Research Proposal Progress Report

Survey for grapevine leaf roll-associated viruses in *Vitis vinifera* in Georgia and North Carolina (SRSFC Project # 2010-02)

Principal Investigators:

Phillip M. Brannen
Extension Plant Pathologist – Fruits
Department of Plant Pathology
2106 Miller Plant Sciences
University of Georgia
Athens, GA 30602
Phone: (706) 542-1250
FAX: (706) 542-4102
Email: pbrannen@uga.edu

Michael Deom
Professor of Plant Pathology
Department of Plant Pathology
3309 Miller Plant Sciences
University of Georgia
Athens, GA 30602
Phone: (706) 542-1270
FAX: (706) 542-4102
Email: deom@uga.edu

Progress Report: (SRSFC Project # 2010-02)

Objectives:

The objectives of this project are: (1) to determine the prevalence of grapevine leafroll viruses (GLRaVs) in North Georgia *V. vinifera*, *V. aestivalis* and interspecific hybrid vineyards in order to establish a baseline distribution of the disease and its epidemic range, and (2) to further determine the specific impact of the disease on yield and quality parameters.

Justification:

Wine grapes have been planted for some time in the Southeast, but the industry is expanding into new areas. Georgia's industry is smaller and newer compared to the other states(>20 wineries with sales of >50,000 cases per year), but a recent study by the Carl Vinson Institute (Univ. of Georgia) predicted over $585 million in business revenues from the winery industry over the next 20 years. Almost as important as the wine itself is the tourism that Georgia vineyards generate. Common weekend excursion destinations, wineries usually have tasting rooms, villas, restraints, and occasionally entertain weddings. The natural beauty of these vineyards makes them ideal targets for travelers and thus provides income for North Georgia.

With the current value of the Georgia wine grape industry firmly established, it has become clear that diseases can have a substantially negative economic impact. Among these, grapevine leafroll disease (GLD), caused by nine or more grapevine leafroll-associated viruses (GLRaVs) (Rayapati et al., 2008), is an emerging issue in the Southeast. GLRaVs are a highly complex group of viruses belonging to the family Closteroviridae, produce "leafroll" symptoms, and are numbered sequentially as GLRaV-

1, -2, and so on in the order of their discovery. Of these, grapevine leafroll-associated virus-3 (GLRaV-3) appears to be predominant worldwide (Gugerli, 2003).

Leafroll symptom expression varies with species and variety; it is more prominent with red-fruited *V. vinifera* varieties such as Cabernet franc and Merlot than with white-fruited varieties like Chardonnay (Rayapati et al., 2008). Typical symptoms include a reddening of the interveinal area of the leaf blade with the leaf veins and adjacent tissues remaining bright green (Fig. 1). White-fruited varieties can show a loss of green pigmentation in the interveinal regions of leaves. Leaves may also exhibit downward curling and vine vigor can be reduced.

The negative effects of GLD on vineyard productivity have been studied in detail. GLD can significantly reduce vigor, yield and grape quality (Kovacs et al., 2001), and both berry color and Brix (sugar content) are reduced (Martinson et al., 2008; Rayapati et al., 2008). GLD accounts for about 60% of the global grape production losses due to virus diseases (WSU publication). In severely infected vineyards, crop losses of 30-50% have been reported (Martinson et al., 2008). Loss of vigor can also make vines more susceptible to winter injury and other environmental factors, which may increase winter-kill in colder climates.

