2015 Final Technical 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 was to assess the distribution and damage caused by grapevine leafroll viruses (mostly GLRaV-3) in a 'Chardonnay' vineyard in Southwest Michigan and to investigate the presence and role of potential vectors in the spread of the virus. The vineyard was also infected to a with tobacco ringspot virus (TRSV) which is vectored by dagger nematodes. This scenario provided an opportunity to study the interaction of the two viruses in terms of spatial distribution and effects on yield and fruit composition. The specific objectives of the study were to:

1) Study the effect of GLRaV-3 on yield and fruit composition in 'Chardonnay' grapevines
2) Study the spatial patterns 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.

Abstract
The goal of this 2-year project was to gain a better understanding of the impact of grapevine leafroll virus disease in Michigan. In the study vineyard, 95-99% of sampled vines tested positive for GLRaV-3 and 19% for Tobacco ringspot virus (TRSV). Fruit yield decreased with increasing severity of foliar leafroll symptoms (up to 88% loss) and Brix decreased by 2-3°. There was an inverse spatial correlation between leafroll and TRSV symptoms. The grape mealybug (*Pseudococcus maritimus*) was common in SW Michigan and found in NW Michigan for the first time. GLRaV-3 was detected in sampled mealybugs, confirming their role as vectors.

Literature review
Virus diseases are widespread in vineyards throughout the world. Plant viruses are mostly spread via vegetative propagation, although insect and nematode vectors are involved in the spread of some grapevine viruses (Pearson and Goheen, 1988). 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) (Agrios,

2005). In 2011 and 2012, leaf samples from symptomatic grapevines in Michigan vineyards were tested by ELISA for 12 different viruses. Of these, 41% were positive for one or more viruses, including Grapevine leafroll viruses 1, 2, 3, 4-9; Grapevine fleck virus, Grapevine fanleaf virus, and Tobacco ringspot virus. Some grapevines had mixed infections with up to four viruses (Schilder, 2012). 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 or suspected for grape leafroll decline (GLD) symptoms. GLRaV-3 was found to be the most prevalent. A similar survey by Martin et al. (2005) also found GLRaV-1, -2, and -3 in Washington and Oregon vineyards. In the Finger Lakes region of New York, GLRaV-1, -2, and 3 were detected in nearly two-thirds of the vineyard blocks tested (Fuchs et al. 2009). Researchers in Missouri (Milkus et al. 1999) also found GLRaV-1 and GLRaV-3 with some French hybrids and American cultivars 100% infected with GLRaV-3. These studies show that grapevine leafroll viruses are widespread.

Grapevine leafroll viruses are primarily transmitted via planting material, but several species of mealybugs and scale insects have been shown to be vectors, including the grape mealybug (*Pseudococcus maritimus*), vine mealybug (*Planococcus ficus*), and obscure mealybug (*Pseudococcus viburni*) (Skinkis et al., 2009). Soft-scale insects may also transmit grapevine leafroll viruses, but less efficiently than mealybugs. Mealybugs and scale are sucking insects that ingest virus particles in sap of infected plants while feeding and can pass them on when they move to healthy vines. Disease spread can be rapid: when vectors are present, vineyards can go from 10% virus infection to greater than 90% infection in less than 10 years (Skinkis et al., 2009). This may explain the rapid spread of symptoms (in 3-4 years) from almost no observable symptoms to the majority of the vines infected in the 'Chardonnay' vineyard at Tabor Hill. Evidence of mealybug was found under the bark of some vines late last fall (Schilder and Russell, unpublished). Also, symptoms are not always consistent from year to year and stressed vines may express symptoms more readily (Skinkis et al., 2009). Mealybugs have multiple generations per year and are difficult to eradicate due to their protective waxy covering and presence under the bark. Therefore, knowing how to recognize potential insect vectors as well as leafroll symptoms may help growers manage the disease better since without intervention, the virus can spread rapidly with a vineyard and become a threat to neighboring vineyards (Skinkis et al., 2009).

Fig. 1. A) Leaf rolling symptoms of Grapevine leafroll virus and B) Vine decline and death as a result of Tobacco ringspot virus in 'Chardonnay' vines.

