FY2012 Final report to the Virginia Wine Board

"Documentation of Grapevine leafroll-associated viruses and other major grape viruses in wine grape varieties and native grape species in Virginia, and examination of the movement of the grape leaf roll disease to develop management strategies"

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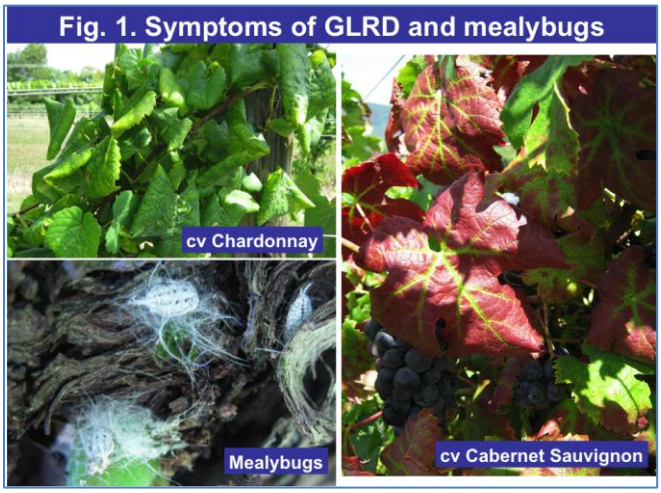
27 July 2012

Objectives

1. Document the prevalence and spatio-temporal pattern of grapevine leafroll disease and associated viruses in Virginia *V. vinifera* and inter-specific hybrids.
2. Determine whether native *Vitis* species serve as asymptomatic hosts and therefore reservoirs of leafroll viruses for newly established and replanted vineyards.
3. Develop observational data as to the presence of mealybugs as a potential vector.
4. Determine the movement of GLRaVs from infected vines to newly planted clean vine within the same row, and examine a potential management tool to restrict the movement of the vector.
5. Determine the association of viruses within a vine (mixed infection) and its potential effects.

Introduction

- Commercial vineyards in the Commonwealth of Virginia have been surveyed during 2009 and 2010 seasons for the presence of grapevine leafroll disease (GLRD) and its potential vector, mealybugs (Figs. 1 and 4).
- Typical symptoms of GLRD are inter-veinal reddening with green veins in red-fruited cultivars and mild yellowing in white-fruited cultivars (Fig. 1). In advanced stages, infected leaves of both cultivars show downward rolling of leaf margins, hence the disease is called GLRD.
- GLRD affects vineyard life span and causes yield reductions, depending on the severity of infection. It also affects the quality of berries by reducing sugar accumulation and by creating uneven ripening.
- At least ten different viruses, called grapevine leafroll-associated viruses (GLRaVs) and designated as GLRaV-1 to -10 in the order of their discovery, have been reported in grapevines affected with GLRD.

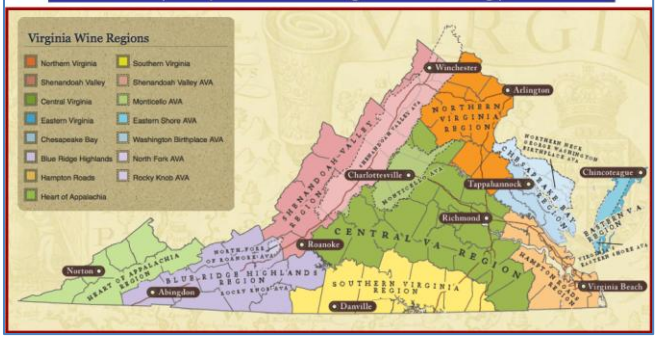


Materials and Methods

A) Survey (objectives 1-3 and 5)

- Several vineyards were selected from each of the five major grape growing regions of Virginia: 1) Northern piedmont; 2) Central; 3) Eastern; 4) Southwest; and 5) Southern Piedmont (Fig. 2). One to three vineyards planted with different cultivars were selected from each region.

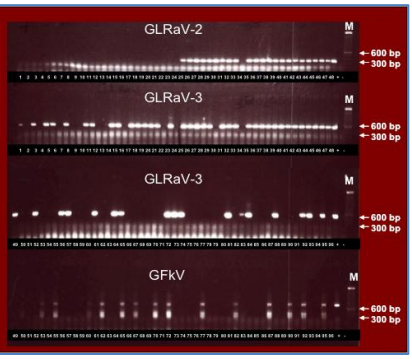
Fig. 2. Virginia wine grape growing regions
(adapted from virginiawine.org)



