Project Title: **Grape Wine Cluster Activity 17 - Grape & Wine CGCN: Development of sustainable management practices for leafhoppers on grapes.**

Reporting Period: 2019-20

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Short Plain Language Summary:

CGCN project #2, contains five research objectives contributing to the sustainable management of leafhopper pests of grapes. The aim of the *Anagrus* egg parasitoid studies conducted in ON and BC is to utilize morphological and molecular diagnostic techniques to identify alternate leafhopper hosts for the parasites, determine if a 3rd *Anagrus* wasp species has arrived in BC, and understand why Virginia creeper leafhopper (VCL) populations have decreased dramatically in certain areas of the Okanagan. Protocols have been developed and large numbers of leafhoppers and *Anagrus* parasitoids have been collected from the Niagara region of ON (Parent, Renkema) and from the Okanagan (Lowery) and Fraser Valleys (Abram) of BC. Molecular diagnostics (Gariepy) have been completed on a large number of leafhopper and *Anagrus* specimens; some *Anagrus* will now be sent to Dr. S. Triapitsyn, UC Riverside and leafhoppers to Ottawa (Kits), for species confirmation. The 2nd objective of this study, an extension of the 1st, is now to collect *Anagrus* and their leafhopper hosts from plants during the spring and summer with the goal to identify plants that might be used to enhance parasitism of VCL or western grape leafhoppers in Okanagan vineyards.

Building on previous research showing a strong feeding deterrent effect to leafhoppers for certain fungicides, feeding choice test bioassays have been completed (Lowery) to date on 9 surfactants, 9 plant essential oils, and 9 commercial products formulated from plant essential oils. Leafhopper control for those that were strongly deterrent to VCL nymphs in the lab will now be assessed in greenhouse and field spray trials. Supplies are in place and protocols developed for planned laboratory insecticide efficacy screening trials (Lowery). A replicated field spray trial was conducted in 2019 to assess the effectiveness of PureSpray Green horticultural oil in comparison to Vegol spray oil. Should restrictions be lifted in time, we hope to carry out additional spray trials in 2020. The final objective is to develop a model of leafhopper development and improve the accuracy of damage thresholds in relation to short periods of deficit irrigation. Leafhopper populations will be sampled from replicated deficit irrigation study plots established (Bowen and Bogdanoff) in 2019, and irrigation will be established for an additional deficit irrigation study in 2020 (Lowery). Temperature and phenology-based leafhopper development models are also being developed to help producers determine appropriate timing to apply control measures (leaf removal, sprays).

Variance – there are concerns that reduced levels of student assistance and quarantine restrictions related to the virus pandemic will prevent completion of all the outlined objectives.

Summary:

Leafhoppers are economically important pests of grapevines in Canada. Feeding by nymphs and adults causes the death of individual leaf cells, resulting in reduced photosynthetic activity and delayed ripening of fruit. Control is currently achieved largely with applications of insecticides, but predators and parasitoids play a significant role and there are opportunities to provide alternatives that are organic or more sustainable. An explanation for the greater abundance of Virginia creeper leafhopper (VCL) in BC compared to elsewhere in Canada is the reduced numbers of its Mymarid egg parasitoid *Anagrus daanei*. A recent dramatic decrease in numbers of VCL in certain areas of the Okanagan suggests that another species of egg parasitoid might have arrived, or survival of *A. daanei* might have improved with the arrival of another leafhopper species that serves as an alternative winter host. Comprehensive parasitoid and host plant association surveys in BC and ON will help determine if parasitoid species can be introduced to affected regions, or if host plants supportive of alternative leafhopper hosts can be utilized to enhance parasitism. Identifying *Anagrus* species based on morphological characteristics is difficult due to their small size and similar appearance. For this study, we will utilize genetic molecular techniques in addition to morphological characteristics for the accurate identification of egg parasitoids and their leafhopper hosts. New insecticides for the control of leafhoppers are desirable to prevent resistance development, as replacements to neonicotinoids that are of concern for their effects on non-target species, and as materials more suitable for organic or sustainable production (e.g. less toxic to *Anagrus* parasitoids). Horticultural oils are of interest as stand-alone products or to enhance the activity of insecticides. Efficacy data generated in the laboratory and field will contribute to the registration of novel pesticides more suited to the sustainable management of leafhoppers. From previous research we showed that certain fungicides, surfactants, and plant essential oils were highly antifeedant or repellant to grape leafhoppers. Additional study is required to evaluate the deterrent activity of several newly available commercial materials (e.g. BioRepel with 10% garlic oil) derived from plant essential oils and to assess their activity in the field. Ovipositing leafhoppers also assess grapevines for suitability based on nutrition and water status, and studies from California showed lower densities on vines undergoing moderate deficit irrigation for 3-6 weeks beginning at berry set. An average reduction in oviposition of 44% was also achieved, with additional mortality recorded during development of the nymphs. For this study we plan to evaluate the effect of deficit irrigation on leafhopper populations and vine physiology and fruit yield and quality. The various components of this proposed research will contribute to the development of a sustainable management program for leafhoppers on grapes in Canada.