In a recent survey of New York's Finger Lakes grape growing regions, 68% (Martison et al., 2008) and 33% (Wilcox, 1998) of surveyed *vinifera* and hybrid, and *labrusca* grapes, respectively, were infected with GLRaV-3; a similar Canadian study showed that 50% of surveyed vineyards were infected with GLRaV-3 (Kovacs et. al., 2001). Though GLD has been present in Virginia since the late-seventies (Wolf, 2008), it has increased dramatically in the last few years; the reason for this increase is not known. In Georgia, a younger area of grape production, the disease is either highly scattered or has not often been observed. In fact, it was not identified in Georgia till 2008, when GLD was and confirmed on a Cabernet franc vineyard in Rabun County, Georgia. Of great interest, nearby Cynthiana/Norton (interspecific hybrid with strong *Vitis aestivalis* heritage) plants also showed symptoms, and these were confirmed positive for GLRaV-3 (Fig. 1). In this case, a grape variety with strong native ties showed symptoms, but in other native grapes, such as *Vitis rotundifolia* (muscadine), it is not known whether the virus can infect, and if so, symptomless hosts might be found among native southeastern grapes which often surround *V. vinifera* vineyards. Though GLD is thought to have originated with *V. vinifera* grapes from Euro-Asia, it has been found in Concords (derived from native *V. labruscana*) in New York, and infected Concord vines are often infected without asymptomatics (Martinson et al., 2008). To date, no alternative host beyond *Vitis* species have been found.

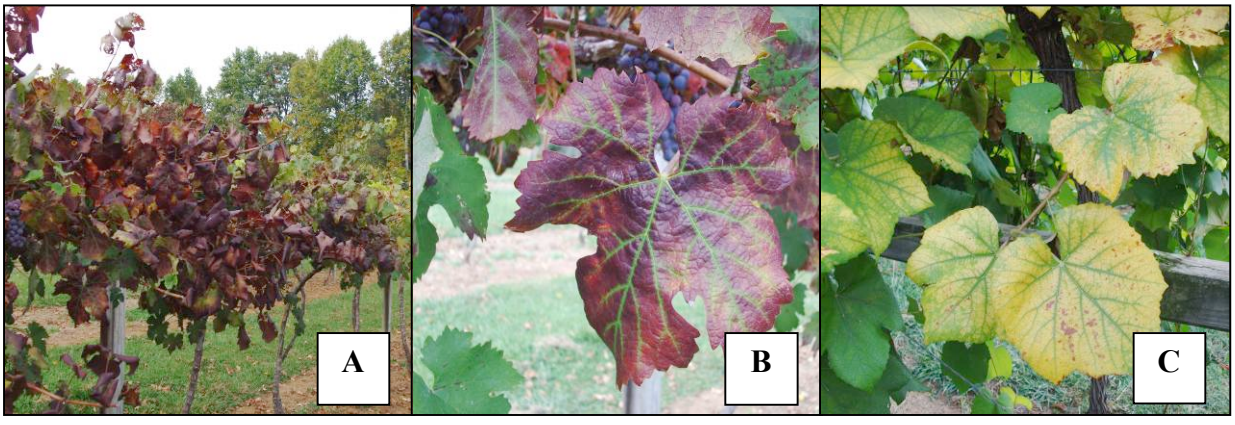

Figure 1. Grapevine leafroll disease (GLD) fall symptoms observed in Georgia on Cabernet franc vines infected with GLRaV-3 (2008). On red *Vitis vinifera* vines, leaves turn red to reddish-purple while main veins remain green (A and B). Nearby 'Cynthiana'/'Norton' grapes also tested positive for GLRaV-3, but symptoms were different, consisting of light green to yellow leaves with darker green main veins (C).

An initial determination of where GLD is present will be critical to future studies of the epidemic spread of the disease. Known methods of dissemination for GLRaV are vegetative propagation, grafting, and transmission by mealybugs (*Pseudococcidae*) and soft scales (*Coccidae*) (Rayapati et al.; 2008). Although adult male mealybugs do not feed and adult female mealybugs are relatively immobile, there is evidence that both adults and instars can be disseminated by wind. In a Washington State study, grape mealybugs (*Pseudococcus maritimus*) were found on insect traps which were placed 8 m away from an infested vineyard (Grasswitz and James, 2008). These insects have been shown to transmit four GLRaVs (GLRaV-1, -3, -5, and -9) (Martinson et al. 2008).

