Development of a Novel, Sensitive Detection Method for Grapevine Red Blotch Virus

Project #: 001900

Pillar Number: 001900

Norgen Biotek

Final Report

Reporting Period: January 1 – August 31, 2017

Date of Submission: September 21, 2017

Program Co-Ordinators: Dr. Y. Haj-Ahmad
Pam Roberts

2. Executive Summary

Grapevine red blotch-associated virus (GRBV) is a plant pathogen that affects grapevines, causing grapevine red blotch disease. Recently, GRBV has been found to be spreading into Canada, and has been detected in vines in both Ontario and British Columbia. Red blotch disease can result in delayed fruit maturity, uneven ripening or failure to ripen, as well as a significant reduction in berry sugar content, therefore having significant economic impacts on the grape and wine industries. The ability to accurately, inexpensively and rapidly diagnose GRBV in the Niagara Region is extremely important in preventing the spread of red blotch disease within vineyards, and to new vineyards in the Niagara Region. The focus of this project is therefore to develop a novel and robust method to allow for the sensitive detection of GRBV from grapevines. The 3 main objectives of this project are to: 1) develop and validate a PCR-based molecular detection system for GRBV detection; 2) develop and validate a robust DNA isolation method that will work on all the different grape varieties grown in the Niagara Region to isolate high quality, inhibitor-free DNA even from low titer samples; 3) standardize the sampling of the grapevines, including where to take the samples from and how to store the samples prior to DNA purification.

This project consisted of 11 different milestones / tasks, and all 11 of these have been successfully completed. First, work was undertaken to synthesize and clone a positive GRBV control that could be used in the remaining tasks. The next task involved designing and validating an end-point PCR based detection method for GRBV detection. Next, work was undertaken to design and validate a TaqMan PCR based detection method for GRBV detection. The next task involved designing and validating a SYBR Green based detection method for GRBV detection. Next, work was undertaken to determine the optimal PCR detection method from the 3 developed and tested methods. For this, various grape vine samples were obtained that were known to be GRBV-positive and GRBV-negative, and all the previously developed methods were tested. Through this a decision was made to use the TaqMan PCR method moving forward, as this method was found to be the most sensitive and specific, even allowing for the detection and differentiation of two different strains of GRBV.

Next, work was undertaken to optimize the DNA isolation method. First, four different homogenization methods were tested and it was determined that the bead homogenization method was the most optimal method to be used with the grape leaves. Next, work was carried out to develop a robust DNA isolation method, and it was determined that Norgen's current Plant/Fungi DNA Isolation Kit resulted in the best DNA yield and quality when compared with all the different conditions tested. Work was then carried out to determine an optimized method to obtain samples from grapevines. Through this work, a standardized method to sample the grapevines was determined, and this involves taking 16 leaf disk samples from leaves collected from 4 different quadrants on each vine. Furthermore, the location on the leaf to take the samples was tested and optimized, and it was determined that collecting leaf disks from near the petiole and mid vain was the optimal site and resulted in the highest virus titer. Work was then carried out to test how samples could be pooled for doing the actual testing. Therefore different ratios of infected:non-infected leaf disks were tested from 1:1 up to 1:16, and it was determined that 1 positive GRBV leaf disk could be detected when pooled and tested with 15 GRBV non-infected leaf disks. Work was also carried out to determine the stability of the virus in the plant in order to develop methods for storage and handling of the samples prior to DNA isolation and GRBV detection. Through this work the leaf disk sample collection method and storage conditions were optimized and established, and involve the collection of leaves in the vineyard followed by subsampling of the leaf disks back in the lab. Furthermore, it was found that the leaf disks could be processed immediately or stored for up to 2 weeks at -20°C before processing without any negative effect on the GRBV detection. Therefore, the entire process of leaf sampling and storage, DNA isolation and GRBV detection with Taqman was developed. Lastly, the full method was validated by comparison to currently used GRBV detection methods, and it was found that Norgen's novel method of GRBV detection was more sensitive that other currently used methods.

