AIP – Activity 3: : **Investigation of Grapevine leafroll associated viruses infecting grapes in BC**

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Grapevine viruses, and particularly *Grapevine leafroll associated viruses* (GLRaV-1, -2, -3, and -4), are known to negatively affect grapevine (*Vitis vinifera* L.) and are regarded as one of the most important biotic constraint to grapevine health worldwide. However, little was known about the status of grapevine viruses in short season grape-growing regions such as the Okanagan Valley, British Columbia (BC). Research conducted by Scientists at the Summerland research and Development Centre (SuRDC) during the 2013-2018 research project have provided valuable information on different areas, including:

1. Molecular characterization of eight different viruses in BC [GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV), Grapevine Pinot gris virus (GPGV), and Grapevine red blotch virus (GRBV)]. In total, over 4,000 composite samples (~20,000 individual vines), collected from over 160 vineyard blocks, were tested. This large-scale survey has tremendously contributed to map the virus distribution in BC and particularly in the Okanagan and Similkameen valleys and has identified highly infected regions such as specific areas in the South Okanagan valley.
2. Spatial patterns of GLRaV infected grapevines in five red *V. vinifera* vineyard blocks and one white *V. vinifera* block located in the North, Central and South Okanagan Valley showed variable degrees of increase in disease spread ranging from 0 to 19.4% over four growing seasons. Contrary, no GRBV disease spread was recorded during three consecutive seasons in one red *V. vinifera* vineyard block.
3. Field surveys to determine infection rates of newly established vineyards (planted in 2016 or 2017) were conducted during the 2017 growing season and showed 9 out of 12 vineyard blocks to have acquired GLRaV-3 within the first year after planting. Infection of newly established plants was shown to come from adjacent GLRaV-3 infected blocks having grape mealybug present. These results emphasize the critical importance of vector management to minimize the impact of GLD in a growing region even if certified virus free planting material is available.
4. Insect taxonomic studies along with DNA-sequencing confirmed the presence of grape mealybug, European fruit lecanium scale, cottony maple scale, and a still unidentified soft scale species in the Okanagan Valley. Successful establishment of grape mealybug and cottony maple scale insect colonies under laboratory conditions was achieved during 2017. This work confirmed both insect species present in the Okanagan Valley to be capable of vectoring GLRaV-3.
5. Field surveys conducted during 2017 in over 50 vineyard blocks and surrounding areas along with DNA-sequencing, allowed confirmation of the presence of the buffalo treehopper (*Stictocephala bisonia*) and willow sharpshooter (*Graphocephala confluens*) in vineyards of the Okanagan, which are suspected vectors of the newly discovered GRBV. Work was conducted during 2017 to establish buffalo treehopper colonies under controlled conditions with the aim to conduct virus transmission experiments.
6. Mealybug and soft scale population dynamics were monitored over four years (2014 to 2017) at five different locations in the south Okanagan using a combination of double sided sticky tape trapping of crawlers, 90 seconds visual counts of large nymphs and adults on the trunk and cordon, and counts of small nymphs on leaves under compound microscope. Although, seasonal variation was observed in the mealybug and soft-scale development, overall, two generations of grape mealybug

and one generation of European fruit lecanium scale were recorded in the Central and South Okanagan. First generation grape mealybug crawler populations were usually high during mid-March, whereas the second generation was commonly observed during mid-July in the South Okanagan.

7. During the 2017 growing season, pheromone traps (Suterra LLC, Oregon) specific for capturing winged male grape mealybug were placed in two commercial vineyards in the south Okanagan. No winged males were detected and, thus, pheromone traps were shown to be unsuccessful for monitoring mealybug populations under Okanagan Valley conditions.
8. Field trials to evaluate the effects of both organic oils (Purespray green, Intelligro) and chemical insecticides (Movento, Bayer CropScience, Calgary, Alberta) on grape mealybug and soft-scale insect vectors were conducted in 2017 based on the population dynamic studies. Preliminary results showed dormant oil sprays targeting first generation grape mealybug crawlers significantly decreased the second generation population in comparison to the control vines sprayed only with water.
9. The effects of GLRaV on vine health, and fruit and wine quality have been studied in four vineyard blocks (1 Cabernet Franc and 3 Merlot). GLRaV-3 consistently reduced soluble solids (°Brix) at harvest. In addition, sensory and PCA analyses of Cabernet Franc +/- GLRaV-3 wines showed virus-infected wines to have significantly less red fruit flavor and aroma. Additionally, GLRaV-3 had minimal effect on bud hardiness.
10. Research, led by Dr. Patricia Bowen and in collaboration with Professor Olaf Niemann (Department of Geography, University of Vitoria), was started during the 2017 growing season to study the potential of drone-based hyperspectral imaging to efficiently detect a spectral signature for GLRaV and GRBV infections in both red and white *V. vinifera* cultivars.