The extent of mealybug infestation in southern vineyards has not been determined. Anecdotal evidence in Virginia indicates possible associations of mealybugs and GLD spread, but this has not been confirmed (Wolf, 2008). Surveys in New York in 2008 did find mealybugs, which were positive for GLRaV-3 and GLRaV-1, indicating for the first time that spread by mealybugs might be important in that state (Martinson et al., 2008). The vine mealybug (*Planococcus ficus*) was not detected in three vineyards in NC where vines of GLD had been observed; however, high populations of the grape mealybug (*P. maritimus*) have been found in one vineyard in NC (Raul Villanueva, unpublished data). Mealybugs have not yet been observed in Georgia, but mealybug management through insecticides is not recommended in New York and other places (Martinson et al., 2008). However, where levels are of economic or pathological concern, mating disruption might possibly offer an effective management strategy (Boyd, 2009).

Grape Leafroll Disease is an emerging threat, and one that is known to reduce grape and wine quality. However, GLD should be manageable if the status of the disease complex in the Georgia area is fully understood. Initially, we aim to assess the prevalence of the disease, as it is essential that we obtain data on the extent of GLD in vineyards in order to develop a plan for management strategies at the regional level. The proposed research addresses one of the priorities of the Viticulture Consortium- East by helping to improve the understanding of the biology of pests and diseases and, allowing for better integrated crop management for the southern region, which is economically viable, environmentally sound and socially acceptable. It will also target the Extension and Research Priorities of the National Grape and Wine Initiative (NGWI) through increasing available website knowledge for pests and diseases of wine grapes. Information developed through this

grant will be utilized for future granting efforts which will incorporate mealybug prevalence and native grape species in the epidemiology of GLD in the Georgia.

The completion of this survey will aid Georgia wine growers in identifying and managing a potentially devastating disease. Controlling GLD will lead to both increased yields and quality of grapes and wine. This will increase viability of Georgia product and give an edge in the national market. With healthier plants, our state vineyards will be better suited to compete in the wine industry. Furthermore, keeping the virus at a minimum will cause less plants to show symptoms and thus protect the pristine image of vineyards. This will protect the valuable tourism industry that accompanies native wine.

Methodologies:

We visited multiple vineyards in Georgia between the beginning of August to the end of October in order to observe GLD symptoms. In addition, samples were received from multiple vineyards from North Carolina during the same timeframe. A visual inspection of vineyards was conducted to gain an overall estimate of GLD infection. The most helpful symptoms are a deep reddening of vine leaves, a downward curl of leaves, and irregular maturing of grape clusters. After visual inspection, the vines that showed the symptoms most expressive of GLD were sampled. The exact number of samples depended on the vineyard size; the minimum was three, ranging upwards of ten samples. Each selected vine was sampled by taking seven to ten symptomatic leaves, petioles included. These were stored immediately in a cooler and then a cold room for additional processing. We are now at this point in the methodologies for this years sampling.

For the next steps (conducted in December 2010), RNA will be extracted from small pieces of the petiole (from the distal part away from leaf blade), where 0.25g will be measured out and cut. These petiole bits will be placed in a BIOREBA sample extraction bag that includes 5ml of extraction buffer (1.59g/L Na_2CO_3, 2.93g/L $NaHCO_3$, 2% PVP-40, 0.2% bovine serum albumin, and 0.05% Tween 20 – filter sterilize). Tissue will be grinded with BIOREBA sample grinder (until no hard pieces remaine). The extract will immediately be transferred to eppendorf tubes and stored at -80°C. To conduct RT-PCR, 4µl of sample extract (from above) will be added to 50µl 1X extraction serum (0.1M glycine pH 9.0, 50mM NaCl, 1mM EDTA, 0.5% Triton X-100) with 1% β-mercaptoethanol added fresh to extraction serum, denatured at 95°C for 10 min, and snap cooled in ice. To conduct RT-PCR, 4µl of sample extract (from above) will be added to 50µl 1X extraction serum (0.1M glycine pH 9.0, 50mM NaCl, 1mM EDTA, 0.5% Triton X-100) with 1% β-mercaptoethanol added fresh to extraction serum, denatured at 95°C for 10 min, and snap cooled in ice. 2µl of denatured extract will be added to one-tube, one-step RT-PCR mix. To visualize the results, 10 ul of the completed reaction will be run on 0.8 to 1.2% agarose gel.