Overall, this project was a great success and Norgen is now able to offer GRBV detection testing services to the farmers, grape growers and wineries in the Niagara Region, Ontario, Canada and world-wide.

3. Detailed description of the Project

a) *Objectives and Project Input*

Objectives

The objectives of this project were to:

1. Develop and validate PCR-based molecular detection systems for GRBV detection. Three different methods will be developed and validated, and the most sensitive and robust method will be chosen:
    a. End-Point PCR method – this will be the most inexpensive method for screening
    b. TaqMan PCR method – this method is more specific and quantitative than end-point PCR
    c. SYBR Green PCE method – this method is more specific and quantitative than end-point PCR
2. Develop and validate a robust DNA isolation method that can isolate inhibitor-free DNA from the different grapevine varieties grown in the Niagara Region. Establish an internal control to validate a successful isolation.
3. Standardization of sample collection and sample handing
    - what parts of the grapevine to obtain samples from;
    - size of the samples that should be obtained;
    - optimal number of samples to collect;
    - ideal storage conditions for the sample prior to processing for DNA isolation.

All of these objectives were met during this project.

Project Input

For the completed project, the project inputs are**:**

Scientist Salary (OGWRI cash contribution) - $ 48000

Consumables (Norgen cash contribution) - $16000

Travel - $400

Overhead (Norgen in-kind contribution) - $26,400

Project co-ordinators (Norgen cash contribution) - $4800

Total Project Cost - $95,600

b) *Project Activities and Outputs*

Milestone One:
Synthesize and/or isolate positive control from an infected plant

Activities undertaken:
Work was undertaken to synthesize a positive GRBV control. The full GRBV sequence was obtained from the NCBI data base (KU564256). Two fragments (A and B) were synthesized without interrupting the ORF of 6 genes. To facilitate cloning into the pUC19 vector, restriction sites were generated at the end of the each fragment. The ligated vector was then transformed into competent cells and screened to obtain the correct clone that contained the GRBV insert. The cloned GRBV positive target was then named pGRBaV-NB16.

Outputs created:
The cloned GRBV positive target pGRBaV-NB16 was generated.

Milestone Two:
Design and validate primers for end-point PCR. Determine specificity, sensitivity, cross-reactivity

Activities undertaken:
Two sets of end-point PCR primers were designed to detect different target genes in the GRBV genome sequence, NB16-D-F and NB16-D-R; NB16-A-F and NB16-A-R. The primers were then validated to determine the specificity, sensitivity and cross-reactivity. Specificity and sensitivity were tested against pGRBaV-NB16, the artificial GRBV positive control using optimized PCR conditions. Both sets of primers were found to be highly specific for detection of GRBV. For a sensitivity test, pGRBaV-NB16 was serially diluted to reach down to 20 copies, and again both sets of primers were found to be very sensitive for the detection of GRBV.

In silico analysis was performed for each set of primer developed for the end-point PCR based on computational analysis. The tested primers were found to match 100% with the GRBV genome, indicating the high specificity of the primers.

Outputs created:
Two sets of detection primers were designed and validated for the end-point detection of GRBV. Both sets of primers were found to be specific and sensitive for the detection.

Milestone Three:
Design and validate primers for Taqman PCR. Determine quencher and florescence. Determine specificity, sensitivity, cross-reactivity.

Activities undertaken:
Four different sets of TaqMan primers were designed to detect four different target genes in the GRBV genome sequence. Probes specific for GRBV detection were labelled with the fluorophore FAM, and the probe specific for the PCR control was labelled with the fluorophore HEX. The primers were then validated to determine the specificity, sensitivity and cross-reactivity. Specificity and sensitivity were tested against pGRBaV-NB16, the artificial GRBV positive control using optimized PCR conditions. All 4 sets of primers were found to be highly specific for detection of GRBV. For a sensitivity test, pGRBaV-NB16 was serially diluted to reach down to 20 copies, and again all 4 sets of primers were found to be very sensitive for the detection of GRBV.