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 distributed throughout the vineyard, but there appeared to be fewer vines with grapevine leafroll symptoms where TRSV incidence was high. About 10% of the vines showed symptoms of both viruses. Fruit yields were low and variable due to winter injury in 2014 and 2015. Yields decreased with increasing severity of foliar symptoms, with up to 88% lower yield and a 2-3° Brix reduction in very severely affected vines. Mealybugs were common in this and surrounding vineyards and were identified as the grape mealybug (*Pseudococcus maritimus*). Mealybug was also found for the first time in Northwest Lower Michigan (Old Mission Peninsula), indicating a risk of virus transmission. GLRaV-3 was detected in sampled mealybugs, confirming their role as vectors. This study shows that leafroll viruses can be damaging to grapevines, especially in combination with TRSV, and that mealybugs are common vectors of leafroll viruses in Michigan vineyards. The results also highlight the importance of clean plant material for vineyard establishment and monitoring and management of grape mealybug in order to prevent spread of leafroll viruses. Further research is needed on mealybug biology and management as well as development of protocols for testing sites for dagger nematodes and nepoviruses before vineyard establishment.

Time line

This was a 2-year project conducted from January 1, 2014 until December 31, 2015.

Work accomplished during period (2014 and 2015) including methods (by Objective)

Accomplishments

1) *Study the effect of GLRaV-3 alone and in combination with TRSV on yield and fruit composition in 'Chardonnay' grapevines*

Grapevines were selected and marked in a virus-infected 'Chardonnay' study vineyard in SW Michigan in 2014 (30 vines) and 2015 (56 vines). Vines were placed into one of 6 categories according to differing degrees of leafroll symptom development: "apparently healthy", "mild", "moderate", "moderately severe", "severe", and "very severe", and "very severe + TRSV symptoms". In 2015, a "TRSV" category was added, which included severely declining vines only. Five to eight vines were chosen in each category. Leaf and petiole samples were taken from each vine in early October and tested for the presence of leafroll-associated viruses (1, 2, 3, 4-9) and TRSV by ELISA (enzyme-linked immunosorbent assay) and PCR in 2014 and only by PCR in 2015. Most (95-99%) of the vines were positive for GLRaV-3 with only two vines in the "apparently healthy" category being negative. The majority of vines with TRSV symptoms were positive for TRSV. In 2014, vines were also tested

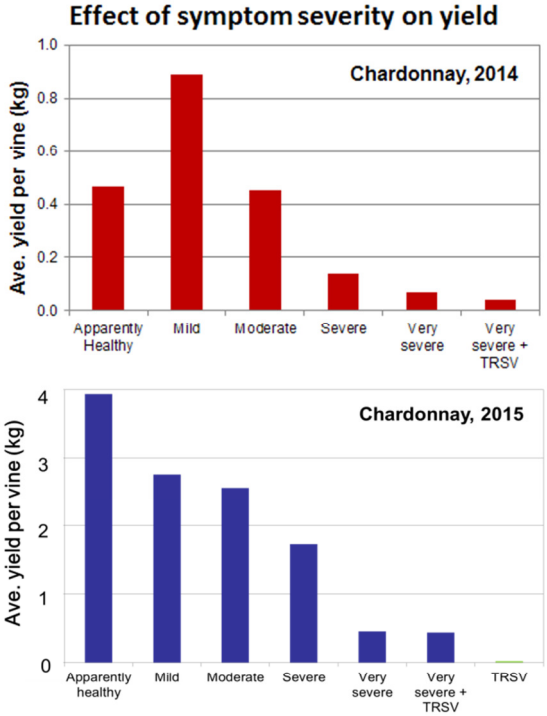

Fig. 2. Effect of grapevine leafroll symptom severity on yield in 'Chardonnay' grapevines after two hard winters. TRSV = Tobacco ringspot virus.

for other important grapevine viruses: GRBaV, GV-A, GV-B, GV-D, and GV-E by PCR in Dr. Naidu's lab; only GV-E was found and discovered to be a unique strain, only present in Michigan. All fruit was harvested from the study vines and yield measurements were taken (total weight, cluster weight, berry number and berry weight). While mildly infected vines on average had higher yield than apparently healthy vines in 2014, there was a lot of variability due to severe winter injury, and apparently healthy vines had a lot of new growth with little fruit. There was again severe winter injury the following winter but overall yields in 2015 were not as low as in 2014. In both years, there was a clear decline in yield with increasing symptoms, with about 60% yield loss in severely symptomatic vines and 80% yield loss in the very severe + TRSV vines. Vines with severe TRSV symptoms had very few clusters with tiny berries. Yield loss was primarily the result of a reduced number of clusters, although clusters were also smaller. Clusters were frozen and later analyzed for Brix, TA, pH, and anthocyanins. In both years, a 2- to 3-degree brix reduction was observed as symptoms increased in severity, and a slight increase in titratable acidity and reduction in pH. Overall, the study showed that GLRaV-3 can significantly reduce yield and juice quality, and that the degree of loss is correlated with symptom severity.