- Due to uneven distribution of viruses in vines, leaf samples were collected from different parts of each vine and pooled for virus testing. Samples from three neighboring vines in each row were selected randomly from each vineyard. Seven leaves per vine were collected randomly and petioles from all samples (7 petioles x 3 vines = 21) were used for extractions.
- Petioles were extracted and tested by single tube-one step RT-PCR using species-specific primers (Rowhani et al., 2000, Rayapati et al., 2006) (Fig. 3).
- Wherever necessary, amplicons were cloned and sequenced and sequences compared with corresponding sequences in GenBank for confirmation of viruses.
- Samples are tested using single tube-one step RT-PCR to detect GLRaVs, with emphasis on GLRaV-2 and -3 and Grapevine fleck virus (GfkV) (Fig. 5)

Fig. 3. RT-PCR detection of three grapevine viruses

GLRaV-2 = Grapevine leafroll-associated virus 2,
GLRaV-3 = Grapevine leafroll-associated virus 3,
GFkV = Grapevine fleck virus.
'+' = positive control
'-' = Negative control
M = 100bp molecular weight DNA marker



- In addition, Winchester's AREC facility has been equipped with modern molecular-biology equipment and tools. Thus, all of current samples have been processed in the AREC.
- we have been testing for other grapevine viruses, including GLRaVs (1, 4, 5, and 9), GVA, GVB, and RSPaV (Rupestris stem pitting virus).

B) Insecticide assays (objectives 4)

- A field containing 3 rows (13 panels per row, 3 vines per panel) of Cabernet Sauvignon (planted in 1990, confirmed GLRaV-3 infected, grape mealybugs observed, divided canopy Lyre training system) had 2 vines in each panel removed and replaced with virus-tested certified vines of Cabernet Franc in 2009 (Fig. 2).

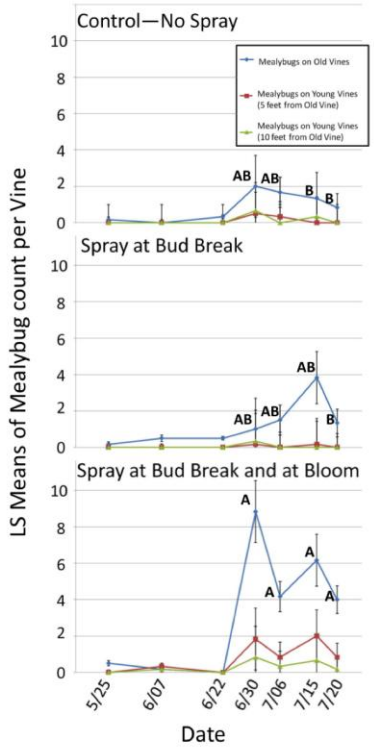
    - New plants were strategically placed so that none of the new vines were next to other infected vines besides the one within the respected panel.
- Three treatments were applied: 1) a neonicotinoid insecticide applied at delayed dormant stage (Assile, 2.5oz/ac); 2) a neonicotinoid insecticide at delayed dormant stage (Assile, 2.5oz/ac) plus a pyrethroid treatment at bloom (Baythroid XL, 3oz/ac); and 3) a control (no spray).
    - There was a total of 6 repetitions of treatments. The experimental design was a randomized block design using a section of the vineyard as a block.
- Visual assessments of GLRaV symptoms and mealybug presence were conducted throughout the 2009, 2010, and seasons. Mealybugs were counted throughout the season by visual inspection of each vine, where the rater spent 5 minutes per vine. GLRaV infections were confirmed using petiole samples from vines using a one tube-single step RT-PCR protocol with primers specific to a portion of the HSP70 homolog of GLRaV-3.
- In 2012, the old interplanted vines were removed and replaced with new certified Cabernet franc vines. The experiment has been continued with 1) a neonicotinoid insecticide applied at delayed dormant stage and at bloom (Movento, 3 oz/ac); 2) a neonicotinoid insecticide at delayed dormant stage (Assile, 2.5oz/ac) plus a pyrethroid treatment at bloom (Baythroid XL, 3oz/ac); and 3) a control (no spray).

Results

A) Survey (Objectives 1-3 and 5)

- We visited 107 vineyards, collected over 1,500 samples from over 400 sampling locations (usually by variety) (Fig. 6 at the end of this report). (note: the combined number from 2009-2011 is used in this report)
- Found 67 (63%) of vineyards to be positive, and 7.3% and 24.6% of samples are positive with GLRaV-2 and -3, respectively.
- Many of the positive samples were from vines planted before 1990's.
- Samples from 60 wild grapevines tested were found negative for the two GLRaVs and GFkV.
- Grape mealybug (*Pseudococcus maritimus*), Gill's mealybug (*Ferrisia gilli*), and Striped mealybug (*Ferrisia virgata*) were identified in vineyards. As far as we know, this is the first state report for Gill's and Striped mealybug in VA. The ability to transmit leafroll virus by Gill's and Striped mealybugs are unknown.
- Mixed-infection by different grape viruses has been assessed since the winter of 2012. Preliminary results indicated a presence of all but one (GLRaV-9) grapevine leafroll

Figure 4 **Mealybug counts on treated and untreated vines in 2011**



Different letters indicate significant difference between treatments per sampling date (P=0.05, bar = standard error) based on Tukey HSD mean separation (JMP 9, SAS Institute). Prior to ANOVA, data were transformed using square root.

viruses among sampled vines, and wide spread of Rupestris stem pitting virus.