Objective 01-1: Determine the *Anagrus* species parasitizing leafhopper eggs on grapevines in BC and ON and search for their alternate winter plant hosts. (Lowery, Gariepy, Parent, Abram; Kits, Renkema; Triapitsyn).

The goal of this objective is the production of a DNA database of group-specific molecular markers for the rapid and accurate identification of *Anagrus* parasitoid species and their leafhopper hosts using molecular markers for parasitized leafhopper eggs collected from grapevines and alternate hosts in ON and BC. A reference collection of slide-mounted *Anagrus* and pinned leafhoppers will be prepared as reference material for morphological determinations. *Anagrus* parasitoids of Virginia creeper leafhopper (VCL) in areas of recent population decline will be determined and alternate hosts investigated.

Previous research during the 2018-19 fiscal year produced DNA barcode primers (TG) that were tested on

Anagrus and leafhopper specimens collected from dormant and summer hosts in BC (TL) and ON (JP, JR), while J. Kits has barcoded and provided molecular sequence data for approximately 120 specimens of 40 species of Typhlocybinae leafhoppers that will serve as reference sequences for the Anagrus/leafhopper host associations. The moderate success achieved using universal DNA barcode primers led to the development (TG) of another set of COI barcode primers that bind to *Anagrus* with more specificity. Similarly, initial success analyzing leafhopper samples in 2018 using universal DNA barcode primers was modest and required a newly designed primer set that bind more specifically to the Cicadellidae. A DNA barcode library for *Anagrus* and leafhoppers has been initiated and will be populated with specimen information as it arrives for processing (TG).

To date, approximately 600 *Anagrus* from BC and ON have been analyzed; however, we continue to have issues obtaining high quality DNA sequences. This could be due to degradation of samples or due to issues with primer specificity. A new set of degenerate primers were developed for *Anagrus* to help solve any issues with primers not binding efficiently to the target section of DNA, which may allow broader amplification of different *Anagrus* species from diverse geographic locations. The degenerate primers were designed by modifying the original *Anagrus* primers but with a few degenerative bases in regions where there appears to be some variation between species within the genus. Initial PCRs with these primers have been successful and sequencing is in progress. Once an effective set of primers that yield high quality DNA sequences has been developed, we will re-sequence the samples that failed to produce adequate DNA barcodes and will continue to populate the DNA barcode database with the information. Samples collected in 2019 in ON have been received and are in the queue for processing, as are additional samples collected in 2019 from BC.

For the collection of *Anagrus* from winter hosts in ON (JR), samples of dormant wood from trees and shrubs along the edges of four Niagara-region vineyards and at a few other sites were collected approximately weekly from mid-February to early April, 2019. Samples were weighed and placed in sealed containers in the laboratory, and emerging *Anagrus* wasps were collected three times weekly from glass vials that were attached to the containers. Anagrus (12 of possibly 2 sp.) were saved in ethanol in a refrigerator and sent to London RDC for molecular identification (by TG).