Success Story - A success story presents a significant result or an important milestone achieved. It is intended to showcases achievements in applied research. Focus on research results, successful technology transfer, potential for pre-commercialization, and/or potential impact. A Success Story is not a progress report for each activity (suggested length 2 – 3 paragraphs).

Research conducted in this five year study presents for the first time detailed information about the current status of grapevine viruses and their insect vectors as well as the effect that viruses have on grapevine health and fruit and wine quality in a Canadian grape-growing region.

DNA-based molecular tools were developed and/or implemented for detection of up to 16 different grapevine viruses and absolute quantification of two major viruses, *Grapevine leafroll associated virus* 3 and Grapevine red blotch virus. Grapevine viruses reported for the first time to occur in BC vineyards are *Grapevine leafroll associated virus* 2, *Grapevine leafroll associated virus* 4, Grapevine fleck virus, Grapevine red blotch virus, and Grapevine Pinot Gris Virus. This represents a significant milestone as no previous capacity for molecular diagnostics and evaluation of samples from large-scale field diagnostics was available in an AAFC laboratory.

This study showed Grapevine leafroll disease (GLD) spreading at rates of up to 10% increase per year in the South Okanagan Valley and identified the presence of two GLRaV-3 insect vectors in BC vineyards, grape mealybug (*Pseudococcus maritimus*) and cottony maple scale (*Pulvinaria innumerabilis*). This information is critical as a combination of vector control and availability of certified virus free planting material is needed to effectively manage GLD. This study provided for the first time potential control strategies for insect vectors by applications of organic products and/or chemical

insecticides.

A comprehensive five year study on the effects of virus infection on vine health (including bud hardiness), and fruit and wine quality has significantly contributed to a better understanding of the impact that these viruses have on grapevines grown in a short season grape-growing region. This information will significantly contribute to the development of best practices to mitigate the impact of these viruses.

2. **Objectives/Outcomes**

3.1. Mealybug and soft scale vectors of grapevine leafroll viruses.

- **Description:** Accurately identify the soft scale and mealybug species infesting grapevines in south-central BC and determine their ability to transmit grapevine leafroll virus.
- **Outcome:** Produce distribution and abundance maps of soft scale and mealybug species, and in collaboration with Dr. Bob Foottit, AAFC-ECORC, and by genetically sequencing and identifying collected specimens provide an accurate means of identification for future management and research. Determine the ability of soft scale and mealybug to transmit leafroll virus and establish their transmission efficiencies.
- **Deliverables:** During the 2017-2018 research year, colonies of grape mealybug (*Pseudococcus maritimus*) and cottony maple scale (*Pulvinaria innumerabilis*) were successfully established on grapevine plants and maintained under greenhouse conditions at the Summerland Research and Development Centre. Transmission experiments using batches of crawlers (mobile first stage) of grape mealybug and cottony maple scale has resulted in positive transmission of *Grapevine leafroll associated virus 3* under greenhouse conditions. These results confirm the capability of grape mealybug and cottony maple scale, both present in Okanagan vineyards, to effectively transmit *Grapevine leafroll associated virus 3*

  Field surveys were conducted during the 2017 growing season to determine the presence and monitor treehopper and sharpshooter (suspected vectors of Grapevine red blotch virus) insect populations in the Okanagan and Similkameen Valleys. In total, 53 vineyard blocks (sites) were surveyed bi-weekly from June to October 2017 using the sweep net method. Additionally, samples were also collected in surrounding vineyard vegetation. Of the 53 vineyard blocks, treehoppers were found in seven sites in both Okanagan and Similkameen Valleys.

  Molecular identification of selected treehopper specimens was conducted based on mitochondrial cytochrome oxidase I (*COI*) gene. DNA results indicated the presence of buffalo treehopper

(*Stictocephala bisonia*) in BC vineyards showing 98.8% nucleotide sequence homology with reference sequences from public databases. Morphological characterization needs further clarification to distinguish it from the three-cornered alfalfa treehopper. Preliminary experiments have shown that the buffalo treehopper is capable of acquiringGrapevine red blotch virus; though, its potential to transmit this virus needs further investigation. Similarly, three specimens of sharpshooter were subjected to DNA barcoding based on partial COI gene and sequence analysis identified these species as Willow sharpshooter (*Graphocephala confluens*) with 100% homology at the nucleotide level with reference sequences from public databases. Attempts to establish buffalo treehopper colonies under greenhouse conditions are being evaluated.

