Final report to the Virginia Wine Board – 2013 FY
Proposal #13-1746-02

PROJECT TITLE: Understanding Grapevine Virus Complex, and Development of Grapevine Leafroll Disease management

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OBJECTIVES:

1. Determine the association of viruses within a vine (mixed infection) and its potential effects
2. Development of mealybug management strategies
3. Determine the effect of grapevine leafroll virus infection
4. Development of better diagnostic methods for grapevine viruses

Reports for each objective

1) Determine the association of viruses within a vine (mixed infection) and its potential effects

During 2009-2013, we sampled around 1,375 (675 of which are used in individual virus testing, the other 700 used in intensive field sampling for virus spread/pattern analysis) cultivated grapevine samples comprising 39 different wine grape varieties. In our previously used molecular assay, we have detected Grapevine leafroll-associated viruses (GLRaV-2 and -3) and grapevine fleck virus (GFkV), because both GLRaV-2 and -3 are very common among wine grape production worldwide and GFkV is known to cause detrimental damage when combined with GLRaV-3. We have since updated our molecular assay to have the ability to detect

grapevine leafrol disease with more accuracy, thanks to recent genomic data becoming widely available. Thus far, 10%, 26%, and 1% of vines were positive for GLRaV-2, GLRaV-3, and GFkV, respectively. With just those three viruses, 64% of the total vineyards surveyed were positive for at least one infected grapevine.

In 2012 and 2013, we expanded our detection into more varieties of viruses. During 2013, we have tested over 577 samples that were collected during between 2009 and 2013 for several viruses that are known to cause serious threat to wine grape production (Table 1). We have tested for GLRaV-1, -4, -5 and -9, and Respestris stem pitting associated virus (RSPaV-1), grapevine virus A and B (GVA and GVB). RSPaV, GVA and GVB are among the Rugose Wood Complex viruses that cause slow decline of grapevines. Also, GVA can be transmitted by mealybugs, the same vector as GLRaV-3.

Table 1 shows the total numbers of positive grapevines found so far in VA as well as the number of those vines that are involved in cases of mixed infection. Our current results support that GLRaV-3 was the most common virus from the leafroll-complex (26.3%) and RSPaV-1 (57% positive) was the most commonly found virus in the state and is involved in slightly more mixed infection cases than GLRaV-3. Testing for new viruses, such as Grapevine vein clearing virus (GVCV) and Grapevine red blotch-associated virus (GRBaV), is underway. No cases of GVCV have been confirmed, but 16% of samples have tested positive for Red blotch, making it more common than GLRaV-2 (Table 1). This study, although not yet finished, is showing that Virginia has a large number of infected vineyards and better management strategies need to be implemented across the state.

Table 1. Current results of virus survey out of 577 total grapevine samples tested.

Virus	Number of Positive Vines	% of positive	Number of mixed infections	% of mixed infection
GLRaV-1	7	1.2%	7	100.0%
GLRaV-2	61	10.6%	47	77.1%
GLRaV-3	152	26.3%	120	78.9%
GLRaV-4*	6	1.0%	6	100.0%
RSPaV-1	328	56.8%	109	33.2%
GVA	26	4.5%	25	96.2%
GVB	12	2.1%	11	91.7%
GFkV	5	0.9%	4	80.0%
Red Blotch	93	16.1%	40	43.0%
Vein Clearing	0	------	------	------

*GLRaV-4 includes strains 5, 6, 9, De, Pr, and Car as they are now all considered one virus species

In addition to wine grapes, a total of 100 wild grapevines were sampled. Some of these are taken from a field adjacent to vineyards, and others are taken from mountains. None of wild grape samples was positive for any viruses. This is a promising result since recently, a wild grapevine in California (*Vitis californica*) has tested positive for GLRaV-2, GLRaV-3, GVA, and GVB. This also indicates that we need to maintain our leafroll management in order to avoid escape of viruses to wild grapes.

