

Progress Report Submitted to:  
The Pennsylvania Wine Marketing and Research Board  
July 22, 2019

For the project entitled: **Survey for Grapevine Leafroll Viruses in Pennsylvania**

Principal Investigators:

Bryan Hed: Agriculture – Special Operations, Penn State University  
Lake Erie Regional Grape Research and Extension Center  
662 Cemetery Road, North East, PA 16428;  
Phone: 814-725-4601, ext. 203; fax: 814-725-8135; E-mail:[bxh38@psu.edu](mailto:bxh38@psu.edu)

Dr. Michela Centinari: Department of Plant Science, Assistant Professor of Viticulture  
Penn State University  
Phone: 814-867-0514; email: [mzc22@psu.edu](mailto:mzc22@psu.edu)

Dr. Cristina Rosa: Department of Plant Pathology and Environmental Microbiology,  
Assistant Professor of Plant Virology, Penn State University  
Phone: 814-867-5372; email: [czt2@psu.edu](mailto:czt2@psu.edu)

In July of year one (2017) this project began with an attempt to collect information from 105 wine grape growers in Pennsylvania through an online questionnaire. Growers were asked what varieties they grew, if they were seeing symptoms of grapevine leafroll disease (GLRD; pictures of symptoms were included in the questionnaire), and if they would be willing to cooperate in the collection of tissue samples from their vines and analysis for leafroll virus 1 and 3, the most common of the leafroll viruses in North American vineyards. In this initial phase of the project, sample collection focused on four cultivars of *Vitis vinifera* (Cabernet franc, Pinot noir, Chardonnay, and Riesling) and one French hybrid cultivar, Chambourcin, that were deemed among the most important cultivars in the PA industry. Twenty-eight cooperators were growing these cultivars and were selected for tissue collection. Selected growers were individually contacted via email and arrangements were made to collect leaf petiole samples from their vineyard blocks. Selected growers were from all parts of the state, but were most numerous in Berks, Lancaster, and Erie counties, representing the southeastern and northwestern areas of the Commonwealth, where most wine grapes are grown. Of these 28 growers, 22 reported they had seen leafroll-like symptoms in their vineyards.

In 2017, samples were collected from each of 42 vineyard blocks from 16 locations. Ten samples were collected from each vineyard block and each sample consisted of 21 petioles from 3 adjacent vines in a row (7 petioles per vine). Samples were collected from symptomatic and non-

symptomatic vines, in a semi-randomized manner. After collection, samples were transported back to the laboratory and stored at 4°C until serological analysis: samples were analyzed for GLRaV-1 and 3 with enzyme-linked immunosorbent assay or ELISA (AgriTest), using the manufacturer standard protocol.

In 2018, samples were collected from an additional 21 vineyard blocks from 8 locations for a two-year total of 63 vineyard blocks from 24 locations. Of those 63 blocks, 40 were from southeastern PA, 15 were from northwestern PA, and the remaining 8 blocks were from other parts of the state. The same sampling protocol and analysis were used in all blocks. In all, a total of 605 samples were collected from symptomatic and non-symptomatic vines, and subjected to the ELISA test for grapevine leafroll virus 1 and 3.