In silico analysis was performed for each set of primers and probes developed for the TaqMan real-time PCR based on computational analysis. The tested primers and probes were found to match 100% with the GRBV genome, indicating the high specificity of the primers.

Outputs created:
Four sets of TaqMan primers were designed and validated for the detection of GRBV. All 4 sets of primers were found to be specific and sensitive for the detection.

Milestone Four:
Design and validate primers for SYBR Green PCR. Determine specificity, sensitivity, cross-reactivity

Activities undertaken:
Two sets of primers were designed to detect two different target genes in the GRBV genome sequence using SYBR Green technology, NB16-A and NB16-D. The primers were then validated to determine the specificity, sensitivity and cross-reactivity. Specificity and sensitivity were tested against pGRBaV-NB16, the artificial GRBV positive control using optimized PCR conditions. Both sets of primers were found to be highly specific for detection of GRBV. For a sensitivity test, pGRBaV-NB16 was serially diluted to reach down to 20 copies, and again the primers were found to be very sensitive for the detection of GRBV.

In silico analysis was performed for each set of primers developed for the SYBR Green PCR based on computational analysis. The tested primers were found to match 100% with the GRBV genome, indicating the high specificity of the primers.

Outputs created:
Two sets of detection primers were designed and validated for the detection of GRBV using SYBR Green technology. The primers were found to be specific and sensitive for the detection.

Milestone Five:
Determining the optimal PCR detection method from the 3 developed and tested

Activities undertaken:
Various grape vine samples were obtained that were known to be GRBV-positive and GRBV-negative. DNA from isolated from cambium samples using Norgen's Plant/Fungi DNA Isolation Kit. The isolated DNA samples were then used in downstream PCR detection reactions using the 2 sets of end-point PCR primers, the 4 sets of TaqMan PCR primers and the 2 sets of SYBR Green primers developed and described above. All the results were compared to determine which primer set from each detection method was the most sensitive and specific for the detection.

Through this work, it was determined that the NB16-A primer pair was the optimal ones for the end-point detection, and this was confirmed by sequencing the amplified bands from the end-point PCR and determining that they all matched with GRBV. It was also determined that qNB16-B were the optimal primers to use for the TaqMan detection and were the most specific and sensitive. Lastly, the NB16-A primers for SYBR Green detection were shown to be the most sensitive and specific of the 2 primer sets tested. A decision was made to use the TaqMan PCR method moving forward, as this method was found to be the most sensitive and specific, allowing for the detection and differentiation of two different strains of GRBV.

Outputs created:
Decision was made as to which was the most sensitive and specific primer pair for end-point detection, Taqman detection and SYBR Green detection. A decision was made to use the TaqMan PCR method moving forward.

Milestone Six:
Test different homogenization methods on different grapevine samples.

Actions undertaken:
Four different homogenization methods were compared for their efficiency and ease of use in high throughput analysis for homogenizing grape leaves. Bio-Raba bag maceration, liquid nitrogen grinding, bead homogenization and a novel microwave method were tested, and it was found that the bead homogenization method was the most rapid and convenient method and that it extracted the highest yield of DNA from the grape leaves.

Outputs created:
It was determined that the bead homogenization method was the most optimal method to be used for GRBV detection.

Milestone Seven:
Sequential testing of current Norgen DNA isolation kits to optimize binding, washing and elution steps to develop a robust isolation method to use on different grapevine samples.

Actions undertaken:
Norgen's Plant/Fungi DNA Isolation Kit was used as a starting point for isolation of DNA from the homogenized leaf samples. In order to try and improve grape DNA isolation efficiency, 4 different binding and washing conditions were tested. Binding Buffer B, Binding Buffer H, Binding Buffer C and Solution

WN were extensively tested for use in the DNA isolation procedure, and the DNA quality and yield was determined and compared for all the different solutions tested. It was determined that the best DNA quality and yield was obtained when Norgen's current Plant/Fungi DNA Isolation Kit was used.