To better examine the extent of GLD, two vineyard blocks have been mapped for symptoms. Each vine in a set area was scored for visual symptoms, zero to five. Zero indicates no GLD symptoms, while five correlates severe symptoms and supposed infection. Anything scored three and higher is suspected to be positive for GLD. Two sites have been selected for its perceived prevalence of GLD. The chosen blocks were

red grape varieties and showed moderate amounts of GLD symptoms. The mapping will continue over multiple years to detect any possible spread throughout the block. To determine if the symptoms suspected to be GLD actually are, twenty samples have been taken: ten from vines with scores not thought to be GLD, ten from those that are. These samples will be treated exactly like the general vineyard survey, following the same extraction and molecular methods.

Certain grape fruit parameters concerning quality will were studied to see the effect of GLD infection. Clusters were taken from one block used as one the mapping sites, and the same twenty vines used before in the mapping provided the samples. Five randomly taken clusters were weighed and averaged from each vine. These were squeezed to obtain grape must. A hydrometer was used to find the Brix content, a measurement that expresses sugar quantity of the grapes. The pH was also determined using an electric pH meter. The results compared suspected healthy vines with supposed GLD infected vines. This test was conducted in October of 2009 and 2010, and will be completed in October 2011.

The current status of mealybugs in Georgia and North Carolina vineyards is unknown. To understand what types are present, if any, and their prevalence, two methods were proposed. While conducting the survey for GLD in vineyards and searching for symptoms, a researcher will also be actively looking for traces of mealybugs. Because mealybugs and their byproducts are difficult to detect in all but the most severe infestations, pheromone traps were also set up in five Georgia vineyards. Scenturian pheromone lures from Suterra were used in conjunction with sticky delta traps. Traps were checked once a month, and pheromone lures were replaced at those times. Five traps were scattered randomly through each of five Georgia vineyards.

Several wild *Vitis* species grow abundantly in Georgia and North Carolina, and these vines could serve as disease reservoirs. While visiting vineyards, researchers have briefly inspected any dense growth near blocks of grape. When wild grapes have been discovered within ten feet into the growth, several leaves were taken and placed into a Ziploc bag. These tissue samples were treated the same as *Vitis vinifera* samples taken from vineyards. They will be tested via RT-PCR to detect the presence of GLRaVs, and the grape species will be identified.

Results:

A tentative GLD survey was conducted in the fall of 2009 in Georgia vineyards. While a severe infection was detected in one vineyard, no other samples tested positive for GLRaVs. In 2010, an extensive survey was conducted throughout Georgia and North Carolina vineyards, for which RT-PCR results are pending. Visual inspection suggests a more widespread epidemic that originally estimated.

The vineyards testing positive for GLD in the 2009 survey was chosen as the first of the block mapping sites. Mapping was completed in the fall of both 2009 and 2010. Two prospective vineyard blocks for the second mapping site were plotted in the fall of 2010; determination of the second site will be evaluated over the winter.

Assays on grape quality parameters were conducted in August of 2009 and 2010 at the first mapped site. Infected grape vines showed lower values in all three categories when compared to healthy vines.

Wild vines of multiple species were found in association with many of the sites, and theses will need to be processed for viral infection as well.

Figure 2. Site 1 Block Mapping 2009

Leaf Roll Score	***Average Cluster Weight (lbs)***	***Brix***	***pH***
Average of GLD infected vines	0.573	14.875	2.9448
Average of healthy vines	0.68	16.5875	3.0348

Suterra mealybug lures were deployed in five different vineyards starting in August of 2010. While no mealybugs were obtained from the pheromone traps, a small population of the insects was discovered at a Georgia vineyard. These were sent to a N.C. State entomologist for identification.