B) Insecticide assay (Objecitve 4)

- One new vine, in 2009, (control: no insecticide treatment) tested positive for GLRaV-3, indicating the spread of GLD via mealybugs; however, neither symptoms nor mealybug was found.
- Movement of female grape mealybugs to newly planted vines was confirmed in 2010, and mealybugs were found on newly planted vines regardless of treatments.
- In 2010, Mealybug counts were high early in the season (both before and at bloom); it became increasingly harder to find the insect as the season progressed. However, in 2011, mealybugs were found in high numbers later (3 weeks after bloom) in the season (Fig. 4), indicating potential effects of seasonal environmental conditions on mealybug population changes.
- Mealybug counts differed significantly among treatments later in the season, suggesting a link between treatments and mealybug survival. i.e., potential negative impact of treatments on beneficial insects
- Slow movement of mealybugs between vines in the same panel is suggested by significantly different counts among vines (eg. Old, Young (5ft.), Young (10ft.)) (Fig. 4).
- In 2011, we added another trial using systemic insecticides. The results have shown a decrease of mealybug population over time (Fig. 7), indicating newer neonicotinoid insecticides were good options for mealybug control.
- The insecticide trials have been expanded to use two locations at AREC, and another two at commercial vineyards in 2012 season.
    - Preliminary results showed that low mealybug counts in all the locations, indicating the effect of environmental conditions. Warm winter temperature is suspected to be the cause since beneficial insects might have been active during winter months.

Figure 5. Grape mealybugs on a Cabernet sauvignon vine

Figure 6. Number of vines tested per variety, shown as a bar graph. Number of infected vines was color-coded as Dark gray (negative = no infections), Blue (GLRaV-3), Green (GLRaV-2), and White (Grapevine fleck virus) area.

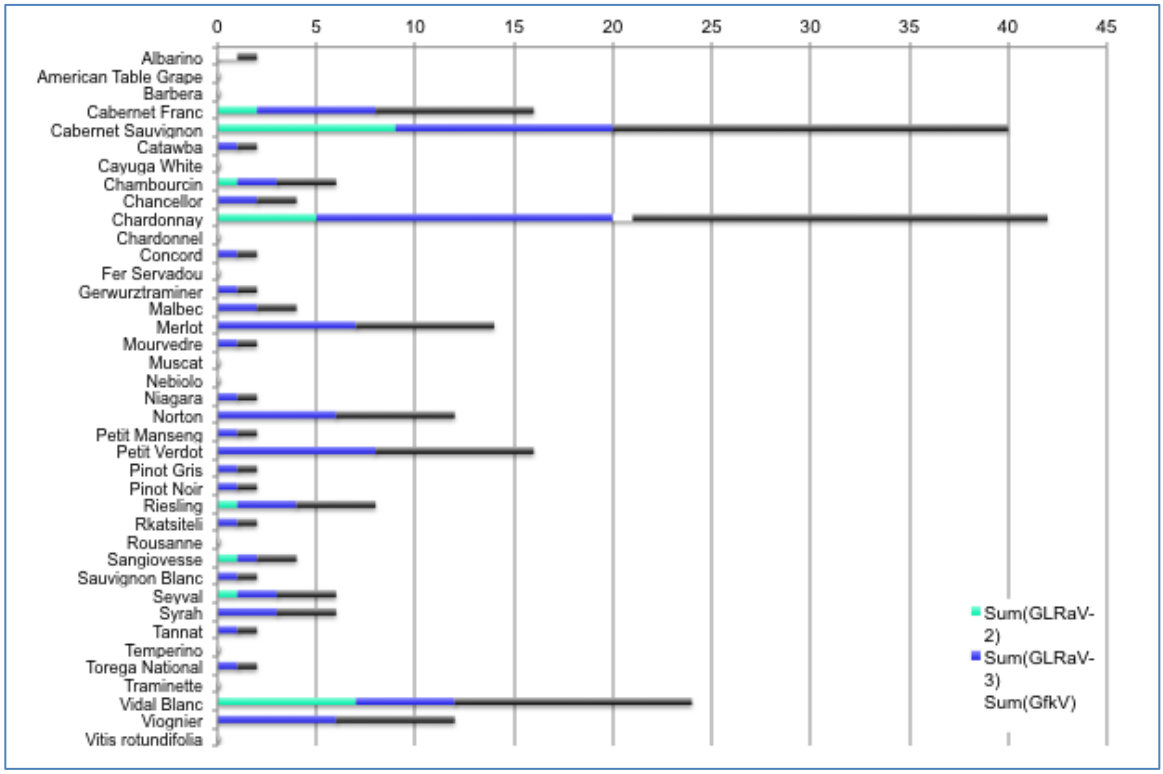
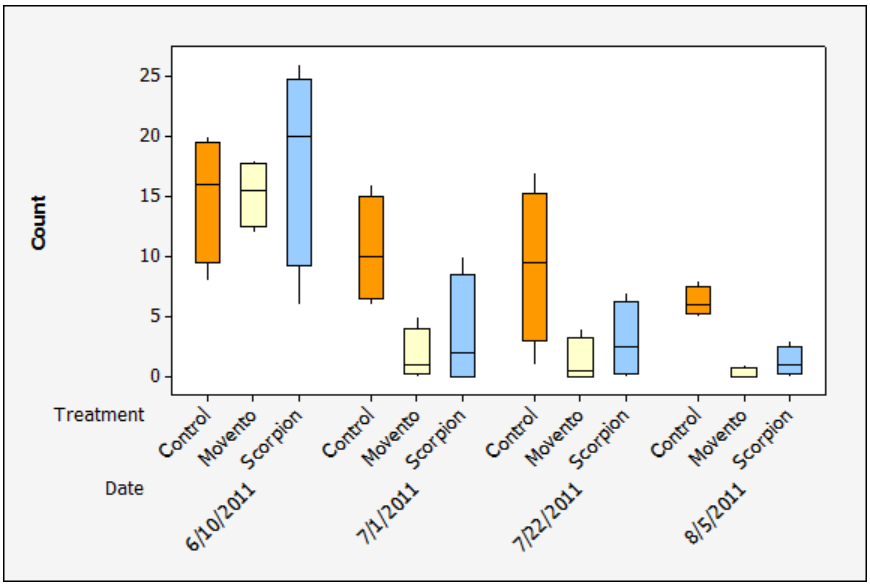