Outputs created:
It was confirmed that Norgen's current Plant/Fungi DNA Isolation Kit is the optimal method to use for DNA isolation from the grape leaf samples, resulting in a high yield and quality of DNA.

Milestone Eight:
Obtain different samples from different locations on grapevines and test isolation and detection developed above to determine best way to sample and if sensitive enough for low titers

Activities undertaken:
In order to determine the best way to sample the grapevines, samples were taken from three different vineyards at a Niagara-on-the-Lake farm and were collected from three different varieties. First we determined the optimal number of leaf disk to collect and test for DNA isolation and GRBV detection. In order to do this, each vine was "divided" into 4 quadrants (upper left, lower left, upper right and lower right), and various numbers of leaf disks were collected from the different areas. For the testing, 8 to 16 leaf punches were taken and tested for DNA isolation. The previously developed bead beating method was used to homogenize the samples, and the DNA was then isolated using Norgen's Plant/Fungi DNA Isolation Kit. The quality and yield of the purified DNA was tested by 18S rRNA detection. Through these experiments we established an optimized collection method that involves collecting up to16 leaf disks (ideally 4 from each quadrant). The DNA quality remained excellent for 18S rRNA detection without PCR inhibition when using the 16 leaf disks. Secondly we determined where the leaf disk samples should be collected on the leaf. Nine different collection sites were selected on the leaf and tested for the detection of GRBV. The result showed that near the petiole and mid vain was the optimal site to collect the leaf disks and resulted in the highest virus titre for optimized detection.

Outputs created:
Developed a standardized method of how to obtain samples from the grapevines (16 leaf disks, 4 collected from each quadrant), as well as the optimal site on the leaf to collect the leaf disks (near petiole and mid vain).

Milestone Nine:
Test pooling of samples (1:1 infected to non-infected up to 1:100 infected to non-infected). Determine sensitivity and how to do pooling for actual testing

Activities undertaken:
Once the optimized method of sampling was determined in Milestone Eight above, work was undertaken to determine the sensitivity of the detection and how samples could be pooled for the actual testing. In order to do this a number of different dilution experiments were carried out. Known GRBV positive leaf disks were serially mixed with GRBV non-infected leaf disks in varying ratios up to a total of 16 disks (1:1 infection to non-infected up to 1:16 infected to non-infected). The DNA isolation method based on the optimized bead homogenization method was then carried out and the GRBV detection assay based on Taqman real-time PCR was performed. From this work it was found that 1 positive GRBV disk could be sensitively detected in the mix with 15 GRBV non-infected leaf disks, indicating that pooling 16 leaf disks to detect GRBV is acceptable.

Outputs created:
An optimized sample pooling method was established that allows for the detection of 1 infected leaf disk in a pool of 16 leaf disks.

Milestone Ten:
Test to determine stability of virus in plant to develop methods for storage and handling of samples prior to DNA isolation.

Activities undertaken:
In order to test this, the previously developed leaf disk sample collection method was tested in two ways: 1) collect the leaf disk directly into the bead homogenization tube on site in the vineyard; or 2) collect the leaves and do subsampling in the lab (pooling and punching the leaf disk and transfer to the bead homogenization tube). Both methods demonstrated efficiency and convenience for sample processing. Storage conditions were also tested by storing the collected samples (leaf disks) at -20˚C for 2 weeks and comparing the sensitivity of GRBV detection to the results when the samples were processed immediately without storage. No sign of dilution or PCR inhibition was found when the samples were stored at -20°C until the process.

Outputs created:
Leaf disk sample collection method and storage condition were optimized and established. The most convenient method is to collect the leaves in the vineyard and then do the subsampling back at the lab. The leaf disks can be processed immediately or stored up to 2 weeks before processing without any negative effect on the GRBV detection.

