AIP - Activity 4: : Epidemiological and diagnostic studies of grapevine trunk diseases to develop and implement effective disease management strategies.

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Methodology:

Development and implementation of DNA-based detection and quantification tools for grapevine trunk diseases in the field.

Implementation and improvement of a detached cane assay (DCA). This methodology allows the screening of large amount of control products (chemicals and/or biologicals) against plant pathogens on plant material under contained conditions (greenhouse). This methodology is an effective and rapid method for products' screening and selection of best performing products to conduct field trials.

All these products have potential to be commercialized/registered in Canada

Pest Control Product:

Implementation and improvement of chemical and biological products to control grapevine trunk diseases.

Discovery of 10 biological agents (*Trichoderma* and *Bacillus* spp.) that can be potentially used for the control of grapevine trunk diseases.

These biological agents have potential to be commercialized/registered in Canada

Implementation of trunk renewal under BC conditions to mitigate the impact of grapevine trunk diseases in vineyards. Several growers have adapted and implemented this methodology since 2016 in the Okanagan valley

Grapevine trunk diseases (GTD), caused by a wide range of taxonomically unrelated fungal pathogens, cause important economic losses to the grape and wine industry worldwide and are considered one of the major threats to the future sector's economical sustainability. This AIP research (2013-2018) builds on work conducted in the previous funded DIAP project (2010-2013) and focuses primarily on understanding the epidemiology of GTD in BC vineyards, a key component to develop and implement effective control strategies in both vineyards and nurseries in Canada.

A three year study to determine seasonal patterns of GTD inoculum in vineyards in the Okanagan Valley was completed in 2017. Spore samples from five vineyard blocks located in North, Central and South Okanagan were collected weekly (3 locations) or daily (2 locations) using volumetric spore trap devices from May 2014 to October 2017. Climate parameters (T, RH and rainfall) were also collected

from each vineyard during the study. Protocols for total DNA extraction from spore trap samples were developed. Additionally, different sets of either species specific or multi-species primers were successfully implemented for absolute quantification of GTD inoculum using the digital droplet PCR (ddPCR). Overall results showed GTD spores to be present in all five vineyard blocks during the growing season (March-April to September-October); however, GTD species detected and total amount of spores varied with location. Overall, a significant higher amount of spores was generally first detected during early spring (March-April) and usually but not always correlate with large rainfall events. Interestingly, GTD spores were also detected during dry conditions, primarily in July and August. Because GTD infect grapes mainly through pruning wounds, results from this research are of significant importance as show high GTD inoculum levels in the vineyard short after pruning is conducted. These results suggest end of winter or early spring (March/April) as potentially the best time to start pruning wound protection to minimize infections caused by GTD in the Okanagan Valley.

Currently, no chemical or biological products are registered to control GTD in Canada. Laboratory and greenhouse control trials were completed in 2017. In total, 12 different products (chemical and biological) were tested to assess their efficacy and duration in protecting pruning wounds against GTD fungi. Preliminary results from these trials identified 4 chemical and 1 biological product to effectively reduce GTD infection (>80% control) under greenhouse conditions. Additionally, during 2017, 8 different *Trichoderma* and 2 *Bacillus* species were identified and characterized from different hosts in the Okanagan and were screened under laboratory conditions to determine their potential as biological control agents against GTD. Preliminary results from *in vitro* studies showed all *Trichoderma* and *Bacillus* isolates from BC to significantly reduce GTD fungal growth and hence, they could potentially be used as biocontrol agents. Field trials have been proposed in the next CAP funding cycle (2018-2023) to evaluate these products and biological agents under field conditions with the aim to provide the industry with registered products to control GTD in Canada.

First year data from pruning trials started in winter of 2016 was collected and analyzed during 2017. The main goal of this experiment is to determine whether pruning time has an effect on reducing infections by GTD fungi. Preliminary results indicated that pruning in December or January can reduce GTD infection up to 60%. Pruning trials were repeated in 2017 and a third year will be conducted in 2018.

Similarly, trunk renewal trials started in winter of 2016. The main goal of these trials is to evaluate trunk renewal (surgery) as an effective management strategy against GTD under BC conditions. Preliminary results from the first year trial showed 100% of the renewed plants in winter to survive. Yield data is expected to be recorded in 2018 and trials are expected to continue under the next CAP funding cycle.

Proof of concept of a recently developed YVD-DNA-macroarray diagnostic tool continued during 2017 by testing commercial plant samples. Results obtained within this activity show the YVD-DNA-macroarray as a robust, sensitive and specific diagnostic tool with the potential to be used in commercial diagnostics. A total of 150 plants from two different nurseries from different geographical locations were tested throughout the time of this project. Overall results showed 92% of the plants (138 plants) to be infected with at least one GTD fungal pathogen. No statistical differences on infection levels were observed between the two nurseries with a total of 90 and 95% of the plants infected from nursery A and B, respectively.