Parasitism rates and leafhopper abundance was also evaluated in three organic vineyards in Niagara. Grape leaves were collected weekly from mid-June to early September, 2019, with 5 leaves from each sample examined. Eggs were categorized as parasitized or healthy, and a leaf disc with an egg was saved in ethanol and sent for molecular identification (by TG). Ten leaves from each sample were held under an overturned pot with a glass vial, into which emerged *Anagrus* adults were trapped and preserved for molecular identification. Leafhopper abundances in vineyards were also assessed by vacuum sampling one panel per sample point per vineyard. Samples are currently being assessed and leafhoppers identified.

In the lower mainland of BC, other than a few (9) transient adult leafhoppers, during this first year of sampling (2019 PA) there was no evidence of colonization of grapes by VCL or Western Grape Leafhopper (WGL) in three organic vineyards. There was also no evidence of damage and growers reported never having to apply an insecticide of any kind. Similarly, leafhoppers were also not found on their alternate host, Virginia creeper vine, at three sites in the Chilliwack area. This finding could reflect unsuitable environmental conditions for the leafhoppers, or perhaps that parasitism rates are exceptionally high.

Numerous adult leafhoppers were collected (PA) from Himalayan blackberries, Geum sp., Pacific Ninebark, and cherry (all Rosaceae) at several lower mainland, BC, sites throughout the season. From dormant leaf and stem material a total of 250 suspected *Anagrus* spp. emerged, along with a number of immature leafhoppers which will help establish tentative host associations. These specimens are preserved in 95% ethanol and are currently being sorted for molecular and morphological identification. Collections of dormant wood of blackberry, rose, ninebark, and cherry were made again in March to assess emergence of overwintering leafhoppers and parasitic *Anagrus* wasps, but this material could not be processed due to the shut-down of operations.

In the search to determine if *A. tretiakovae*, a 3rd species that parasitizes leafhopper eggs on grapes, might have moved north from WA, dormant rose material was collected (TL) in Osoyoos near the American border on Feb. 10, 2020, and again in early March and placed in extraction chambers. *Anagrus* that emerged from these winter hosts have been preserved in ethanol for identification by molecular (TG) and morphological methods (ST).

At the Summerland location having reduced leafhopper numbers on grapes and Virginia creeper, *Anagrus* and leafhopper hosts were extracted from dormant woody plants and from potential summer hosts for the second year (TL). Leafhopper numbers and parasitism rates were assessed again on leaves (20/plant) sampled from grapevines and Virginia creeper. A similar procedure was followed at a second site in north Oliver, with large numbers of *Anagrus* and leafhoppers from both locations preserved for future identification. Parasitism rates and leafhopper counts have been mapped with the intent that information can be used to pinpoint the location of possible summer and winter alternate hosts of *A. daanei* that parasitizes eggs of the VCL. Visits were made (TL) in spring 2020 to a third location south of Oliver that was identified as having very few leafhoppers on grapevines. Contact was made with participating homeowners and wineries, *Anagrus* and leafhopper hosts were reared from sampled dormant woody material, and GPS coordinates and mapping of the locations of sample materials carried out. Due to the pandemic and restrictions, it is uncertain that we will be able to collect samples this summer from these study areas.

Variance – Delayed receipt of funding in 2018, increased (25%) student pay, and the quarantine restrictions imposed in 2020 will have a significant negative impact and undoubtedly effect our ability to meet the outlined objectives.

Objective 02-2: Evaluate if provision of alternate summer hosts (mint leafhopper) of *Anagrus erythroneurae* can enhance parasitism of the western grape leafhopper in commercial vineyards. (Lowery, Kits, Gariepy, Triapitsyn)

Initially proposed to evaluate if provision of alternate summer hosts for *A. erythroneurae*, specifically the mint leafhopper, can enhance parasitism of WGL in commercial vineyards, to better suit industry needs the objective has been adapted to look for alternate spring and summer herbaceous hosts of *A. daanei* that parasitizes eggs of the VCL. Funding had also arrived too late in 2018 to establish a new long-term planting. Completion in 2018 of a previous research project of vineyard groundcover vegetation that included a treatment consisting of a mix with dwarf catmint, a known host for the mint leafhopper, showed the lingering presence of a persistent insecticide that inhibited leafhopper colonization of the study plots. This variance is also due in part to the 25% increase in student pay that has forced a 25% cut in student help. A positive outcome for this new objective is more certain with the limited assistance available.

