Ontario GRAPE & WINE RESEARCH Incorporated

# FINAL Report

OGWRI Report.

Project number: 3441

**OGWRI title:** Improving sparkling and still wine quality: preventing high volatile acidity, honey off-flavour and other faults that reduce wine quality through natural Canadian indigenous yeast isolates.

Researchers: Dr. Belinda Kemp & Dr Debra Inglis
Reporting Period: April 1, 2019 and March 31, 2020
Submission date: 23rd February 2020
Program Coordinator: Dr. Belinda Kemp (PI) and Debra Inglis (Co-PI)

# 1. Executive Summary

In wines, a limited amount of "sweet/honey" flavour contributes to the complexity of wine, but at high levels, is considered a fault (Campo et al. 2012). It has been observed by sparkling producers in Canada Ontario that sparkling wines in Ontario produced with Pinot noir grapes (susceptible to sour rot) can have an obvious "sweet/honey" flavour. With sparkling wine production on the rise across Ontario, it is critical that this issue is addressed. Recently, two specific aroma compounds have been identified in wine that cause this "sweet/honey" off-flavour, namely Ethylphenyl acetate (EPhA) and Phenylacetic acid PhAA (Campo et al. 2012). Both compounds have also been reported to contribute to "sweet/honey" off-flavour in wines made from grapes that contain some sour rot (Campo et al. 2012). It is unclear as to when these problem compounds originate, and the direct linkage to sour rot development in grapes. It could be that the grapes have high levels of the precursor, and the sour rot microbial complex acts on the precursor contributing to the "sweet/honey" off-flavour when sour rot develops in the fruit. Alternatively, commercial yeast could be forming these compounds during fermentation when acetic acid levels are high in the starting must (such as when sour rot grapes are present) through esterification reactions with other fermentation metabolites.
PhAA is a plant-growth regulator so it could possibly be produced in grapes susceptible to sour rot as a growth response to an alteration of the surface of the grapes. No studies to analyze the grapes before, and after, sour rot to measure the precursor PhAA and the metabolite EpHA have been carried out. We hypothesize that these compounds are high in grapes as they begin to break down internally from sour rot, and then are transferred to the must and ultimately the wine. Studies have not been carried out on Ontario varieties in Canada prone to sour rot used in red table wines and sparkling wines (Pinot noir). Consumer threshold levels have been identified in wines made from the Portuguese red grape variety Trincaderia. However, the "sweet/honey" off- flavour also appears to be increased by the common nitrogen additive Diammonium phosphate (DAP) during fermentation and lees aging. It is essential we test grapes harvested for sparkling wine that are known to be susceptible to sour rot, for the precursor and the metabolite (Torrea et al. 2011, Campo et al. 2012). The thin-skinned Pinot noir is particularly susceptible to sour rot, widely planted across Canada in Ontario and used in sparkling wine production.

The overall objectives of this project are to identify if two "sweet/honey" off-flavours caused by ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA) are present in Pinot noir grapes as a result of sour rot infection as well as in sparkling and still wines fermented from those grapes. We will test consumer acceptance of the compounds in red and sparkling wines; and test if natural indigenous yeast isolates from Canadian vineyards can remove the compounds along with acetic acid. An indigenous yeast isolated from an Ontario vineyard will be trialed for commercial scale red wine production.

**Project objectives/goals and the anticipated outcomes of the project**

| Milestone Description | Intended Outcome 2019-20 | Actual Outcome 2019-20 |
|---|---|---|
| 1.Method development of a GC-MS method to identify and quantify EPhA & PhAA in grape juice and sparkling wine in year 1 only. | Test precision, accuracy, levels of quantification and detection using GC-MS using new columns, a range of concentrations and deuterated compounds. | The method has successfully been developed and is currently being used on the grapes during ripening, juice and wines. |
| | | |
| 2. Carry out sensory analysis | Identify sensory and statistical | Sensory analysis has been completed |