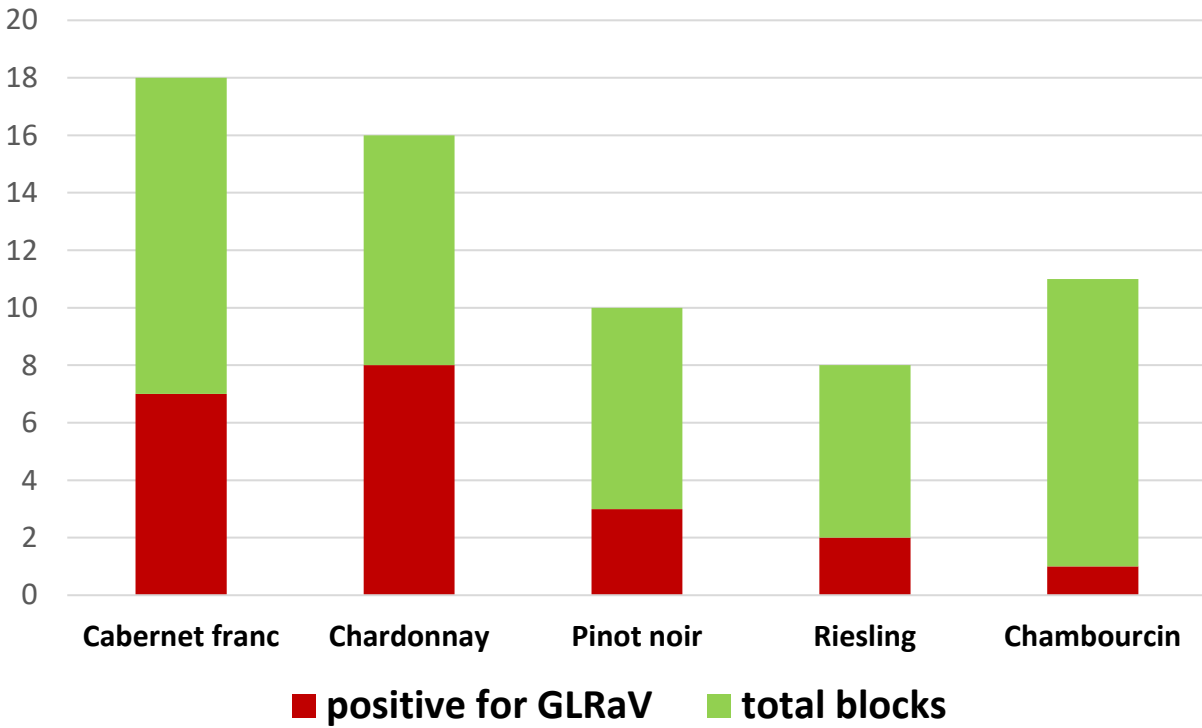
After year two, 21 (33%) of the blocks had tested positive for leafroll virus. Nine percent or 1 of 11 Chambourcin blocks sampled, contained vines that tested positive for leafroll virus 1 and/or 3. Among the 52 *V. vinifera* blocks sampled, about 20 (38.5%) contained vines that tested positive for leafroll virus 1 and/or 3. Specifically, 25, 30, 39, and 50% of the Riesling, Pinot noir, Cabernet franc, and Chardonnay blocks were positive for leafroll virus, respectively (Figure 1). At the sample level, 0.9, 7.5, 11.2, 13.5, and 22.8% of Chambourcin, Riesling, Pinot noir, Chardonnay, and Cabernet franc samples were positive for one or both of these leafroll viruses (Figure 2). Therefore, within these varieties, Cabernet franc appears to be the most heavily infected statewide, with 42 of 184 samples testing positive. It should be noted however, that sampling was not entirely random; symptomatic vines were targeted for some of the sample collection in each block. For this reason, the results may be overestimations of the overall incidence of GLRaV infected vines in the participating vineyard blocks, particularly for Cabernet franc and Pinot noir that tend to show more vivid leaf symptoms and are therefore more easily targeted. On the other hand, the targeting of symptomatic vines for at least some of the samples in each vineyard would also tend to improve the accuracy of the *incidence of GLRaV infected blocks in the study*, in that it is less likely we would have missed finding an infected vine in a vineyard that had at least some presence of these viruses. Chambourcin, the only hybrid, appears to be the least infected, with just 1 of 110 samples testing positive. Incidentally, that one positive Chambourcin sample was collected from a vineyard immediately adjacent to a heavily infected Cabernet franc vineyard block.

At one location where we were able to collect data on all four *V. vinifera* cultivars and where there were many vines positive for leafroll virus among all cultivars, there was good correlation among red varieties between vines that showed symptoms (red, curled leaves) and vines that tested positive. However, among white varieties (Riesling and Chardonnay) the correlation was poor. This may indicate that it is harder to visually identify suspicious vines among white cultivars than it is among reds.