As an extension to the research outlined above to determine the reasons for decreased VCL in select Okanagan locations, *Anagrus* parasitoids and leafhoppers were also collected in 2019 from several herbaceous plant species. Plant material for these collections was placed in sealed plastic containers having an attached clear glass vials for collection of emerged specimens. Material has been preserved in ethanol for species determination by morphological (ST, JK) or molecular (TG) means. In addition to Solomon's seal and strawberry, of particular interest are *Anagrus* reared from winter hardy beans planted in vine rows as a cover crop

Variance: The focus will shift more to sampling and collecting plant material throughout the spring and early summer for the collection of *Anagrus* parasitoids of leafhopper eggs. *Anagrus* and associated leafhopper hosts will be preserved and identified using molecular and morphological techniques. Plants identified as being hosts for one of the species that parasitizes eggs of the leafhopper pests (i.e. *A. daanei*, *A. erythroneurae*, or *A. tretiakovae*) could be preserved or enhanced in order to increase parasitism rates in vineyards. This change in focus is required as a result of the 25% increase in pay rate for students instituted during the summer of 2019. The loss of 1/4 of the student help requires a reduction in total time allocated to certain projects. Moving forward, due to the virus pandemic it is uncertain when field and lab research will resume.

Objective 03-3: Evaluate in the laboratory the deterrent effects of new plant-based essential oils to Virginia creeper leafhopper and conduct field spray trials with these and with essential oils identified previously. (Lowery, Nield)

Building on previous work that demonstrated effective deterrence of leafhopper feeding by certain commercial fungicides belonging to the strobilurin class, the objectives of the current research is to evaluate in the laboratory the deterrent effects of new commercial plant-based essential oil products and surfactants to VCL and to conduct field spray trials with these and with essential oils identified previously. This research requires the ongoing maintenance of a laboratory colony of VCL and potted grapevines required for colony maintenance and for use in the lab bioassays.

Utilizing the previously successful feeding choice test lab bioassay, during 2019-20 deterrent effects on VCL feeding were determined for six commercial plant essential oil products and six surfactants. Combined with trials completed previously, the laboratory choice test bioassays are ahead of schedule and nearing completion. A small additional number of choice test bioassays will be conducted when materials are available and following removal of restrictions on research relating to the pandemic. Several materials show great promise, such as the surfactants Silwet® and Widepread Max®. As soon as we are able, these and certain formulated plant essential oil products will be included in greenhouse trials to help determine anti-ovipositional effects and in a field trials to measure levels of leafhopper control.

Choice test feeding bioassays with VCL have now been successfully completed for 9 plant essential oils, 9 oils or commercially available formulated plant essential oils, as well as 9 surfactants. Several were shown to be highly deterrent/repellent, with some such as the OMRI-approved surfactant Silwet being highly effective at the recommended field rate. Only 13% of nymphs remained on the treated leaf discs after 24 hr. Progress is being made analyzing and compiling the data for inclusion in a scientific manuscript.

Variance – to date there are no variances to report and the laboratory component of the research is ahead of schedule and nearing completion. There is concern that the reduced level of student help and the current quarantine restrictions will impede progress and completion of the planned greenhouse and field trials.

Objective 04-4: Assess the efficacy to leafhopper nymphs of horticultural summer oils and insecticides more compatible with integrated pest management. (Lowery, Nield)

The objective of this research is to assess in laboratory and field trials the efficacy to leafhoppers of horticultural summer oils and insecticides more compatible with integrated pest management under field conditions. A replicated field spray trial was successfully conducted to assess the efficacy of PureSpray Green horticultural oil and Vegol, a formulated canola oil. Results showed a reduction in numbers for a single application of 2% PureSpray Green of about 60%. We were hoping to repeat the spray trial again in 2020 to evaluate more frequent applications and higher spray volumes. The parent company, PetroCanada, is currently applying for registration of this product for the management of leafhoppers on grapevines in Canada. A separate field trial with Silwet and one other organic insecticide material that were planned for this summer are in doubt due to the restrictions related to the virus epidemic.