| | | |
|---|---|---|
| using forced-choice ascending series difference test methodology using wine samples spiked with EPhA and PhAA at different concentrations to identify perception and rejection thresholds of EPhA and PhAA in sparkling wines & red Pinot noir table wines. | methods suitable for sparkling wine using recognised methods from ASTM international standards.<br>Identify range of reference standards for EPhA & PhAA and spiked levels in wines. | with consumer panel and results were presented in BC Grape Growers and Winemakers conference in July 2019 and at the Australian Wine Industry Technical Conference (AWITC) in July 2019. |
| 3. Harvest grapes & produce base wines for sparkling wines and still red wines. | Grape & juice analysis of sugar levels, pH, acidity levels, EPhA, PhAA, ethyl acetate & acetic acid weekly leading up to harvest.<br><br>Winemaking consultancy for sour rot fruit. | Grape/juice and wines were analysed for °Brix levels, EPhA, PhAA, acetic acid, pH, ethyl acetate, total acidity leading up to harvest 2019 (Pinot noir). Completed for 2019.<br><br>Sparkling base wines: To be bottled for sparkling 2nd fermentation in May 2019.<br>Pinot noir red wines: To be filtered and bottled in March 2019.<br><br>Consultancy with industry partners for winemaking with sour rot was completed. |
| 4. Chemical and sensory analysis including consumer preference analysis of Pinot noir red wines. | Standard wine chemical analysis + EPhA, PhAA, acetic acid, ethyl acetate.<br><br>Difference testing (triangle test), descriptive analysis (DA) of still red Pinot noir wines. | All standard chemical analysis of juice for sparkling wines and red Pinot noir table wines have been completed.<br><br>Sensory analysis to be carried out on red wines later in 2020 and for sparkling wines in 2021. |

## 2. Detailed Description of the Project

### Project

The overall objectives of this project are to identify if two "sweet/honey" off-flavours from ethyl phenylacetate (EPhA) and phenylacetic acid (PhAA) are present in Pinot noir grapes because of sour rot infection, as well as in sparkling and still wines fermented from those grapes. Test consumer acceptance of the compounds in red and sparkling wines, and test if natural indigenous yeast isolates from Canadian vineyards can remove the compounds along with acetic acid. Furthermore, an indigenous yeast isolated from an Ontario vineyard will be trialed for commercial scale red wine production.

### Objectives

- Quantify EPhA, PhAA, ethyl acetate and acetic acid levels from clean and sour rot infected fruit in grapes prior to harvest to measure baseline values of taint and precursor compounds in grapes. Produce Pinot noir sparkling and still red wines with varying amounts of sour rot to determine if the taint compounds EPhA, PhAA, ethyl acetate and acetic acid are present.

- Determine the potential of the indigenous yeast isolated from Ontario to reduce acetic acid, EPhA and PhAA in sparkling and still red wines.
- Establish the consumer detection and the consumer rejection threshold levels of EPhA and PhAA associated with “sweet/honey” off-flavour in Pinot noir sparkling and still red wines to determine at what concentrations the compounds affect wine quality.
- Differentiate and describe sparkling wines made from grapes with varying sour rot levels fermented with a standard commercial yeast versus the Brock isolated yeast

**Progress**

Grapes during ripening, at harvest and post-fermentation in 2019 have been analysed for the standard juice and wine chemical analysis including acetic acid and ethyl acetate. All sparkling wine grapes during ripening, juice at harvest and base wine has been analysed for EPhA and PhAA concentrations. The red Pinot noir juice and wine is still undergoing analyses of the two “off-flavor” compounds. Sensory analyses of the sparkling wines will be undertaken after 2nd fermentation/disgorging in 2021 and the red wines later in 2020.

**Results to date**

Acetic acid, EPhA and PhAA were all found to increase as the percent of fruit with sour rot infection increased in the sparkling wine treatments (0, 10%, 20%, 30% & 40% of sour rot by volume (L)) {Figure 1 & 2}. CN1 yeast was found to consume acetic acid from juice to base wine (Figure 1). Acetic acid concentrations are significantly different (P = 0.05) between EC1118 and CN1 yeast treatments.

