Forrest Nutter, an epidemiology professor at Iowa State University is collaborating with us on the statistical analysis of spatial patterns of virus infection and winter injury in relation to virus infection. Based on visual inspection of the vineyard maps, grapevine leafroll symptoms appear somewhat inversely related to TRSV symptoms. Patterns of grapevine leafroll symptom severity also suggest expanding foci of infection, such as when the infection is spread by mealybugs from virus-infected planting material. Vine death appears to be mostly associated with TRSV infection and possibly lower areas of the vineyard.

3) Identify and quantify mealybugs and other potential vectors in a GLRaV-3 infected vineyard.
In late June of 2014, two vineyards where vines had previously tested positive for Grapevine leafroll virus were sampled for possible insect vectors of the virus. The 'Chardonnay' vineyard in SW Michigan was included, while the other was a mixed variety planting in Jackson County, MI. In each vineyard every 10th vine was sampled for the presence of live mealybugs, evidence of previous mealybug presence (wax, webbing, shed cuticle) on the trunk, cordon and shoots. Trunks were sampled by stripping a ~ 3 x 20-cm piece of loose bark and recording the number of live mealybugs and/or evidence of mealybug presence. Cordons were similarly sampled but a smaller (~ 1 x 5-cm) strip of bark was examined. The presence of ants and other insects observed during assessment was noted. All live mealybugs were collected for species verification. We were unable to find mealybugs at the vineyard in Jackson County, but there was a very high population of boxelder bugs (*Boisea trivittata*) throughout the vineyard and we collected samples of those for virus testing. Boxelder bug samples were stored individually as described above. Mealybug samples were sent to USDA-APHIS for species identification and were determined to be *Pseudococcus maritimus*, the grape mealybug. In August 2014, we returned to the Berrien Co. farm and sampled 8 additional vineyards in a similar fashion to that described above. We assessed 5 vines per row on each of 5 rows in each vineyard block. At this sampling, we counted live mealybugs, signs of mealybug infestation, and also recorded where ants were present. In addition we collected 3 fully expanded leaves from each sampled vine so that these could be tested for the presence of the leafroll virus. We found mealybug infested vines in every vineyard we sampled at the Berrien county farm. The relative abundance of mealybug infestation in vineyards on that farm is shown in Table 1. Across all sampled vineyards, there was a strong positive correlation (r=0.78) between the number of vines with mealybug infestation and the number of vines with ants (Fig. 6). This was not surprising since many ants are known to have a mutualistic relationship with mealybugs. This finding suggests that efforts to control the spread of mealybugs and grapevine leafroll virus will need to take ant management into account.

4) Assess role of potential vector(s) of GLRaV.
A total of 50 grape mealybugs from the 'Chardonnay' vineyard in Berrien Co. and 31 boxelder bugs from the mixed variety planting in Jackson Co. were transferred to individual micro-centrifuge tubes and frozen in a -80°C freezer. In December, mealybug and boxelder bug samples were sent on dry ice to Dr Naidu's laboratory at Washington State University to be tested for the presence of grapevine leafroll virus by PCR. Testing is currently in progress. If the insects contain the virus, this suggests that they are playing an active role as as vectors.

Table 1. Relative abundance of mealybugs, evidence of meaybugs and the presence of ants in vineyards at a Berrien Co., MI farm.

Vineyard	Number of sampled vines	Total number of vines with mealybugs	Percent of vines with live mealybugs	Percent of vines with evidence of mealybugs	Average number of mealybugs per vine	Total number of vines with ants
Chardonnay 2	5	4	80	100	4.75	4
Seyval Blanc	15	11	73	87	5.55	13
Chardonel	15	7	47	67	3.71	7
Chardonnay 1	25	10	40	96	2.70	11
Chancellor	25	7	28	60	3.57	1
Vignoles	15	4	27	53	1.75	5
Riesling	25	6	24	76	1.00	8
Pinot Noir	15	3	20	73	2.67	3
Total/average	**140**	**52**	**42.4**	**76.5**	**3.21**	**52**

Communications Activities, Accomplishments and Impacts

Understanding the economic impact and risk of spread of grapevine leafroll virus will help growers make well-informed decisions regarding the value of virus-tested planting material as well as potential removal of infected vines/vineyards that have become uneconomical due to virus infection. The project will also help grower recognize virus symptoms and potential vectors. In addition, vector management and removal of infected vines will help curb virus spread. The project has already led to greater awareness of virus and mealybug infestation issues in Michigan. Various growers requested virus testing of their vines in response to our presentations, which also led them to reassess the damage that leafroll disease may be causing. Some growers have since applied Movento (spirotetramat, a systemic insecticide) to control mealybug infestations. While Movento is a very effective insecticide, it will not provide 100% control of mealybugs, but it can be an effective tool to stop grapevine leafroll viruses from spreading to neighboring uninfected or newly planted vineyards when applied at suitable timing. Dormant oil sprays may also be helpful. Based on this work, we will be formulating recommendations for management of grapevine leafroll and tobacco ringspot viruses in Michigan as well as assist in the decision-making process on vineyard removal. In New York, for instance, researchers recommend removing the entire vineyard when 25% of the vines are infected.

We have presented information on this project at the pre-harvest IPM meeting in Baroda, MI in August 2014, the Northwest Orchard and Vineyard show in Jan. 2015, and the Southwest Horticulture Days in Feb. 2015. We will also present the results at upcoming grape grower meetings during the 2015 growing season and the WERA-20 (Fruit Virology Working Group) meeting in Beltsville, MD in July 2015. Furthermore, we will write at least one MSUE News article on this topic during the 2015 growing season. In addition, we will present a paper on the results at the American Phytopathological Society meeting in Pasadena, CA in August 2015. A scientific paper for Plant Disease is currently in progress.

Budget narrative

The project was conducted consistent with the budget proposed by the principal investigator and approved by the State of Michigan. Matching funds were obtained from Project GREEEN in the amount of $15,800 and a second year has been applied for and is pending. In-kind matching support ($4,000) was provided by Tabor Hill Winery for maintenance costs of the 1.5 acre vineyard that we will use for our study. Dr. Naidu Rayapati, grape virologist at Washington State University tested both plant and insect samples for the presence of at viruses, which represents matching support of about $5,000 in postdoctoral labor and supplies.