3.2. Management of grapevine leafroll vectors.

- **Description:** Monitor soft scale and mealybug development in order to determine appropriate timing of sprays against the young, active crawler stages. Assess the effectiveness of early season sprays of horticultural oils and insecticides against the immature crawler stages.
- **Outcome:** Production of a developmental or phenology model for the timing of control, and provision of information to industry regarding appropriate methods to best manage grapevine leafroll vectors using chemical and non-chemical controls.
- **Deliverables:** Mealybug and soft-scale population dynamics have been monitored over four growing-seasons from 2014 to 2017 at five different locations across the south Okanagan Valley using a combination of the double sided sticky tape method, 90 seconds of visually counting large nymphs and adults ontrunks and cordons, and inspection of leaves under compound microscope. During the 2017 growing season, pheromone traps (Suterra LLC, Oregon) specific to evaluate winged male grape mealybug populations were placed in two commercial vineyards known to harbor grape mealybug in the south Okanagan. Overall, seasonal variation was observed in mealybug and soft-scale development; however, two generations of grape mealybug and one generation of European fruit lecanium scale were recorded in the Central and South Okanagan in 2017. First generation grape mealybug crawlers were high during mid-March, whereas the second generation was observed during mid-July under South Okanagan conditions.

No winged male grape mealybug insects were captured using pheromone traps in two vineyards during the 2017 growing season, which confirms that winged male populations are not common under Okanagan valley environmental conditions. These results show that contrary to other grape-growing regions, pheromone traps are unlikely to serve as a monitoring tool for grape mealybug in the Okanagan Valley.

Based on the population dynamics recorded during 2015-2016, two vineyard blocks were selected in the South Okanagan to conduct, in collaboration with Dave Nield (Minor Use Program, AAFC), efficacy trials using early season horticultural oils (Purespray green, Intelligro) and registered systemic insecticides (Movento, Bayer CropScience, Calgary, Alberta) targeting the crawler stages at both early and late season. Treatments in the efficacy trials were designed following a randomized complete block design (200 vines per plot = 4 rows by 50 vines/row, 10 vines per treatment). Each treatment was replicated four times. Treatments included horticultural oil alone, systemic insecticide alone, dormant oil plus systemic insecticide, Malathion alone, and a water only spray as a control. Horticultural oil at a 2% concentration was applied on vines at late dormancy period (April 20th, 2017). Systemic insecticide Movento was applied with the maximum recommended concentration of 0.195ppm (585ml/3000 L.) when second-generation crawlers were first observed (June 6th, 2017).

Malathion 85E at 0.2% concentration was applied on vines later in the season to target scale insect crawlers (July 12th, 2017). All sprays were carried out using recapture sprayer.

Crawler populations were measured following a combination of double sided sticky tape method and 90 seconds visual count as previously described.

In both trials, dormant oil spray targeting first generation grape mealybug crawlers showed a decrease in the second generation population in comparison to no-spray control vines. To conclude the efficacy of individual and combined effect of all treatments, population measurements during the current growing-season (2018) will be necessary. The present data could be used to design additional treatments targeting mealybug and scale insect populations.

3.3. Identification, characterization, incidence, and geographical distribution of grapevine leafroll associated viruses (GLRaVs) in BC vineyards.

- **Description:** Identification and characterization of the different GLRaVs present in all grape-growing regions of BC. Conducting field surveys to determine the incidence and significance of grapevine leafroll disease (GLD) in BC. Vineyard mapping to study GLD rate of spread within and between vineyards in time. Deposit domestic GLRaVs BC isolates in the Canadian National Plant Virus Collection housed at AAFC-PARC (Curator, M. Bernardy).
- **Outcome:** Knowledge of the different GLRaVs occurring in BC. Study of the different genetic populations of GLRaVs in BC. Knowledge of disease spread if any within vineyards and between vineyards with regard to insect vectors present in BC. This information will assist growers to understand potential risks associated with this disease and its insect vectors and will contribute to decide on the appropriate management strategy to be followed.