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

All vines in a 10-row section of the vineyard (~900 vines) were examined and rated for symptoms of leafroll and tobacco ringspot virus as well as for winter injury in 2014. Many vines were killed to the ground and some had already been removed by the farm crew. Others had dead cordons but had vigorous regrowth from the ground or buds on the stem. In 2015, after another hard winter, the vineyard looked worse with even more vines entirely dead or partially dead. Samples were taken from every 10th vine on a grid pattern and tested for the presence of GLRaV and TRSV. A high percentage of vines were infected with GLRaV-3 (95-99%) and some were infected with both viruses. Overall, 25% of the vines in the vineyard were dead or had been replaced with new vines since 2013, mostly due to TRSV which may predispose vines to winterkill. Leafroll symptoms were clustered to some extent. Symptom were less severe in areas where there was more TRSV, probably due to missing and stunted vines which may have interrupted movement of mealybugs. Plants were to be mapped again by symptoms after harvest in 2015, but the grower had started removing the vines in response to virus infection and poor recovery from winter injury.

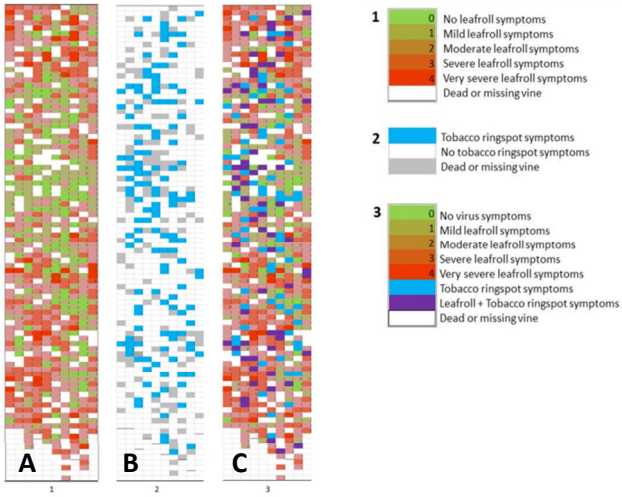

Fig. 3. Vineyard map of leafroll severity (A), TRSV symptom incidence and vine death, and both (C).

3) Identify and quantify mealybugs and other potential vectors in an infected vineyard.

In 2014, the study vineyard was examined for mealybugs and other potential vectors by checking along the trunk, cordon and shoots for insects. Loose bark was also removed to search for mealybugs. Evidence of mealybug infestation was found


Fig. 4. Grape mealybug adults and nymphs excreting sugary "honeydew".

on 72% of 90 vines examined in10 rows. Detected insects were counted and their location recorded in terms of position on the vine. Ants were also commonly associated with mealybugs and actually "farm" and protect mealybugs for their sugary excretions. Thus ants can be an indicator of the presence of mealybugs beneath the bark. A second vineyard where leafroll virus appeared to be actively spreading was identified in Jackson, MI. Ten rows of this vineyard were examined as described above. No evidence of mealybugs was found however a large number of adult and nymph box elder beetles were present. Representative samples of the mealybugs were sent to an insect taxonomist in Florida and the USDA's Systematic Entomology Lab in Beltsville, MD for identification to species. Specimens were identified as the grape mealybug, *Pseudococcus maritimus,* which is common in other grape-growing regions of the United States. In October 2015, 15 vineyards on six farms (five in SW Michigan and one in NW Michigan) were inspected for mealybugs and other potential vectors on 10-20 vines per vineyard. White fluffy mealybug debris (evidence) was found in all vineyards scouted, on 10-90% (average 40%) of the vines in individual vineyards. Actual mealybugs were also observed but were rare as it was rather late in the season. The discovery of mealybugs on the Old Mission Peninsula was a first as they were not known to be present there. They were associated with a spreading GRLaV-3 infection in a row of Pinot noir vines, which were subsequently taken out.

Fig. 5. Mealybug evidence (white fluffy deposits) seen after stripping off grapevine bark near base of vine.

4) Assess whether potential vector(s) contain GLRaV.