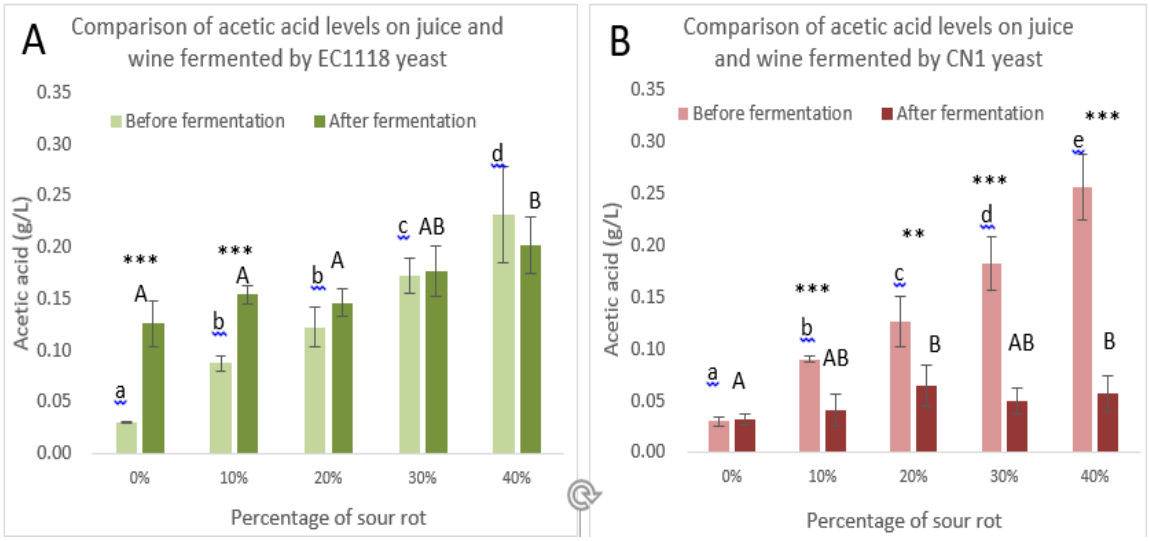


**Figure 1.** Comparison of acetic acid (g/L) on juice and wine fermented by EC1118 yeast (A) and by CN1 yeast (B). Tukeys HSD tests were done between treatments; bars with different letters show significant difference ($\alpha<0.05$). ** ($\alpha<0.01$) and *** ($\alpha<0.001$) show significant difference within the two bars of the same percentage of sour rot.

EpHA and PhAA increased according to the percentage of juice (L) from whole bunch pressed sour rotten clusters. In the consumer sensory study in year 1 of this study, we

established that in sparkling wine, the concentration of EPhA in sparkling wine that consumers could detect was 604 (µg/L). All base wines made with EC1118 yeast are below the level that can be detected by wine consumers. However, the concentrations at which consumers reject wines on the basis of the sensory characteristics is when they are present togther at 140 ug/L (EpHA) and 700 µg/L (PhAA). Base wines produced rom EC1118 yeast are all below these concentrations but they are liable to change as nitrogen (specifically Diammonium phosphate/DAP), and aging on lees increases the concebtrations of the two compounds. Further statistical analyses of the juice and CN1 base wine concentrations of EpHA and PhAA are underway following the recent completion of the GC-MS analysis.

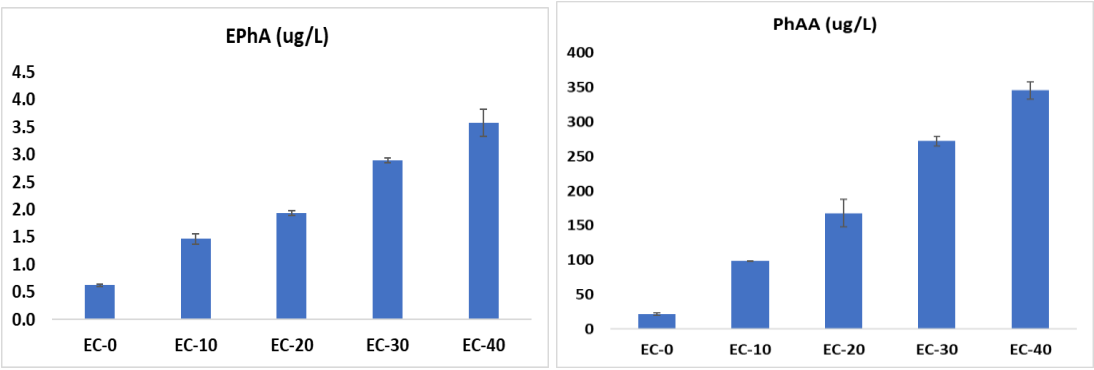


**Figure 2.** Concentration of EpHA and PhAA in 2019 sparkling base wines made using EC1118 yeast for primary fermentation.

*a) Project Activities and Outputs*

**Identify key activities undertaken to achieve the project objectives**

A PhD student started at Brock University on 2nd September 2019 and was working on the winemaking and chemical analysis. Sadly, due to personal health issues, Liying returned to China and withdrew from the PhD at Brock University. A Brock University graduate has now been accepted as an MSc student and will start 2nd September 2020. In the meantime, the PI (Belinda Kemp), and research assistant are carrying out the winemaking and laboratory analysis for the 2019 wines (Rachel Gerroir). The 2019 base wines have been made, and will be bottled in early 2020 at Fielding Winery. The Pinot noir red still wines will be bottled in March/April. Full consumer sensory analysis has been completed with 50% (sparkling wines) carried out at Brock University and 50% (red wines) performed by sensory scientists using their consumer panel at Vineland Research and Innovation.