In addition to GTD fungi identified from nursery material, *Fusarium* species were isolated from over 33% of ready-to-plant nursery propagated material. Fusarium is not considered an important fungal pathogen on grapes but its high prevalence in nursery plants needed to be studied. Accordingly, work was conducted during 2017 to determine the role that *Fusarium* species play on young vine decline. In total, 4 different *Fusarium* species were identified and characterized using both morphological and

molecular studies. Moreover, pathogenicity assays were conducted in one year-old plants and showed *Fusarium* species to be pathogenic to grapes and cause similar symptoms to those caused by the well-known young vine decline fungal genus *Ilyonectria*. These findings expand our knowledge on the fungal species involved in young vine decline and introduced with the nursery planting material

Success Story

The research conducted during these five years provides for the first time information about the epidemiology of GTD fungal pathogens in a grape-growing region in Canada. This research has revealed the abundance and seasonal patterns of these important grape pathogens in the Okanagan Valley and accordingly, determined high and low risk infection periods throughout the year. This information is critical to develop and implement effective management practices and can be used by the industry to better timing spray applications (chemical and/or biological) to achieve the highest control.

Currently, neither chemical or biological products nor cultural practices are registered or available in Canada for growers to control GTD. Research conducted in this project presents for the first time potential control products and cultural practices for Canadian growers to be adapted for control of GTD. Though control strategies need further evaluation under field conditions, this study is the first step in providing solutions to control GTD in Canada.

We have developed and implemented a robust, sensitive, specific, and rapid detection molecular detection tool for GTD (DNA-macroarray). This tool is the first of its kind for GTD pathogens with the advantage of screening over 70 different fungi in one test. We have shown not only the advantages of this molecular tool against current diagnostics available for GTD at the nursery level but also have successfully adapted it to evaluate the health status of grapevine nursery material coming into Canada. With the need of clean plants for a sustainable growth of the Canadian grapevine industry, tools like the DNA-macroarray have significant potential within a health plant program.

2. **Objectives/Outcomes**

4.1. <u>Study the epidemiology/disease cycles of major grapevine trunk disease (GTD) pathogens in BC</u>

- **Description:** Study the epidemiology and disease cycles of grapevine trunk diseases fungal pathogens identified in a disease survey conducted from 2010-2012. Use epidemiological data to investigate or adapt various trunk disease control strategies in the vineyard.
- **Outcome:** Identify key infection periods within GTD pathogen disease cycles. Identify effective commercial fungicide that should add grapes and GTD pathogens to the Canadian label. Identify cultural practices that can help minimize and manage GTD.
- **Derivable:** Five volumetric spore trap devices (Burkard, England) along with weather stations (HOBO or Campbell Scientific) were located in five different vineyards, including West Kelowna (North Okanagan), Summerland and Okanagan Falls (Central Okanagan), and Oliver South and Osoyoos (South Okanagan). Spore traps and weather data were collected daily (West Kelowna and Oliver South) or weekly (Summerland, Okanagan Falls, Osoyoos) from May 2014 until October 2017. DNA extraction protocols and multi-species specific primers, developed and implemented during the early stage of the project, were used for absolute quantification of GTD pathogens (Botryosphaeriaceae spp. and Diatrypaceae spp.) using the digital droplet PCR (ddPCR) system. Data analysis (2014-2017) from weekly spore traps was completed during 2017-2018. To date, one year data analysis (2014-2015) from daily spore traps has been completed. Full data analysis from these two sites is expected to be completed by April-May of 2018. Overall results from both weekly and daily spore trap samples

showed GTD spores (Botryosphaeriaceae spp. and Diatrypaceae spp.) to be present in all five vineyards throughout the growing season (March-Aprill to September-October). However, species prevalence and inoculum quantity varied depending on location and year.

Summerland: Low levels of Botryosphaeriaceae (BOT) spores were overall detected in 2014. In 2015, a significant first discharge of BOT spores was detected the first week of April and the first, third and last week of May. High levels of BOT spores were also detected the first week of September. Very low levels of BOT spores were detected during 2016 and 2017. Diatrypaceae (DIA) spores were detected in high levels from April to August/September in all three years (2014-2017). Highest spores count was detected in August in 2014, July in 2015 and May in both 2016 and 2017.

Okanagan Falls: High levels of BOT spores were detected throughout 2014, 2015, 2016 and 2017 growing seasons (March/April to September/October). Highest counts of BOT spores were recorded on the last week of May in 2014, last week of June and last week of September in 2015, and first week of March in 2016. Very low levels of DIA spores were detected in 2014. DIA spores were detected from April to August in 2015 and 2016 with highest count recorded in April of 2016.