- **Deliverables:** During the 2017 growing season several experiments were conducted to determine the *Grapevine leafroll associated virus* 3 infection rates in newly established vineyards in the Okanagan Valley.

  In total, 1,141 composite samples (equivalent to 5,705 individual vines) and 42 targeted individual vine samples were collected from 12 vineyard blocks in the Okanagan Valley established between 2016 and 2017. Collected samples were assessed for GLRaV-3 infection in laboratory testing by ELISA (CFIA) and RT-PCR (SuRDC). Out of the 1,141 composite samples, 275 (24%) were found positive for GLRaV-3, indicating a high incidence of the virus in the newly established vineyard blocks in the Okanagan Valley.

  Among the 12 vineyard blocks surveyed, three blocks accounted for 79.2% of new infections (218 positives out of 275 samples) indicating the majority of the new GLRaV-3 infections to be confined to these three vineyards. Four blocks showed no positives for GLRaV-3, whereas the remaining five blocks showed less than 5% infection levels.

  Nine newly established (2016 and 2017) vineyard blocks in the Okanagan Valley were assessed for Grapevine leafroll disease (GLD) infection based on both visual and laboratory testing of GLRaV-3. Distances from neighbouring plantings to the test block, vine spacing and row spacing were measured, and the test block area within the larger block was flagged for future identification. Visual assessments were performed on the blocks surrounding the test block to determine mealybug and soft-scale populations.

Although GLD infection patterns needs further assessments in the 2018 growing season, preliminary analysis suggests that GLRaV-3 infection in newly stablished blocks came from neighbouring (adjacent) infected blocks that that had grape mealybug and/or soft-scale insects present. These results will help design strategies in determining the width of buffer zones to minimize secondary spread of GLD. This study highlights the importance of managing insect vectors before clean propagated "certified virus free" material can be used to plant new vineyards or re-plant virus-infected vines that had been removed.

Droplet Digital PCR was developed and standardised for detection and absolute estimation of viral copy numbers for GLRaV-1, -2, -3 and GRBV.

Isolates of GLRaV-1, -2, -3 and -4; Grapevine red blotch virus, Grapevine fanleaf virus, and Grapevine Pinot Gris virus are being propagated and maintained in *Vitis vinifera* cultivars under greenhouse conditions at Summerland Research and Development Centre. These isolates are being used as positive controls for routine diagnostic purpose as well as submitted to National Plant Virus Collection program.

Three vineyard blocks with Grapevine red blotch virus infections were mapped to monitor for disease spread and the incidence in the entire block by visual assessment was recorded. Testing of a selected number of infected plants was conducted by PCR using Grapevine red blotch virus.

During the 2017 growing season, and in collaboration with Dr. Pat Bowen (SuRDC) and Professor Olaf Niemann (Department of Geography, University of Vitoria), one Chardonnay and one Merlot block, both infected with GLRaV-3, and two Cabernet Franc blocks infected with Grapevine red blotch virus, were used for drone-based hyperspectral imaging to assess whether a spectral signature for GLRaV-3 and GRBV infection could be developed for diagnostics. Two different flights at 30 and 10 m altitude were conducted in all blocks. Data analysis is currently ongoing. This preliminary work will establish the bases of future research proposed in the CAP funding program to determine the feasibility of using hyperspectral imaging for disease detection in the field.

3.4. <u>Quantify the effects of Grapevine leafroll viruses on vine growth, fruitfulness and winter hardiness.</u>

- **Description:** The effects of the various GLRaVs on vine physiology will be determined (2013-2017) in commercial vineyard blocks having both infected and healthy vines. Measures of fruitfulness, shoot growth, pruning weights, leaf chlorophyll content and photosynthetic activity will be measured throughout the season and buds tested for levels of cold hardiness in winter. Comparisons will be made for at least 30 pairs of diseased and healthy vines per site, with several sites chosen for each virus to allow for evaluations of differences between rootstocks or varieties. Data for any particular site will be collected over two seasons. Depending on the number of sites and infected vines, statistical analyses will be performed using pairwise T-tests or more sophisticated analytical procedures.
- **Outcome:** knowledge on the effect that GLRaVs has on vine health
- **Deliverables:** During 2017, in a "tier one" premium Cabernet Franc block an experiment pairing a (+)GLRaV-3 with 2 flanking (-)GLRaV-3 vines, replicated 20 times, was set up to examine the spread of Grapevine Leaf Roll Virus and its effects on fruit composition and wine quality. This block has always been cropped at low levels by thinning fruit to one cluster per shoot to maintain high fruit quality, and because of this there were no differences in yield and crop load among viral infected and healthy vines. As in past years, the rate of photosynthesis was again reduced for virus infected vines when

measured after veraison, when viral symptoms began to appear. After harvest canopies of GLRV infected vines had significantly more red leaves than those of healthy vines. Viral testing on October 2017 found 4 vines to be newly infected with GLRaV-3, which represents an infection rate of 5% per year since 2013. There were no difference in hardiness between (+)GLRaV and (-)GLRaV vines. Researchers hope to maintain this experiment for another 5 years to chronicle the long term effects of GLRaV-3 on vine health, fruit composition and wine quality.