Figure 7. The number of mealybugs over time in response to two systemic insecticide treatments. There was a significant (P < 0.05) interaction between time and treatments, indicating reduction in number of mealybugs counts over time on treated vines are significantly larger (=less mealybugs) than untreated check.



Summary and future research objectives

We are about to complete our 3-year survey project (the last part of samples will be processed during August 2012). What we found was an overwhelmingly high number of leafroll viruses in commercial vineyards. More than 60% of vineyards were found to be positive with at least one of leafroll virus. As we indicated in the new proposal, we expand our analysis to examine for other grapevine viruses. So far, Respestris Stem Pitting virus is one of the common viruses in VA commercial vineyards. Our field study reveled that some of the insecticide treatment (which was listed in our pest management guide) can results in increase of mealybug population. This was probably due to suppression of beneficial insects by these treatments. Some of the systemic materials seem to provide a better efficacy, and we need to collect more data on these materials in coming years.

Our next steps in this research area are to answer these questions: 1) What is the meaning of the high rate of leafroll virus infection? How to determine economic thresholds?; 2) How is the mix infection of learoll and other viruses affect crop quality or quantity?; 3) Is the systemic insecticide the best solutions for vector control?; 4) How to gain better understanding of leafroll virus lifecycle to determine the best IPM strategy; and 5) Development of quick and robust virus identification methods that meet VA grower's needs.

This project was also an educational opportunity for Mr. Taylor Jones, who joined my lab in 2010. He has been maintaining a very good GPA (3.6) and actively participating in the survey as well as field aspects of the project. He is expected to finish his master's program in the fall of 2012. He indicated to continue on to his PhD program with me, and I am expecting him to be involved in future objectives of this research project.

Lastly, the results of this project has been presented at various stakeholder meetings including the VVA winter conference and IPM workshops. In addition, we have been presenting our results at various scientific meetings. I would like to list presentations by both Mr. Jones and me in 2011 about grapevine leafroll. All presentations were received highly among our colleagues.

NITA M., T. Jones . (2011) "A survey for grapevine viruses in Virginia vineyards" 62nd ASEV National Conference p.141.

NITA M., T. Mekuria*, N. Rayapati. (2011) "Limited effects of insecticidal treatments on the spread of grapevine leafroll diseases." Phytopathology 101:S85.

NITA M., T. Jones (2011) Limited effects of foliar insecticidal treatments on the control of mealybugs on grape. 87th Annual Cumberland-Shenandoah Fruit Workers Conference.

NITA M., T. Jones (2011) Grapevine Leafroll Disease research update. Virginia Vineyards Association meeting, February 3, 2011, Charlottesville, VA.