Protocols have been developed for determining in the laboratory the efficacy of organic and novel insecticides to Virginia creeper leafhopper nymphs based on treated leaf disc bioassays. Materials have been chosen for inclusion in these screening trials and companies have been contacted regarding provision of test materials and support for eventual product registration. Potted grapevines and a healthy Virginia creeper leafhopper colony are in place and we are ready to switch from our antifeedant/repellent trials to these efficacy trials as soon as the current restrictions are eased and technical assistance is available. Results and information will be provided to the Interior Minor Use Committee and collaborating companies.

Variance – the reduced level of student assistance resulting from the 25% pay increase implemented in 2019 will be applied mostly to other objectives and will hopefully not impact this project unduly, but there is concern about the duration of the quarantine restrictions.

Objective 05-5: Improve the accuracy of damage thresholds in relation to water stress and determine the effect of timed, short term deficit irrigation on leafhopper populations and vine physiology. (Pat Bowen, TL, Carl Bogdanoff)

The objective of this study is to develop a model of leafhopper development and improve the accuracy of damage thresholds in relation to short periods of deficit irrigation. Building on research from California showing a reduction in leafhopper numbers with imposed periods of water stress, leafhopper populations are to be assessed in replicated deficit irrigation trials that were established by Bowen and Bogdanoff in 2019. Leafhoppers were absent from these plots during 2019, however, due to a previous application of a long-lasting systemic neonicotinoid insecticide. It is hoped that leafhoppers will re-establish in the plots for 2020. Plans are in place to establish a second experiment at SuRDC in 2020 that will allow for differing levels and durations of irrigation. The irrigation system will be established for this trial this summer, but it is currently not certain that we will be able to collect leafhopper counts or carry out measurements of vine growth, etc.

Research to develop temperature-based and phenological models of leafhopper development have been initiated in the laboratory. Dr. Elizabeth Wolkovich, UBC-Vancouver, was recently awarded a grant to study phenological and temperature-based developmental models for grapevines. We have investigated collaborating on this project, but will also implement these recordings for the sites at SuRDC. Collection of data on leafhopper population development matched to phenology and temperature data would eliminate or reduce the need to monitor leafhoppers to determine the appropriate time to apply control measures (e.g. early emergence of nymphs in the case of leaf removal) growers could utilize temperature data monitored in their vineyard blocks.

Variance – The reduced amount of available student labour and current quarantine restrictions will impede completion of the outlined objectives. Dr. Pat Bowen and Carl Bogdanoff, leads for this project, are planning to retire before the project end-date.

Outputs

Date	Category/Sub-Category/Type	Description	Status
2020/05/	Article/Paper	Acheampong, S., E. Lord, and D.T. Lowery. 2020. Monitoring of *Drosophila suzukii* in Okanagan Valley Vineyards, British Columbia, and Assessment of Damage to Table and Wine Grapes. The Canadian Entomolgist. (accepted for publication in May)	Accepted for publication.
2020/02/27	Speaker / Presenter	Lowery, T. 2020. Leafhopper Research Update: adding to the toolkit. Annual Growers Meeting, South Valley Sales, Oliver, BC. 2020/02/27.	Invited
2019/03/01	PhD Thesis	Vukicevich, E. 2019. The Effect of Vineyard Groundcover Vegetation on Soil Fungi and Plant-Soil Feedback. PhD Thesis, University of British Columbia, Okanagan. Kelowna BC. Accepted unanimously with no revisions Mar. 1, 2019.	
2019/04/	Article/Paper	Vukicevich, E., Lowery, D.T., Bennett, J., and Hart, M. 2019. Effect of groundcover vegetation and soil properties on soil fungi in multi-year survey across five semi-arid vineyard sites. Frontiers in Ecology and Evolution. http://dx.doi.org/10.3389/fevo.2019.00118	
2019/02/13	Article/Paper	Vukicevich, E., Lowery, D.T., Eissenstat, D., and Hart, M. 2019. Changes in arbuscular mycorrhizal fungi between young and old *Vitis* roots. Symbiosis. 10 pps. Published online 13 Feb., 2019. doi.org/10.1007/s13199-019-00598-3.	
2019/1/	Newsletter article	Lowery, T. 2019. Soft Scale and Mealybug Population Assessments: seeking collaboration from growers. SuRDC Wine Grape Research Newsletter. Posted on the BCWGC and GGBC websites, Jan. 2019. 2 pps, 3 photos.	
2019/02/22	Speaker/Presenter	Lowery, T. 2019. Vineyard groundcover workshop, Growers Supply Hort. Show. Oliver, BC, Feb. 22, 2019. Panel member.	invited
2019/02/22	Speaker / Presenter	Lowery, T. 2019. Management of Grape Mealybug and Soft Scale. 20th Ann. Growers Supply Hort. Show. Oliver, BC, Feb. 22, 2019.	Invited