In the highly symptomatic leaf roll infected Merlot vineyard, the new experiment set up in 2015 to see if vines infected with both GLRaV-2 and -3 could be rehabilitated with reduced crop load and supplemental fertilization treatments, was mistakenly harvested by the winery/vineyard host. Photosynthesis measurements made during the growing season showed there was no difference in rate due to treatments or to vine vigour before veraison, but then after that as viral leaf symptoms appeared the rate of photosynthesis decreased with increasing leaf redness. Viral titer of leaf samples collected Sept 1 and 27 showed little relationship to vine vigour and were unaffected by treatments. There were no treatment effects on hardiness measured in the fall and mid-winter.

3.5. Determine the effects of GLRaVs infections on fruit and wine quality.

- **Description:** As for the pairs of virus-infected and healthy vines outlined above, the effect of virus infection on fruit quality (maturity, soluble solids, pH, etc.), chemical composition, and wine quality will be evaluated in the laboratory (2013-2017). The chemical composition of fruit from infected and healthy vines will be determined by analysis and quantification of compounds, mostly phenolics, associated with wine sensory quality. Small, replicated amounts of wine produced during the fall in the small lot winery at SuRDC will be tasted and evaluated by panels of experts consisting mostly of commercial wine makers and other industry members for quality.
- **Outcome:** knowledge on the effect that GLRaVs has on fruit quality and on the final product (wine)
- **Deliverables:** In the Cab Franc block, vines infected with GLRaV-3 had reduced soluble solids at harvest for the fifth consecutive year. Also affected in this growing season, Leafroll infected vines had decreased pH and increased titratable acidity. Wines from individual vines were again made from healthy and virus infected vines, and will undergo sensory evaluation in 2019. This past spring, sensory evaluation by industry winemakers of the 2014 and 2015 Cab Franc wines revealed differences in aroma, flavour and mouthfeel attributes. For 2014 the (-)GLRaV-3 wines had more black fruit flavour, body and astringency and less vegetative flavour than (+)GLRaV-3 wines, while only a single difference was found in the 2015 vintage where the (+)GLRaV-3 wines had greater red fruit aroma. This lack of differences between the (+) (-) GLRaV-3 wines in the 2015 vintage was likely due to a late harvest and overripe fruit (28 to 30 brix) from which the wine was made. In both years increased soluble solids in the fruit was positively correlated with increased black fruit flavour in the wine.

3.6. Transfer knowledge to the BC grape and wine industry and provide methods to reduce the introduction and subsequent spread of grapevine viruses.

Updates on the different research activities were presented to the BC industry through talks and presentations at the 2017 annual BCWGC Enology and Viticulture Conference in Penticton (BC). Additionally, talks and seminars have been given on the subject matter to other grapevine industries in Ontario and Nova Scotia.

During 2017-2018, a total of 11 presentations related to the work conducted in this research project were given in scientific conferences, invited presentation at academic, industry and government institutions, and industry conferences. Additionally, three peer review scientific manuscripts were published.

Methodology:

New/improved products and methodologies described above are fully developed and implemented and can be potentially transferrable to the sector, including both public and/or private plant diagnostic laboratories in the case of the molecular tools developed in this study.

These new/improved methodologies could have a significant impact on the grape and wine sector. In order to effectively manage grapevine virus diseases, continuous monitoring and testing are needed to record the health status of vineyards. However, current testing is expensive, costly and not widely available in Canada. The detection and diagnostic methodologies for grapevine viruses developed and improved in this research, if adapted by diagnostic laboratories, will provide grape-growers with more sensitive, rapid, specific and cost-effective testing.

Results from this research have already been adapted by industry members in BC, primarily insect control strategies and monitoring of virus spread, and wider adaptation by industry is expected following continuous technology transfer by SuRDC scientists to industry members.