When we compared samples based on their environment, vines planted prior to 1990 had a significantly higher chance of being infected with either GLRaV-2 or -3 than vines planted after 1990 (Table 2). It can be suggested that older vines were not subjected to the new molecular testing methods of the current era; therefore, the virus screening was not as good as the current standard. Similarly, vines that were infested with mealybugs had a significantly higher chance of

being infected with GLRaV-3, but not with GLRaV-2 (Table 3). This is expected since mealybugs are efficient vectors of GLRaV-3, but not a vector for GLRaV-2. It was also found that visual symptoms are not a good indicator of virus infection (Table 3). There were vines with 100% foliar symptoms that contained no viruses and there were also vines that were symptomless that were, in fact, infected with a virus.

Table 2. Probability of finding vines infested with either GLRaV-2 or -3 based on age of vine.

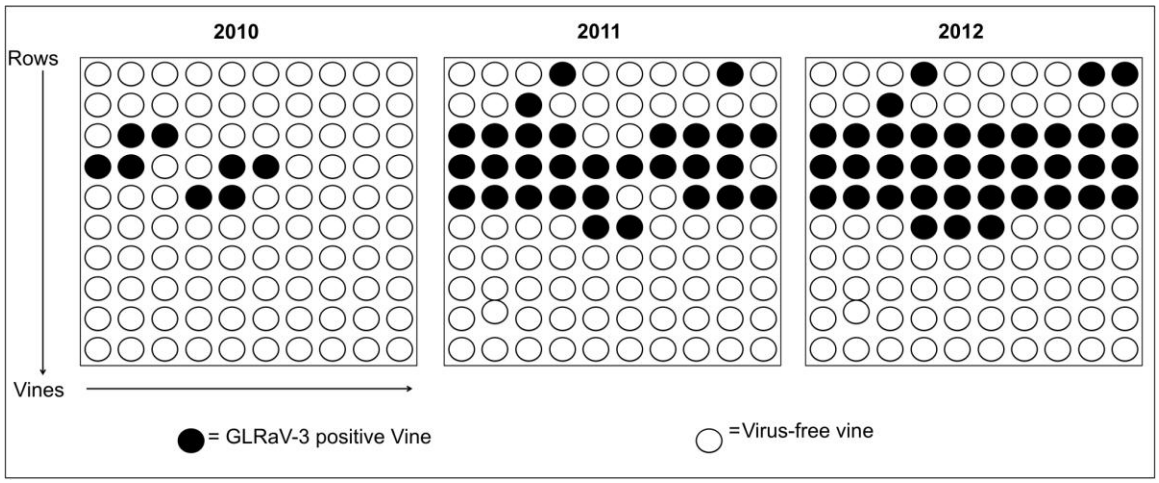
	GLRaV-2		GLRaV-3	
Age group	**LSMean[z]**		**LSMean[z]**	
Pre-1990	18.4%	A	71.4%	A
1990's	9.1%	B	38.6%	B
2000's	5.0%	B	12.2%	C

Table 3. Results from χ^2 tests on probability of GLRaV-2 or -3 infected vine and presence of visual symptoms or mealybugs

Condition	**Virus**	χ^2	***P*-value**
Symptoms	GLRaV-2	0.99	0.32
	GLRaV-3	0.03	0.85
Mealybugs	GLRaV-2	0.23	0.63
	GLRaV-3	16.2	< 0.0001

In addition to simple detection of samples, we have conducted several intensive sampling studies to monitor the movement of GLRaV-3 in a vineyard. One intensive sampling block that was tested (at the Winchester AREC) in three consecutive years showed spread of GLRaV-3 in over three years (Fig. 3). This block, which was two years old at the time of the first sampling, was planted directly next to a leafroll-infected block. At the end of the 2010 season, only 8 vines were infected with GLRaV-3; however, by the end of the 2011 season, the disease had spread to a total of 30 infected vines, a 375% increase. In the 2012 season, only 6 more cases of leafroll were found. It is important to note here that mealybug populations were very high in the 2011 season, which most likely was the cause for the quick spread of the disease. In all three years, there were significant levels of aggregation, meaning that GLRaV-3 tended to spread to nearby, adjacent vines from year to year.