In 2014, a total of 50 mealybugs from the Tabor Hill vineyard and 30 box elder adults and nymphs from Lone Oak vineyard were collected, frozen and shipped on dry ice to Dr. Rayapati at Washington State University who extracted RNA followed by a PCR assay for the presence of grapevine leafroll-associated viruses. GLRaV-3 was detected in some of the mealybugs but not in the box elder bugs. This further confirms that mealybugs are vectors of GLRaV-3 as has been shown in other regions.

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. We have noticed a growing awareness among growers of virus risk to grapes and ability to recognize virus symptoms and mealybug evidence. In addition, there is a growing recognition of the need for virus-tested vines and vector management. Based on the results of this project, several growers have applied the insecticide Movento for control of mealybugs and one grower removed a row of Pinot noir vines to keep leafroll virus from spreading to the rest of his vineyard, potentially averting long-term losses. The Chardonnay study vineyard was also removed based on the high infection rate and losses observed. Other growers have requested diagnostic advice or testing.

We have shared results from this project with grape growers and other stakeholders at extension meetings (Viticulture Day 2015 and 2016; Great Lakes Expo 2014 and 2015, and the pre-bloom grape IPM meeting 2015), the North Central American Phytopathological Society meeting in East

Lansing, MI in 2015, and the WERA-20 (Fruit Virus and Viruslike Diseases Regional USDA Working Group) meeting in Beltsville, MD in July 2015 and Davis, CA in July 2016. The results were also shared via an invited presentation at a Cornell University Webinar on Grapevine Viruses and Clean Plants in March 2015. An article (14 pages) was recently published using information in "Appellation Cornell" entitled: Clean Plants for the Future of the Eastern Wine and Grape Industry" (http://grapesandwine.cals.cornell.edu/newsletters/appellation-cornell) by Tim Martinson et al. (2016). We are currently preparing a grape virus fact sheet (Schilder and Brown-Rytlewski, 2016) to be published through MSU Extension and a scientific paper to be submitted this fall to the journal Plant Disease.

Funding Partnerships

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. In-kind matching support ($8,000) was provided by Tabor Hill Winery for maintenance costs of the 1.5 acre vineyard used for the 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 $10,000 in postdoctoral labor and supplies. The results from this research project have led to a successful application for a 2016 USDA Methyl Bromide Transition grant ($420,460) to investigate management of dagger nematodes and nepoviruses in grapes and other fruit crops.

References

Agrios, G. 2005. Plant Pathology. 5th Edition. Elsevier, Inc., Edmonton, Alberta, Canada.

Endeshaw, S. T., Sabbatini, P., Gianfranco Romanazzi, G., Schilder, A. C., and Neri, D. 2014. Effects of grapevine leafroll associated virus 3 infection on growth, leaf gas exchange, yield and basic fruit chemistry of *Vitis vinifera* L. cv. Cabernet Franc. Scientia Horticulturae 170: 228–236.

Fuchs, M. F. 2007. Grapevine leafroll. Cornell University Fact Sheet. www.nysipm.cornell.edu/factsheets/grapes/diseases/grape_leafroll.pdf

Fuchs, M., Martinson, T. E., Loeb, G. M., and Hoch, H. C. 2009. Survey for the three major leafroll disease-associated viruses in Finger Lakes vineyards in New York. Plant Disease 93:395-401.

Martin R. R., K. C. Eastwell, A. Wagner, S. Lamprecht, and I. E. Tzanetakis. 2005. Survey for viruses of grapevine in Oregon and Washington. Plant Dis. 89:763-766

Mekuria, T. A., Soule, M. J., Jaragula, S., and Naidu, R. A. 2009. Current status of grapevine viruses in Washington State vineyards. Phytopathology 99:S83.

Milkus, B. N., and Goodman, R. N. 1999. A survey of Missouri vineyards for the presence of five grape viruses. Am. J. Enol. Vitic. 50:133-134.

Pearson, R. C., and Goheen, A. C. 1988. Compendium of Grape Diseases. APS Press, St. Paul, MN.

Schilder, A. M. C., and Brown-Rytlewski, D. 2016. Virus and Viruslike Diseases of Grapes. Grape Facts: Michigan State University Extension Fact Sheet (in preparation).

Schilder, A. 2012. Overview of grape diseases during the 2012 growing season. Michigan Grape and Wine Newsletter 2 (14): 4-5.

Skinkis, P. A., Dreves, A. J., Walton, V. M., and Martin, R. R. 2009. Field monitoring for grapevine leafroll virus and mealybug in Pacific Northwest vineyards. OSU Extension Publication EM 8985.