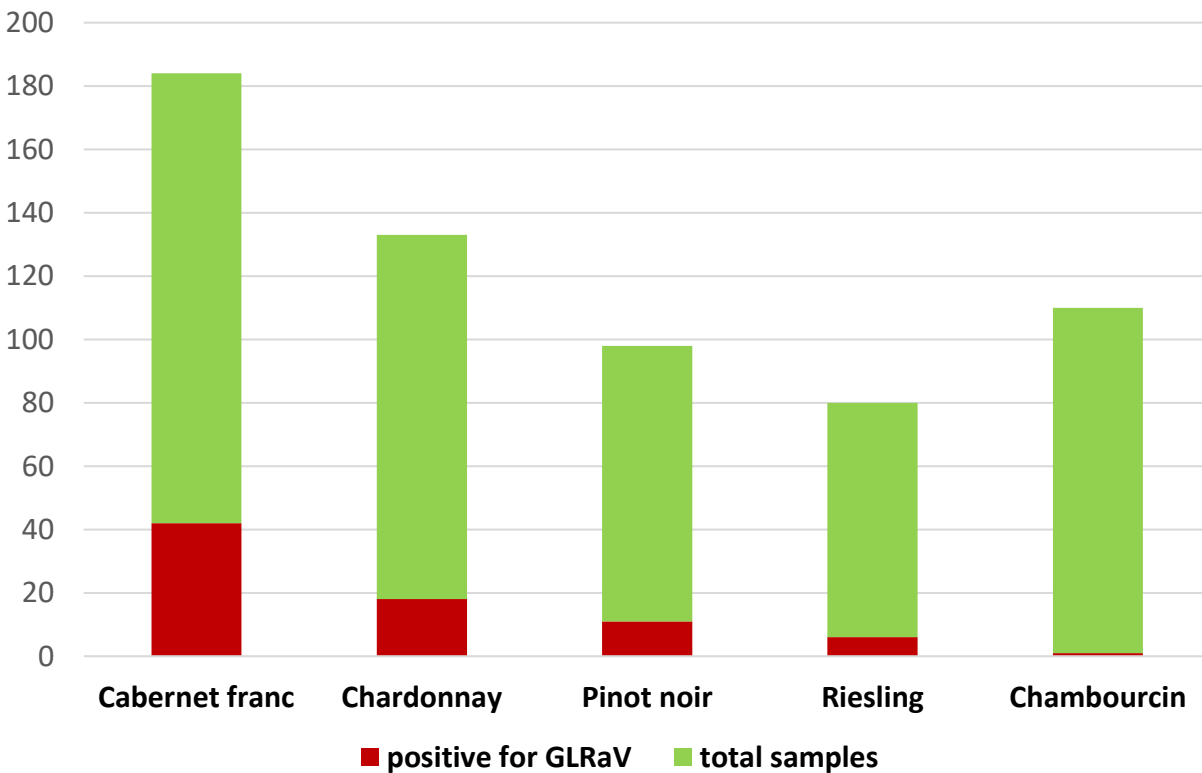
After two years of surveying wine grape vineyards in many parts of the Commonwealth of Pennsylvania, it is clear that grapevine leafroll associated viruses 1 and/or 3 (GLRaV1, 3), are common in wine growing areas of the state (found in a third of all vineyards tested), particularly in older *Vitis vinifera* vineyards and/or vineyards that originated from non-virus certified planting stock. This is important to the wine grape industry because these viruses cause grapevine leafroll disease (GLRD), that can significantly reduce yields, vigor, and cold hardiness, delay fruit maturity, reduce color development (in red grapes), and reduce fruit quality, which can negatively impact perceived wine quality. These effects are predominantly observed on varieties of *Vitis vinifera*, where symptoms of GLRD consist of cupping and discoloration, primarily of older leaves, in late summer and fall. Therefore, wine grape growers are urged to scout their vineyards during the ripening period for these symptoms. However, as visual symptoms are not considered confirmation of the presence of these viruses, growers will need to submit leaf petiole samples for laboratory analysis of vine phloem tissues to confirm the diagnosis.