Figure 3. Yearly observations of GLRaV-3 in a vineyard are showing rapid spread of virus among vines.



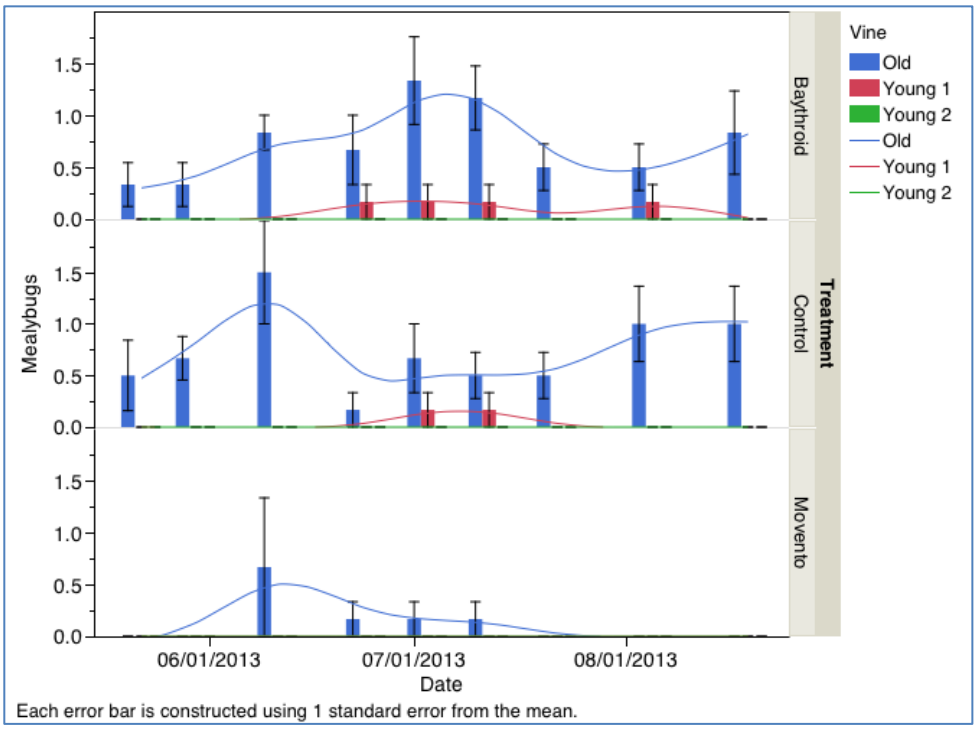
We have also conducted a survey for mealybug species. In 2012, mealybugs were collected from 7 different vineyards in the state and species identification of these insects is currently underway. We have now shown that the Gill's, grape, obscure, and striped mealybugs are present in vineyards in Virginia. Of those, only the grape and obscure mealybugs are known to transmit grapevine leafroll disease. More preliminary data shows that we can detect GLRaV-3 in Gill's mealybug, which suggested it probably be able to transmit the disease. Transmission studies will be conducted during the field season of 2014 to obtain preliminary data. Mealybug rearing for this study is currently underway.

Objective 2) Development of Mealybug Management Strategies

At AHS AREC, we have been conducting two insecticide trials. At Cabernet sauvignon vineyard, which was planted in 1990 and nearly all vines are infected with GLRaV-3, we replanted all but one vine per panel with a new Cabernet Franc vines. The idea here is to monitor movements of the virus and the vector (mealybug). Initially, contact insecticide programs were tested during 2009-2011, and we found out that application of contact insecticide (Baythroid) can actually increase the population of mealybugs in the treated vines.