**Link key activities with defined milestone schedule**

Consumer testing for the detection and rejection thresholds of the honey, off-flavors in sparkling and red wines was completed. The GC-MS method has been developed and used to analyse the juice and wines. The full range of wines detailed in the project, and their percent sour rot with CN1 yeast and EC1118 yeast treatments were produced in 2019 (sparkling base wines and red wines). These three milestones have been completed but not all data has been analysed concerning EPhA and PhAA in the CN1 and EC1118 juice yet.

**Identify the activities in measurable and quantifiable terms**

Laboratory analysis of juice for EPhA and PhAA has been completed. Sensory consumer testing of the two compounds at varying concentrations is now complete and results were presented at two conferences in 2019 in British Columbia and Australia). Sensory analysis for 2019 Pinot noir wines will be completed in 2020 and sparkling wines in 2021. The molecular characterization of CN1 yeast isolated by Brock University has now been completed.

**Identify any challenges encountered to complete the project objectives**

| **Project Challenges** | |
|---|---|
| **Challenges to Date** | **Explanation** |
| Monitoring of EPhA and PhAA compounds in Pinot noir grapes during ripening. | Samples have been analysed but data requires statistical analyses. |
| Consumer sensory panel recruitment | It proved to be difficult to recruit the number of panellists required for both sparkling and red wines so the red wine sensory analysis was out-sourced to Vineland Research and innovation who used their own sensory panel to complete the red wines. |
| Shortage of sour rot grapes for sparkling base wines and Pinot noir red still wines | Due to a low frequency of sour rot in 2019 it was difficult to get suitably rotted grapes. After assistance from Wendy McFadden-Smith we were able to locate a vineyard who offered their grapes to us. |
| Graduate student | The PhD student recruited withdrew for health reasons but a Brock University graduate student has been recruited (2nd Set 2020) who will continue the research project in 2020, 2021 & 2022. |

*b) Reach and Communication*

Target audience for this project is grape growers and winemakers. In December 2019, results to date were presented to winemakers from Ontario, BC, New Brunswick, Nova Scotia and Quebec at Brock University in Fizz Club to 40 winemakers. Winemakers tasted the wines from the EC1118 yeast trial to taste the impact the different percent of sour rot juice had on the 2019 base wines. Further presentation of results will occur at ICCWS 2020 in the form of two posters (abstracts accepted- see below).
The consumer sensory results were presented in poster format in at the BC Winegrowers Conference in July 2019 to 50 winemakers and in Adelaide, Australia at the AWITC conference in July 2019 to 1000 growers and winemakers from across Australia, New Zealand, France, Germany and other wine producing countries.

Results to date of the CN1 yeast were presented by Debra Inglis on 27th January 2020 as part of the CCOVI Lecture Series. The sparkling base wine EPhA and PhAA/honey and

acetic acid results will be presented on 23rd March 2020 in the CCOVI Lecture Series. Both presentations will be permanently available on YouTube.

Funding from OGWRI was present on all presentations by inclusion of the OGWRI logo and in text under the acknowledgement section of all posters and presentations.

***Publications***

Kelly, J.M., van Dyk. S.A, Dowling, L.K., Pickering, G.P., Kemp, B. and Inglis, D.L. *Saccharomyces bayanus* yeast isolate consumes acetic acid during fermentation of high sugar juice and juice with high starting volatile acidity. *Submittted to OenoOne October 10, 2019, accepted with revision February, 2020.*

***Seminars and conferences attended***

Belinda Kemp. (2019). Fizz Club Ontario. Brock University, Ontraio, Canada. 10th December 2019. *(Oral presentation)*

Belinda Kemp. Fizz Club BC. (2019). Summerland Research & Development Centre, BC. 7th August 2019. *(Oral presentation)*

Belinda Kemp. (2019). Fizz Club Tasmania: Sparkling Wine Masterclass. Tasmanian Institute of Agriculture (TIA), University of Tasmania, Launceston, Tasmania, Australia. 26th July 2019. *(Oral presentation)*

Gary Pickering, Hannah Pickering, Stephanie Small-Kelly, Amy Blake, Amy Bowen, Debbie Inglis and Belinda Kemp. (2019). Detection and consumer rejection thresholds of honey off-flavour in sparkling wine. Australian Wine Industry Technical Conference (AWITC). Adelaide Convention Centre, Adelaide, South Australia. 21st-24th July 2019. *(Poster)*