Osoyoos: BOT spores were detected from May to September in 2014, from March to October in 2015 and 2016, and from May to September in 2017. Overall, the highest BOT spore release was recorded during the month of June. DIA spores were detected from March to September in all years with highest release of spores recorded in June (2014 and 2016) and August (2015 and 2017).

West Kelowna: First year data analyses (May 2014 - May 2015) showed BOT and DIA spores to be present in the vineyard from May to September. BOT spores were still detected during October and November but in very low amounts.

Oliver South: First year data analyses (May 2014 - May 2015) showed BOT spores to be present in the vineyard throughout the entire period with the exception of December. Though present, low levels of BOT spores were detected in January and February. First large discharge of BOT spores was detected on March the 1st 2015 followed by a higher discharged on March the 19th. The largest spore discharged recorded followed a rain event of 13.4 mm on March 16th. DIA spores were recorded from May to end of September of 2014. No spores or very low quantity were recorded from early October 2014 to late February 2015. First DIA spores discharge was recorded on March 4th 2015.

Overall, the first release of inoculum was detected at the end of winter beginning of spring when average temperatures were above freezing and correlated, though not always, with rainfall. Significant numbers of spores were also trapped during the summer months. Results of this study will assist to develop more effective control strategies by optimizing chemical and/or biological agent applications as well as identifying the best time for pruning in BC.

GTD are one the main biotic factor limiting both vineyard longevity and productivity in BC. GTD fungi infect grapevines through wounds and openings, primarily pruning wounds. However, there are no products currently available that control the fungus inside the wood once it has infected and colonized the vascular system. Accordingly, the most effective strategy available today to minimize the impact of GTD relies in protecting the pruning wounds. While other countries possess a wide range of control products, there are currently none registered in Canada against GTD. Therefore, *in vitro* laboratory and greenhouse studies were conducted in 2016 and 2017 to evaluate both chemical and biological products against the most prevalent GTD species found in BC as this data is needed to obtain product label extension or new registration. Eight chemical and 4 biological products were screened using a detached dormant cane assay in the greenhouse. Canes were pruned, immediately treated, and inoculated with either a spore or conidial suspension 24 h after treatment. Results showed several products within the triazole and thiophanate chemical groups to achieve a over 80% control under greenhouse conditions. Studies to determine the lifespan of three chemicals and one

biological on pruning wounds were also conducted. Results showed mean percent disease control (MPDC) of chemical products to decrease when pruning wounds were challenged with the pathogen either 7 or 21 days after treatment. Contrary, MPDC significantly increased after 21 days when pruning wounds were treated with a *Trichoderma*-based biological product.

Additionally, studies conducted during 2017, identified and characterized my means of morphological and molecular studies up to 8 different *Trichoderma* and 2 *Bacillus* species from different hosts in the Okanagan, including *Trichoderma atroviridae*, *Trichoderma viride*, *Trichoderma koningii*, *Trichoderma koningiopsis*, *Trichoderma asperellum*, *Trichoderma harzianum*, two novel *Trichoderma* spp., *Bacillus subtilis* and one *Bacillus* sp. In vitro dual culture tests using a total of 30 different isolates (30 *Trichoderma* and 2 *Bacillus*) were conducted to determine the antagonistic activity of all these species against GTD fungi, including *Neofusicoccum parvum*, *Diplodia seriata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Eutypa lata*, and *Phomopsis viticola*. Overall, over 70% mycelial growth reduction was observed with all *Trichoderma* and *Bacillus* species indicating the high potential of these species to be used as biocontrol agents. Greenhouse trials will be conducted in the summer of 2018 to further evaluate these isolates with the goal of conducting field trial in the winter of 2019.

Further studies are required to determine the efficacy of these products in the field under BC environmental conditions. We envision this study as the first step in the process of getting control products registered in Canada to provide grape-growers with control strategies against GTD.

Studies to develop and implement cultural practices (effect of pruning time and trunk renewal) to minimize GTD infection in the Okanagan Valley started in winter of 2016-2017. Trials were repeated in winter of 2017-2018 with data collection expected to start in April 2018.
Pruning trials: Grapevines in a Merlot and a Chardonnay block were pruned at five different times during winter 2016-2017 and Spring 2017 (December 15 2016, and January 15, February 15, March 15, and April 15 of 2017). Pruning wounds made at different times were then artificially inoculated with a 10^5 spores/ml suspension of GTD pathogens *Neofusicoccum parvum* and *Diplodia seriata* on March 16, April 16, May 16 and June 16 of 2017, which correlates with high spore concentrations found throughout the Okanagan Valley based on the spore trapping studies. Overall results showed pruning conducted in December and/or January to significantly decrease (up to 60%) GTD infection compared to pruning done later in February, March and April. Pruning wounds made in March and April showed the highest susceptibility to infection by GTD pathogens. Overall, pruning wound susceptibility decreased significantly as the interval between pruning and inoculation increased. Results of this study will assist to evaluate best pruning time that would significantly reduce the risk of infection by GTD in the Okanagan Valley.