In 2012, all young Cab Franc vines were replaced with new Cab Franc vines, and a new field study has been started. This time, we included three insecticide treatments 1) Assail at bud break then Baythroid at bloom, 2) Movento (spirotetramat) at bud break and at bloom, and 3) no spray check. The experiment was repeated in 2013 and the results can be seen in Figure 4. We found that the Baythroid treatment was not significantly different from the control treatment in respect to mealybug numbers and, as before, the older vines in the planting still maintained the highest numbers of mealybugs. Overall counts in 2012 and 2013 were lower than in 2011, suggesting possible climatic or seasonal changes in mealybug populations.

Figure 4: Efficacy of insecticide treatment on to mealybug population over the course of 2013 season.

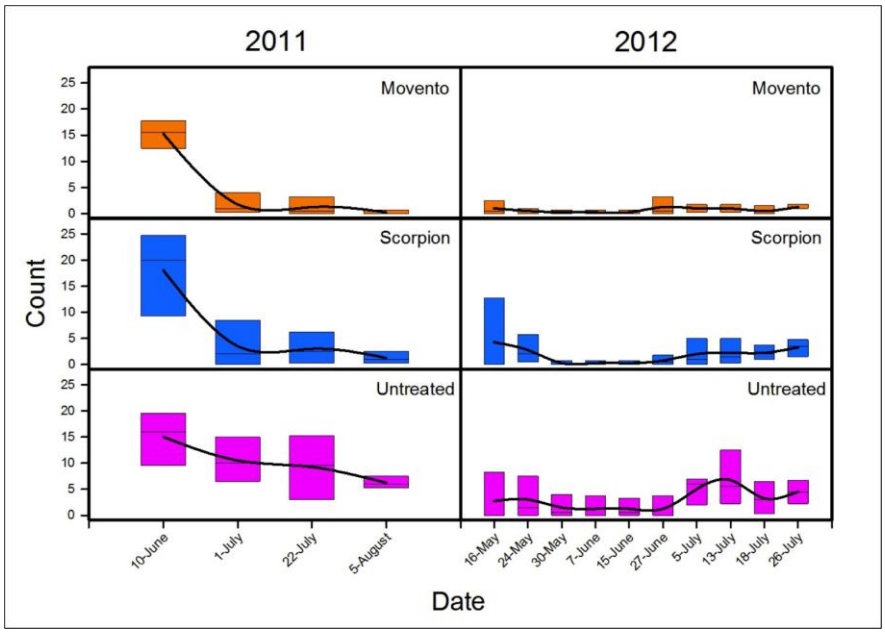


There is another trial at the AREC. We have been using Merlot vineyard where is located right next to Chardonnay vineyard where we found a few new infection by GLRaV-3 in 2010. Since none of Merlot vine was infected in 2011, we have been using this vineyard to monitor the movement of GLRaV-3 and mealybugs in new vineyard. We have used four insecticide programs 1) Lorsban High applied at dormant, 2) Lorsban low rate applied in-season, 3) Baythroid applied in-season, 4) Movent applied in-season, and 5) untreated check. As of 2013, there are some infected vines and a few (only one or two females) mealybugs observed, but no major outbreak has been observed. This experiment is in its last year (2014) and few mealybugs have been observed in the plot once again.

In addition to the AREC vineyard, we have been conducting two studies at two commercial vineyards at Orange, VA. At the first location, three treatments were tested: 1) Scorpion (dinotefuran); 2) Movento (spirotetramat); and 3) no sprayed check. Unlike AREC vineyard, application was made after observation of mealybugs in canopies.

Due to different insecticide treatments applied, the Orange vineyard trial yielded different results from the AREC vineyard. In 2011, mealybug counts ranged from 0 to 26 and an average count per vine across date and treatments was 7.1 (Figure 5). When effects of date, treatment, and their interaction were examined using ANOVA, there was no statistically significant ($P < 0.05$) interaction between date and treatment. Both date and treatment significantly ($P < 0.05$) affected the number of mealybug on vines. The differences in dates were basically the declining trend in number of mealybugs following application of insecticide treatments. There was a significant difference of mealybug counts ($P < 0.05$) between treated and non-treated vines; however, spirotetramat and dinotefuran were not significantly different.