Milestone eleven:
Full validation of the method, write report and make slides

Activities undertaken:
For full validation of the developed method, 45 grape leaf samples were collected from 3 different grape vineyards located in Niagara-On-The–Lake from three different varieties. In this experiment, Norgen's unique and optimized sample collection methods, DNA isolation method and GRBV detection were tested. Furthermore this comprehensive GRBV detection method was compared with GRBV detection results done separately by Eurofins STA Laboratories (Gilroy, CA) and at Brock University (St. Catharines, Canada) using their GRBV detection method. The comparative result showed that Norgen's GRBV detection method showed the highest sensitivity of GRBV detection. The final report is now being written, and slides will be made and presented to the OGWRI Board Members and Technical Committee Members.

Outputs created:
Norgen's novel and newly developed method of GRBV detection was fully validated and compared to other established methods. Norgen's method was found to be more sensitive than the other methods.

c) *Reach and Communication*

Identify primary target audience / beneficiaries of this project i.e. agricultural producers, consumers, youth, farm families, rural Canadians, food processors, educators

- The primary target of this project will be grape growers who might be impacted by GRBV. Infection of a vineyard with GRBV could be extremely detrimental to their crop and livelihood, resulting in a large financial impact not only for the farmer, but also their family and their employees. Entire vineyards have had to be pulled due to GRBV, therefore a system to monitor and detect this pathogen here in the Niagara Region will have the greatest impact on the growers.
- The extended target will be wine processors who need a monitoring system for GRBV infection during processing and production of wine
- Also for the grape growers, this project will provide a guideline of how to collect samples, transport and identify GRBV.

Indicate the total number of people reached (If the project involves workshops/seminars, please indicate how many attended each event)

- Interim project progress meeting was held on April 6th, 2017 at Norgen Biotek for the OGWRI Board Members and Technical Committee Members.
- Research Scientists from AAFC-Vineland, ON provided us with GRBaV related samples required for some tasks in the project
- Task force meeting at Matt's farm was held on July 12th
- Tel. conference was held with Dr. Marc Fuchs (Cornell University, Ithaca, NY) and OGWRI technical board members on Aug. 2nd at OGG meeting room
- Project review meeting was held with Dr. Marc Fuchs (Cornell University, Ithaca, NY) on Sept. 8th 2017 at Norgen Biotek
- Attended Knight Grapevine Nursery open house for GRBV seminar

Indicate how the targeted audience / beneficiaries were reached i.e. research publications, seminars, press releases, promotional material. Please include samples of any communication material developed for the project and indicate the number printed / distributed

- No seminar for growers has been made yet, but this will be done at a future date

Indicate when OGWRI was identified as a supporter throughout the period of the project

- OGWRI was identified as a supporter during the interim project progress meeting
- OGWRI will certainly be identified as a supporter when we have an opportunity to present the results from this project.

4. Project Outcomes (actual vs. expected) at short and long-term

a) *Short-term*

Milestone 1:
Expected outcome – Positive control obtained

Actual Result – The cloned GRBV positive target pGRBaV-NB16 was generated. This positive control has successfully been used in the other milestones to test the developed primers and detection methods.

Milestone 2:
Expected outcome – Design and validation of an end-point detection method for GRBV

Actual Result – Two sets of detection primers were designed and validated for the end-point detection of GRBV. Both sets of primers were found to be specific and sensitive for the detection.

Milestone 3:
Expected outcome – Design and validation of a TaqMan detection method for GRBV

Actual Result – Four sets of TaqMan primers were designed and validated for the detection of GRBV. All 4 sets of primers were found to be specific and sensitive for the detection.

Milestone 4:
Expected outcome – Design and validation of a SYBR Green detection method for GRBV

Actual Result – Two sets of detection primers were designed and validated for the detection of GRBV using SYBR Green technology. Both sets of primers were found to be specific and sensitive for the detection.