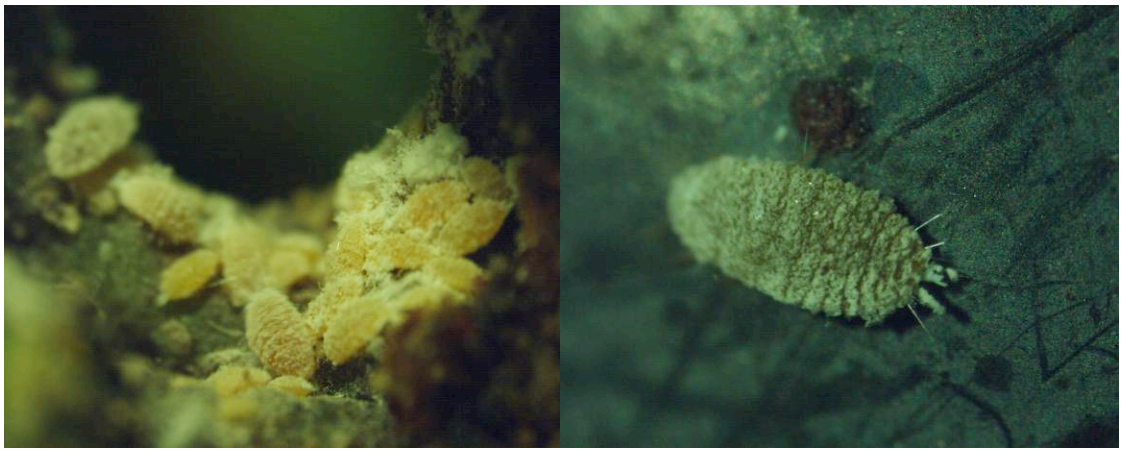

Figure 3. Mealybugs residing inside a cluster of Cabernet Franc grapes.

Conclusions: Much work still needs to be conducted to fulfill the goals of this grant. However, samples have been collected from NC and GA sites, two sites have been mapped, mealy bugs have been found, and wild grapes have been identified in close proximity to many vineyards. The extent of the GLD symptoms observed in both NC and GA has been surprising to some degree, and this does give us a snapshot of the industry issues with GLD at the moment. Many producers have new vineyards (<3 years old) which are showing extensive GLD symptoms. If these vineyards have confirmed GLD, then there will be major issues with vine destruction and replanting in order to establish clean vineyards.

Impact: To date, the impact has been minimal, but we are now starting to understand the degree of the problem which these viruses are presenting in the Southeast. We will know much more over the next year as we continue these studies.

Literature Cited:

Boyd, V. 2009. The smell of success: mating disruption helps augment a multi-pronged attack on vine mealybug. The Grower Volume 42(1).

Grasswitz, T.R., James, D.G. 2008. Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. Entomologia Experimentalis et Applicata 129:268-275.

Kovacs, L.G., Hanami, H., Fortenberry, M. and Kaps, M.L. 2001. "Latent infection by leafroll agent GLRaV-3 is linked to lower fruit quality in French-American hybrid grapevines Vidal blanc and St. Vincent." American Journal of Enology and Viticulture 52(3): 254-259.

Martinson, T., M. Fuchs, G. Loeb, and H. Hoch. 2008. Grapevine leafroll – an increasing problem in the Finger Lakes, the US and the world. Cornell University Cooperative Extension (http://winegrape.ag.psu.edu/docs/winegrapeinfo/attach/Grapevine_Leafroll_Cornell_Report.pdf)

Rayapati, N., S. O'Neal, and D. Walsh. 2008. Grapevine leafroll disease. Washington State University Extension Bulletin EB2027E (http://pubs.wsu.edu)

Rowhani, A., Biardi, L., Johnson, R., Saldarelli, p., Zhang, Y.P., Chin, J., and Green M. (2000). Simplified sample preparation method and one-tube RT-PCR for grapevine viruses. 13th ICGV, Extended Abstracts, pp148.

Wilcox, W.F., Jiang, Z.Y., and Gonslaves, D. 1998. Leafroll virus is common in cultivated American grapevines in Western New York. Plant Pathology 82:1062.

Wolf, A. 2008. Leafroll virus issues. Viticulture Notes (Nov/Dec 2008). (http://www.ext.vt.edu/news/periodicals/viticulture/08novemberdecember/08novemberdecember.html#3)