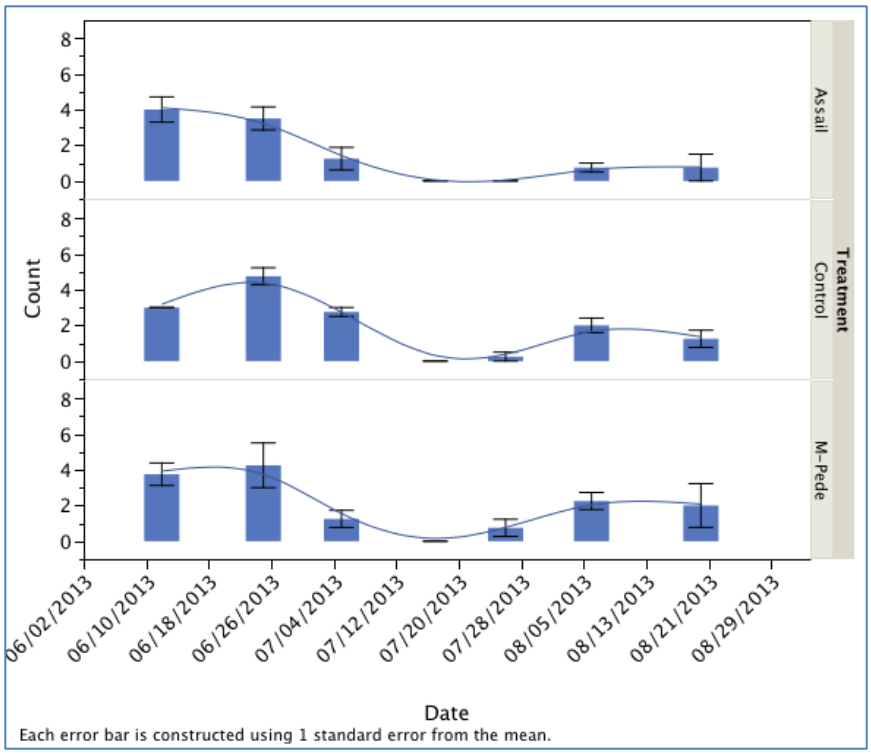
Figure 5. Seasonal changes of mealybug counts on the vines treated with Movento, Scorpion, and an untreated check in 2011 and 2012. The middle line of the box is the mean number, and bottom and top are 25% and 75% data range, respectively. Both Movento and Scorpion were applied using a backpack sprayer onto foliage twice in mid-June in both years.



In 2012, the overall population of mealybugs was lower than 2011 (Figure 5). The range of mealybugs counted per vine varied from 0 to 17, and an average count per vine across date and treatment was 1.9. As in 2011, there was no interaction between date and treatment for mealybug counts, but both date and treatment were significant ($P < 0.05$). The difference in dates was due to small peaks at the beginning of the trial and a peak that happened after 5 July. Although the difference between spirotetramat and dinotefuran was small (~1.2 mealybugs per vine), it was significant ($P < 0.05$), and vines treated with dinotefuran harbored lower number of mealybugs per vine. In 2013, the initial mealybug population was counted, and we found that there was significant difference among treatments. Vines treated with Movento resulted in significantly lower initial mealybug population ($P < 0.05$) than Scorpion-treated vines. This indicates a potential carry-over effect from Movento application, which is a systemic insecticide.