Date	Category/Sub-Category/Type	Description	Status
2018/12/31	Article/Paper	Vukicevich, E., Lowery, T., Hart, M. (2019). Effects of living mulch on young vine growth and soil in a semi-arid vineyard, 58(3), 113-122. http://dx.doi.org/10.5073/vitis.2019.58.113-122	Draft
2018/11/21	Article/Paper	Vincent, C., Lowery, T., & Parent, J. (2018). The entomology of vineyards in Canada. The Canadian Entomologist, 1-19. doi:10.4039/tce.2018.55	
2018/07/12	Speaker/presenter	Lowery, T. 2018. Developing a Sustainable, Integrated Pest Management Program for BC Grapes. SFU Masters of Pest Management lecture, SuRDC, Summerland. July 12, 2018. oral presentation.	
2018/04/	Article/Paper	Ayyanath, M-M., C.L. Zurowski, I.M. Scott, D.T. Lowery, M. Watson, D.T. O'Gorman, K.E. MacKenzie J.R. Úrbez-Torres. 2018. Relationship between *Drosophila suzukii* (Matsumura) and post-harvest disorders of sweet cherry, *Prunus avium*. Phytobiomes 2(1): 42-50. Published online Apr., 2018. doi.org/10.1094/PBIOMES-02-17-0007-R	
2018/11/11	Speaker / Presenter	Lowery, T. 2018. History and management of invasive vineyard pests in the isolated interior of British Columbia. ESA,ESC, ESBC Joint Annual Meeting, Vancouver, BC, Nov. 11-14	Invited
2018/07/15	Speaker / Presenter	Vukicevich E, Lowery DT, Urbez-Torres JR, Bowen P, Hart M. 2018. Exploring the dynamics of the AM symbiosis as roots age. 9th Int'l Symbiosis Soc. Congress, Corvaliss, OR. July 15-20, 2018. Oral presentation.	
2018/03/07	Speaker / Presenter	Lowery, T. 2018. Implementing an Integrated Pest Management Program to Reduce Pesticides. Viticulture Diploma class, Okanagan College, SuRDC March 7, 2018. E. Vukicevich instructor. *Invited oral presentation*.	Invited
2018/02/09	Speaker/Presenter	Lowery, T. 2018. Integrated approach to leafhopper management. 19th Annual Growers Supply Horticultural Show, Oliver, BC. Feb. 9, 2018.	Invited

Issues

The 25% increase in student pay imposed half way through the summer of 2019 will result in a necessary 25% decrease in the overall level of support. As of March, 2020, quarantine restrictions related to the virus pandemic have

been applied.
Impact: Reduced ability to fully complete objectives by project end date and/or alteration or elimination of objectives or sub-objectives, particularly those that require a great deal of labour and time.
Action Plan: Collaborating Scientists have been advised to discuss with their ARDT the provision of a casual term position, which was fortunately provided to Dr. Lowery. Moving forward, research projects might need to be altered somewhat to accommodate the shortfall.