Milestone 5:
Expected outcome – Decision made on which PCR detection method is optimal

Actual Result – All the different end-pint, TaqMan and SYBR Green primers designed in the earlier tasks were tested on actual samples (GRBV-positive and GRBV-negative cambium samples). Through this work, a decision was made as to which was the most sensitive and specific primer pair for end-point detection, Taqman detection and SYBR Green detection. A decision was also made to use the TaqMan PCR method moving forward, as this method was found to be the most sensitive and specific, allowing for the detection and differentiation of two different strains of GRBV.

Milestone 6:
Expected outcome – An optimized method for homogenizing grapevine samples is developed

Actual Result – Through extensive testing, it was determined that the bead homogenization method was the most optimal method to be used for GRBV detection.

Milestone 7:
Expected outcome – Development of a robust DNA isolation method that will work with all different grapevine sample types.

Actual Result – It was confirmed that Norgen's current Plant/Fungi DNA Isolation Kit is the optimal method to use for DNA isolation from the grape leaf samples, resulting in a high yield and quality of DNA.

Milestone 8:
Expected outcome – Standardized method for obtaining samples from plants.

Actual Result – A standardized method was developed of how to obtain samples from grapevines (16 leaf disks, 4 collected from each quadrant of the grapevine), as well as the optimal site on the leaf to collect the leaf disks (near petiole and mid vain).

Milestone 9:
Expected outcome – Development of a method to pool samples to allow for testing of multiple sample at a time in order to reduce the cost of testing.

Actual Result – An optimized sample pooling method was established that allows for the detection o f1 infected leaf disk in a pool of 16 leaf disks.

Milestone 10:
Expected outcome – Standardized method for handling and storage of samples for testing.

Actual Result – The leaf disk sample collection method and storage conditions were optimized and established. The most convenient method is to collect the leaves in the vineyard and then do the subsampling back at the lab. The leaf disks can be processed immediately or stored up to 2 weeks before processing without any negative effect on the GRBV detection.

Milestone 11:
Expected outcome – Fully validated method for detection of GRBV. Final report/slides created.

Actual Result – Norgen's novel and newly developed method of GRBV detection was fully validated and compared to other established methods. Norgen's method was found to be more sensitive than the other methods. Final report is being completed and summary slides will be created.

Public Good/Benefit of Project:

Through this project Norgen has developed a novel method for GRBV detection that is fully validated and is more sensitive than other methods currently being used. Currently, Niagara farmers must send plant samples to California in order to be tested for GRBV. This is time consuming, costly, and often difficult due to trying to ship possibly virus-infected plant samples between countries. Now that Norgen has

developed this detection method, we will be able to offer testing to Niagara farmers here in our own region. This will allow for more rapid, affordable and convenient testing for GRBV. Therefore, the development of this sensitive GRBV detection method will help to contribute to GRBV containment and red blotch prevention in the Niagara Region. One way this method will contribute to GRBV prevention is by testing and ensuring the use of clean, virus-free plants in establishing new vineyards. As well, this detection method will help in the Niagara Region with accurate and rapid detection of infections in the vineyards, and therefore will allow for faster management of the infection once it has been confirmed in the vineyard. This could also help to prevent further spread of the infection by helping to rapidly identify the infection as well as potential sources of infection.

Policy Dialogue:

Through this project, the current or emerging issue of GRBV infection has not been redefined. However, the outcome of the project can be used to justify the need for financial support from the government for wide-spread screening of GRBV. The government could be persuaded using the data resulting from this project to help defray the cost of testing for farmers in order to help to prevent this virus from spreading throughout the Niagara Region. Through this work the government will be able to see how important testing is for farmers and for the grape and wine industry as a whole, and therefore they will be encouraged to subsidize the testing and help to make it more inexpensive so that all farmers are able to test for this emerging threat to the industry. Agriculture is a huge industry in the Niagara Region, and it is extremely important that farmers and growers have access to affordable testing for emerging pathogens that could threaten their farms and their livelihoods.