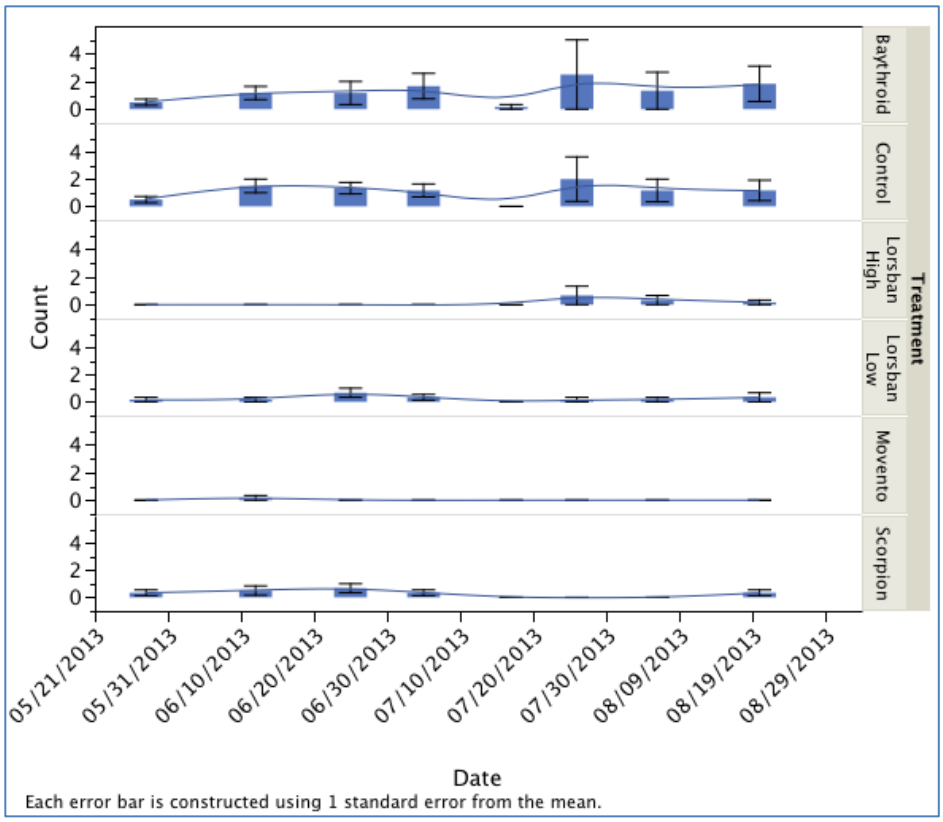
In 2013, a new trial was initiated at the same plot to examine the effects of Assail, M-Pede, and a control (no spray) (Fig. 6). Overall mealybug counts were low throughout the season, and we did not find significant differences among treatments; however, we can observe the same bimodal trend as in AHS AREC result. When we compared the effect of treatment, there was no significant difference ($P < 0.05$) among treatments. This trial is being repeated this year (2014) and is underway with similarly low levels of mealybugs.

Figure 6: Effect of insecticide treatments on mealybug population, Orange, VA Chardonnay 2013



In addition to the Chardonnay vineyard trial, we are conducting another study nearby vineyard, which is also located in Orange County, VA. Here, we have been testing 1) Lorsban High applied at dormant, 2) Lorsban low rate applied in-season, 3) Baythroid applied in-season, 4) Movent applied in-season, 5) Scorpion applied in-season, and 6) untreated check. Although 2012-13 seasons have been low mealybug seasons, the total number of mealybugs counted during the season showed that Baythroid treatment and untreated check resulted in higher number of mealybugs than other treatments, indicating once again the negative impact of in-season broad-spectrum insecticide application to mealybug populations. This experiment is also in its last season of study and mealybug counts have been ongoing since June.

Figure 7. Effect of insecticide treatment on mealybug population, Orange, VA, 2013.



Summary, the objective 2

Our results indicated how quickly mealybugs and GLRaV- 3 could be transmitted to nearby vines, and this rapid movement can happen with some insecticide treatments. The discovery of GLRaV-3 in a newly planted vine six months after planting showed that mealybugs were efficiently transmitting GLRaV-3 to new vines, even though their mobility is somewhat limited.

Our experiments demonstrated that the use of a contact insecticide may not be effective, and could actually increase mealybug populations. At both AREC and Orange locations, we have used Baythroid as one of treatment. In both cases, the mealybug population was not significantly different from untreated check. Moreover, in 2009-2011 studies, we have showed that Baythroid application actually can increase the mealybug population.