Jennifer Kelly, Stephanie van Dyk, Gary Pickering, Belinda Kemp, Debra Inglis. Molecular characterization of locally isolated yeast CN1 and its ability to consume acetic acid during wine fermentation. British Columbia Wine Growers Council (BCWGC) Oenology and Viticulture Conference. Penticton Trade & Convention Centre, Penticton, BC. Canada. 15-16th July 2019. *(Poster)*

Belinda Kemp, Hannah Pickering, Stephanie Small-Kelly, Amy Blake, Amy Bowen, Debbie Inglis and Gary Pickering. (2019). Improving sparkling and still red wine quality: Honey, sweet off-flavour from sour rot. British Columbia Wine Growers Council (BCWGC) Oenology and Viticulture Conference. Penticton Trade & Convention Centre, Penticton, BC. Canada. 15-16th July 2019. *(Oral presentation)*

***CCOVI Lecture Series***

Debra Inglis. (2020). A locally isolated yeast that consumes acetic acid. Is there application in wine production for Appassimento, sour rot infected fruit, ice wine and sparkling. CCOVI Lecture Series, Brock University, Ontario, Canada. 27th January 2020.

Belinda Kemp. (2020). Effect on honey, dusty off-flavours and acetic acid in sparkling wines made from varying amounts of sour rotten grapes. CCOVI Lecture Series, Brock University, Ontario, Canada. 23rd March 2020.

***Accepted conference abstracts***

Shao, L., Kemp, B., Kelly, J., Gerroir, R., McFadden-Smith, W. and Inglis, D. (2020). Influence of isolated indigenous yeast (CN1) on managing sour rot in grapes for Pinot noir sparkling and still red wines. International Cool Climate Wine Symposium (ICCWS), Brock University, Ontario, Canada. 13 - 16th July 2020. *(Poster)*

Shao, L., Kelly, J., Gerroir, R., Inglis, D. and Kemp, B. (2020). Study on honey off-flavor as a result of sour rot and reduction on volatile acidity through indigenous yeast in Pinot Noir sparkling and still wine. International Cool Climate Wine Symposium (ICCWS), Brock University, Ontario, Canada. 13 - 16th July 2020. *(Poster)*

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

*a) Short-term*

The 2019 red wines and base wines for sparkling wines have been made, and chemically analysed. Winemakers were able to taste the influence of sour rot on base wines at Fizz Club in 2019. The consumer sensory testing for the concentrations of the two compounds has been completed, and the CN1 yeast has been characterised by molecular methods.

- Outline the actual short-term outcome compared to the expected
- Identify the public good/benefit of the project

If applicable to the project, please include the following information:

Policy dialogue: Project must indicate if, as a result of the project undertaken, the current or emerging issue has been redefined. Explain and provide a revised description of the policy issue. The project is still ongoing.

Market-trend studies: What further action/response (if any) you need to take based on the results? What trends and factors have been identified?

- Pre-commercialization: Is the project a commercially viable opportunity? Explain the viability or lack of viability N/A
- Value: As a result of the project, are you selling a product, process or technology? What is being sold? What is the unit value of the item sold? How many items have been sold? N/A

*b) Long Term*

- Indicate the key indicators you will be using to measure the project success in the long-term. Please indicate where applicable:
  - The number of jobs created
  - Increased sales
  - Increased use of Ontario products
  - Increased yield or production of Ontario products
  - Any other indicators outlined in milestone schedule

c) *Doing things differently*

- Knowing what you know now, identify what you would have done differently in relation to the Project.

The project is still ongoing.

## 3. Final Comments and Conclusions

The base wines and red wines from 2019 are made and both styles require bottling. The base wines will be cold stabilised and filtered in April and bottled in May at Fielding Winery. The red wines will be filtered and bottled at CCOVI research winery in March/April. Data analysis of EPhA and PhAA is to be completed in March and initial results will be presented for the sparkling juice and base wines on 23rd March 2020 in the CCOVI lecture series. The project is ongoing and an MSc student joins the research team on 2nd September 2020 to work on the 2020 and 2021 vintages.

| **Project Challenges**<br><br>Set out any challenges that have arisen in relation to the Project to date. Provide an explanation of those challenges as well as what you did to address those challenges. Insert additional rows as needed. | |
|---|---|
| **Challenges to Date** | **Explanation** |
| Monitoring of EPhA and PhAA compounds in Pinot noir grapes during ripening. | This activity is now finished and data is due to be analysed in March 2020. |

- Identify any deviations from the project workplan, budget or schedule and discuss the effects of the deviations and the solutions. The project is still on going.
  - Provide a discussion of "lessons learned", recommendations and overall perception of project success. The project is still on going.