Market-Trend Studies:

Based on the results of this project, Norgen will start to offer a GRBV testing service to the farmers, grape growers and wineries in the Niagara Region. This service can also be offered to other grape growing regions in Ontario such as Lake Erie North Shore and Prince Edward County, as well as grape growing regions in other provinces including British Columbia. No new trends or factors have been identified.

Pre-Commercialization:

The results of this project can definitely be commercialized in terms of offering a GRBV testing service to the farmers, grape growers and wineries in the Niagara Region. This is an extremely viable opportunity, GRBV has recently been found to be spreading into Canada, and has been detected in vines in both Ontario and British Columbia. Red blotch disease has a large impact on fruit quality, and can lead to a significant reduction in sugar accumulation as well as uneven ripening or failure to ripen, which can result in off flavours and unripened fruit. Infection of a vineyard with GRBV can have a significant impact on the long-term profitability and sustainability of the grape and wine industry in Niagara. For example, some vineyards in California and Oregon have had to be completely removed due to poor fruit quality caused by GRBV, leading to increased costs and loss of revenue associated with the fruit and the resulting wine. This is clearly a concern in the Niagara Region as this emerging pathogen is starting to spread within the area. Reliable detection of GRBV is therefore extremely important to local grape growers. Currently local growers are sending samples to California for testing, which is time consuming, costly, and often difficult and therefore being able to test their samples locally will be hugely beneficial.

Value:

Norgen is ready to begin offering the GRBV testing service here in the Niagara Region. We have not performed any testing services yet, however we are ready to do so. Norgen will be able to provide specific details of how to obtain the leaf samples from the vineyards, and will perform the subsampling, DNA isolation and subsequent TaqMan PCR detection in the lab. This testing service will be offered at a lower cost than the current testing services available, making it attractive to local farmers. Furthermore, the turnaround time will be much quicker than the current testing services being offered, as the testing will be performed locally instead of shipping samples to California.

a) *Long Term*

The main key indicator that we will use to measure the project success in the long term will be the number of services performed and the revenue generated from these services. As this is a new type of service being offered by Norgen, it will be easy for us to track the growth in revenue generated from these services. Furthermore, we anticipate that as these service contracts increase, we will need to hire additional technicians to perform these services. Therefore we will be creating new jobs and stimulating the economy here within the Niagara Region.

5. Final Comments and Conclusions

Identify any deviations from the project workplan, budget or schedule and discuss the effects of the deviations and the solutions.

The only slight variation from the proposal was that the project timeline was extended slightly. This was due to the fact that grape leaf samples were not available until June due to the growth cycle of the grapevines. Although the project timeline needed to be extended, there were no other deviations and the project was still successfully completed.

Provide a discussion of "lessons learned", recommendations and overall perception of project success

Overall this project was extremely successful and all of the objectives of the project were met. A novel and sensitive method for GRBV detection based on TaqMan PCR was developed and validated. A robust DNA isolation method was developed that allows for the isolation of a high yield and quality of DNA from grape leaf samples. Furthermore, a standardized method of sample collection and handling was determined. A standardized method was developed of how to obtain samples from grapevines (16 leaf disks, 4 collected from each quadrant of the grapevine), as well as the optimal site on the leaf to collect the leaf disks (near petiole and mid vain). An optimized sample pooling method was established that allows for the detection of 1 infected leaf disk in a pool of 16 leaf disks. Lastly, the leaf disk sample collection method and storage conditions were optimized and established. The most convenient method is to collect the leaves in the vineyard and then do the subsampling back at the lab. The leaf disks can be processed immediately or stored up to 2 weeks before processing without any negative effect on the GRBV detection. Through this project, Norgen is now able to offer a complete GRBV testing service to local farmers and grape growers here within the Niagara Region in an effort to help with early detection and containment of GRBV.