Both spirotetramat (Movento) and dinotefuran (Scorpion) treatments worked well in controlling the mealybug populations. With significant population declines in both treatments compared to the untreated check, these two treatments seem to effectively control the population. Spirotetramat may have residual effects on the following years population levels as well. When the same treatments were applied on the same vines two years in a row, the number of mealybugs treated with dinotefuran was numerically lower (difference not statistically significant) than spirotetramat in 2011. The overall counts of mealybugs in 2012 were statistically lower ($P < 0.05$) in spirotetramat-treated vines than dinotefuran-treated vines. Furthermore, the initial count of mealybugs in 2013 showed that vines sprayed with spirotetramat resulted in significantly lower counts of mealybugs than that of dinotefuran.

Use of Assail: Although other neonicotinoid insecticides (Movent and Scorpion) resulted in significant decrease in mealybug population, use of Assail did not resulted in low number of mealybugs in 2013. Also, in the AREC plot, the delayed-dormant application of acetamiprid was tested in 2009-2011, but it did not provide a significant reduction in mealybug numbers.

Objective 3) Determine the effect of grapevine leafroll virus infection

In order to compare wine quality of vines with or without GLRaV-3, we have started a preliminary wine making process using our Chardonnay vines in 2012. At the time of harvest, there were no differences in Brix or pH, thus we are not expecting to see major differences in wine.

To examine potential positive effects of a new bio-based liquid product, ecoAgra Plant Protect, the concentrate was applied foliarly three times to leafroll-infected vines at the end of the 2013 season prior to harvest. This product has been shown to treat Goss' wilt on popcorn and yellow corn, as well as sanitize virus infected lemon trees dying of yellow disease in Mexico and has benefitted other crops such as blackberries, sugar cane, and papaya.

Results

Grapes were harvested and juice samples were sent for analysis. Our results form this study show no significant difference between treated and untreated vines in terms of pH, Brix, and other acids. This trial will also be repeated in the upcoming season.

Objective 4) Development of better diagnostic methods for grapevine viruses

This objective is more for the future studies. We have been investigating the possibilities of using a piece of membrane (= paper) that can trap viral DNA and RNA from the sap of grapevines. This method will help us collecting samples in the future. For example, it will allow us to send a sheet of paper to growers if they have suspicious vines. All they need to do is rub sap from petiole to the paper, and send it back to us. Since DNA or RNA can be very unstable, we are currently relying on the freshness of the sample; however, the structure of the membrane will hold both DNA and RNA in tact for a period of time.

In addition, we have been seeking the way to detect not only presence and absence, but also quantity of GLRaV-3 RNA. It will help us to understand when these viruses will be more prominent within a vine. This information can help us determine the best timing for insecticide application.

Now that it is mid-season, virus titer should be high enough in infected grapevines to start testing this method and developing some preliminary data.

Education and other opportunities: The graduate student, Mr. Taylor Jones, who joined our program in Fall of 2010, and graduated with his MS degree in 2012, is currently taking courses and maintaining a high GPA (3.74) for his PhD work. He is currently involved in working on sample diagnosis for multiple viruses as well as the objectives listed above. He presented his PhD research proposal on March 19th 2014.

Extension and outreach: The progress has been reported as multiple oral and poster presentations in 2012 and 2013 at the VVA winter technical meeting, the national American Phytopathological Society meeting, and the Cumberland Shenandoah fruit worker conference. Also, resultes from our studies has been diectly and indirectly reported to our stakeholders through IPM workshops, vineyard meetings, and newsletter articles.

III. Future Project Plans

1. Determine the association of viruses within a vine (mixed infection) and its potential effects: about to finish in July 2014, with exceptions on a few virtues.
2. Development of mealybug management strategies: Continue insecticide trials in 2014 season
3. Determine the effect of grapevine leafroll virus infection: Continue nutrient/alternative trials in 2014 season
4. Development of better diagnostic methods for grapevine viruses: Test some of membranes in 2014 season.

IV. Funding Expended To Date

We have finished the entire funding as of 30 June 2014.