Trunk renewal: Trunk renewal trials in three different vineyard blocks (Pinot Noir, Chardonnay and Pinot Gris) started in winter of 2016. The main goal of these trials is to evaluate trunk renewal (surgery) as an effective management strategy against GTD under BC conditions. In each block, the trunk of 30 vines (10 repetitions of 3 vines) was cut to 15 cm above ground with suckers left during the summer of 2016 to serve as potential new (renewed) trunks. The hypothesis is to eliminate the infected wood by cutting the trunk to the base and renew healthy (disease free) trunks to train a new vine. Overall results showed all cut vines to survive with abundant suckers. Renewed vines produced 0.41 (Chardonnay), 0.9 (Pinot Gris), and 0.46 (Pinot Noir) average Kg/vine compared with 1.34 (Chardonnay), 2.99 (Pinot Gris), and 1.54 (Pinot Noir) average Kg/vine from uncut vines. These results showed both highest survival rate and yield production after one year of vines being renewed. Data

collection will continue in the following years and higher yields from renewed vines are expected the second year of the trial (harvest of 2018).

4.2. Investigate the utility of molecular plant disease diagnostics using previously developed assays in an array of field testing scenarios.

- **Description:** Implementation of molecular plant disease diagnostics using a previously developed DNA-macroarray assay on different field testing scenarios.
- **Outcome:** Validation of diagnostic technologies under field conditions
- **Derivable:**

The recently developed DNA-macroarray for detection and identification of more than 70 individual fungal pathogens causing young vine decline was succesfully implemented during the course of this project to analyze different substrates, including soil, water, air samples (from spore traps) and ready-to-plant grapevine material from nurseries. The DNA-macroarray was capable of detecting fungal species from all substrates and showed a highest sensitivity than end point PCR. The plant Pathology laboratory developed capacity for testing and screen up to 40 samples in a period of 48 h.

4.3. Implementation of traditional and molecular diagnostic tools with special emphasis in detection at the grapevine nursery level.

- **Description:** Implementation of different traditional and molecular diagnostic tools for detection and identification of fungal pathogens associated with grapevine trunk diseases in grapevine nurseries.
- **Outcome:** Identify potential infection sources at the nursery level and accordingly develop and implement efficient and economically viable control strategies.

Ready to plant material provided by several nurseries from different countries was evaluated. For each plant, total DNA was obtained from roots, rootstock basal end, graft-union, and scion and processed with the DNA-macroarray. During the course of this project a total of 150 plants (600 samples) were screened using the DNA-macroarray and compared with results obtained from traditional isolation techniques and PCR. The YVD-DNA-macroarray successfully detected and identified 17 YVD fungi from nursery propagated material. Seven out of the 17 fungi identified were not known to previously occur in BC. Results showed *Ilyonectria macrodidyma* (Black-foot disease pathogen) to be the most prevalent fungus and it was detected in 90.3% of the plants tested. *Phaeomoniella chlamydospora* was the second most prevalent (77.7%) followed by *Dactylonectria pauciseptata* (61.2%), *Cadophora luteo-olivacea* (59.2%) and *Phaeoacremonium minimum* (36.9%). Among all different plant parts, YVD pathogens were detected primarily at the basal end of the rootstock. The DNA-macroarray was shown to be an accurate, sensitive and a much faster detection tool than other techniques used such as standard PCR or traditional plating.

Results from this objective showed the potential that this detection tool have for multiplexing identification of GTD pathogens and thus, its capability to be implemented at a nursery level to determine the health status of plants leaving the nursery or identify the steps throughout the propagation process where infection may occur. These result can assist to develop best management strategies against GTD fungal pathogens at the nursery level

3. Issues

The passing of Paula Haag, a friend, colleague and Senior Research Technician, in November 2015, negatively impacted the performance of the Plant Pathology lab.
Impact: Delay on sample processing and data analysis of spore trap samples from objective 4.1.
Action Plan: In April of 2016, A new research Technician, Melanie Walker, was assigned to the Plant Pathology Laboratory. Melanie Walker was trained to continue the spore trapping quantification work using droplet digital PCR. To date, 100% of samples has been processed (DNA extraction + ddPCR) and 90% of the data has been analyzed. Data analysis is expected to be fully completed by April/May of 2018.