This project was designed to enable us to measure the incidence of grapevine leafroll viruses 1 and 3 on five grape cultivars important to the wine industry in Pennsylvania, and the two-year survey has accomplished that. Our next goal is to address the impact of these viruses on grapevine productivity and fruit quality in PA, develop measures to reduce their spread and impact, and provide management information that will contribute to the growth and improvement of the wine grape industry in Pennsylvania. This is being accomplished in our second phase of the project that began in 2018. In this phase, we established research plots in two vineyards of Cabernet franc where we have identified healthy and infected pairs of grapevines within the same vineyard. These pairs of vines will be monitored, measured, and compared in subsequent seasons to test disease spread to healthy vines and determine the impact of grapevine leafroll disease on grape quality and productivity in Pennsylvania, with the ultimate goal to mitigate the economic impact of the disease on the PA wine industry.

**Figure 1. Statewide survey: proportion of vineyard blocks positive for GLRaV 1 and/or 3**



**Figure 2. Statewide survey: proportion of samples positive for GLRaV 1 and/or 3**



In the first season of phase two, we measured harvest yield, cluster number, berry weight, cluster weight, fruit/juice composition (brix, pH, and TA), vigor (pruning weight), and bud mortality of 23 pairs of Cabernet franc grapevines (14 pairs in vineyard 1 and 9 pairs in vineyard 2). Pairs consisted of one healthy and one leafroll infected vine, within approximately 5 meters of each other. In both vineyards, yield and cluster number per vine and cluster weight in 2018 were unaffected by the presence of grapevine leafroll virus 1 and/or 3 (tables 1 and 2). However, in vineyard 2 there was a significant effect on berry weight, with berries from infected vines being about 11% heavier. The difference in berry weight could simply be a reflection of the lower level of maturity in berries from infected vines (perhaps berries from healthy vines were more desiccated?). Juice composition was also affected by the presence of leafroll virus, where the juice of infected vines was generally of lower maturity than that of healthy vines, though the differences were not always significant (tables 1 and 2). For example, at location 1 titratable acidity was significantly higher in juice of infected vines than in healthy vines, and at location 2, juice soluble solids were significantly lower in infected vines than in healthy vines, with healthy vines averaging nearly five brix higher than infected vines. Over the past winter we also measured and compared bud hardiness and pruning weight (vine vigor) on these pairs of vines. Those results are presented in table 3; there were no significant differences in vine vigor or bud hardiness between healthy and infected vines in the two commercial vineyards.

Table 1. Cabernet franc grapevines with (V) and without (H) leafroll-associated virus 1 and/or 3 infection: location 1.

	Yield (kg/vine)	Cluster no/vine	Cluster weight (g)	Berry weight (g)	Severity of bunch rot (% fruit mass with rot)	TSS (Brix)	TA (g/L)	pH
H	6.23	44	142.2	1.54	57	18.2	6.33	3.66
V	6.23	45	143.9	1.57	30	17.7	6.81	3.65
<i>P</i> -value	0.995	0.867	0.881	0.412	0.008	0.069	0.031	0.731

Table 2. Cabernet franc grapevines with (V) and without (H) leafroll-associated virus 1 and/or 3 infection: location 2.

	Yield (kg/vine)	Cluster no/vine	Cluster weight (g)	Berry weight (g)	Severity of bunch rot (% fruit mass with rot)	TSS (Brix)	TA (g/L)	pH
H	6.43	82	79.4	1.17 b	No Data	23.7 a	3.21	3.91
V	6.34	73	88.0	1.30 a	No Data	18.9 b	3.33	3.84
<i>P</i> -value	0.876	0.149	0.138	0.045		<0.001	0.205	0.290

Table 3. Cabernet franc grapevines with (V) and without (H) leafroll-associated virus 1 and/or 3 infection: location 1 and 2.

	Vineyard 1		Vineyard 2	
	<b>Pruning wts (kg/vine)</b>	<b>% bud mortality</b>	<b>Pruning wts (kg/vine)</b>	<b>% bud mortality</b>
H	0.95	10.0	1.21	15.6
V	0.87	10.0	1.12	25.6
<i>P</i> -value	0.413		0.532	0.505