ARC Training Centre for Innovative Wine Production

Activity update: 2020-2022

DIRECTOR'S WELCOME AND REPORT

Welcome to this Activity Report from the second iteration of the ARC Training Centre for Innovative Wine Production. It provides an update on our large suite of projects over the last 2.5 years.

As highlights from this time interval, we have:

- Published 75 journal articles, books, book chapters, or conference publications
- Been invited to give or given 48 talks
- Seen a first wave of 5 of our higher degree research (HDR) students successfully complete their studies
- Amassed a combined 27 months of placements with other industry or research organisations

This Centre's projects continue to strive toward addressing challenges for the wine industry, with some topics evolving with industry partner input. This is as it should be in a responsive, industry-led research environment, and particualrly so for a Training Centre. Afterall a key measure of our success is the training of PhD graduates with the skills, knowledge and contacts to positively impact the sector and progress on to their own careers.

In this latter half of 2022, and in spite of the pandemic-related challenges that have beset the last two years, our talented cohort of higher degree research (HDR) students and early career researchers are now at various stages of completion on a range of projects that include:

- A comprehensive comparison of Cabernet Sauvignon wines from multiple regions
- Assessing several grape varieties for their unique microflora
- Exploring the mechanisms behind grapevine bud fruitfulness
- Managing berry heterogeneity
- The grape berry vascular system
- The role of potassium in berry development
- The sugar flavour nexus in berry development
- Rapid assessment and amelioration of smoke taint
- Extraction and evolution of colour and polyphenols in red winemaking
- Filtration methods for efficient wine processing

While sadly there won't be a TC-IWP mark 3 we are already exploring ways to build on some of the learnngs, relationships and outcomes of this Centre in the future. To this end, several of the project areas proposed in TC3 have already secured alternate mechanisms by which they continue.

I invite you to peruse this report to learn about our IWP Training Centre participants and their achievements, which will continue to have an impact in the wine sector for years to come.

Professor Vladimir Jiranek
Training Centre Director

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OUR MISSION AND AIMS

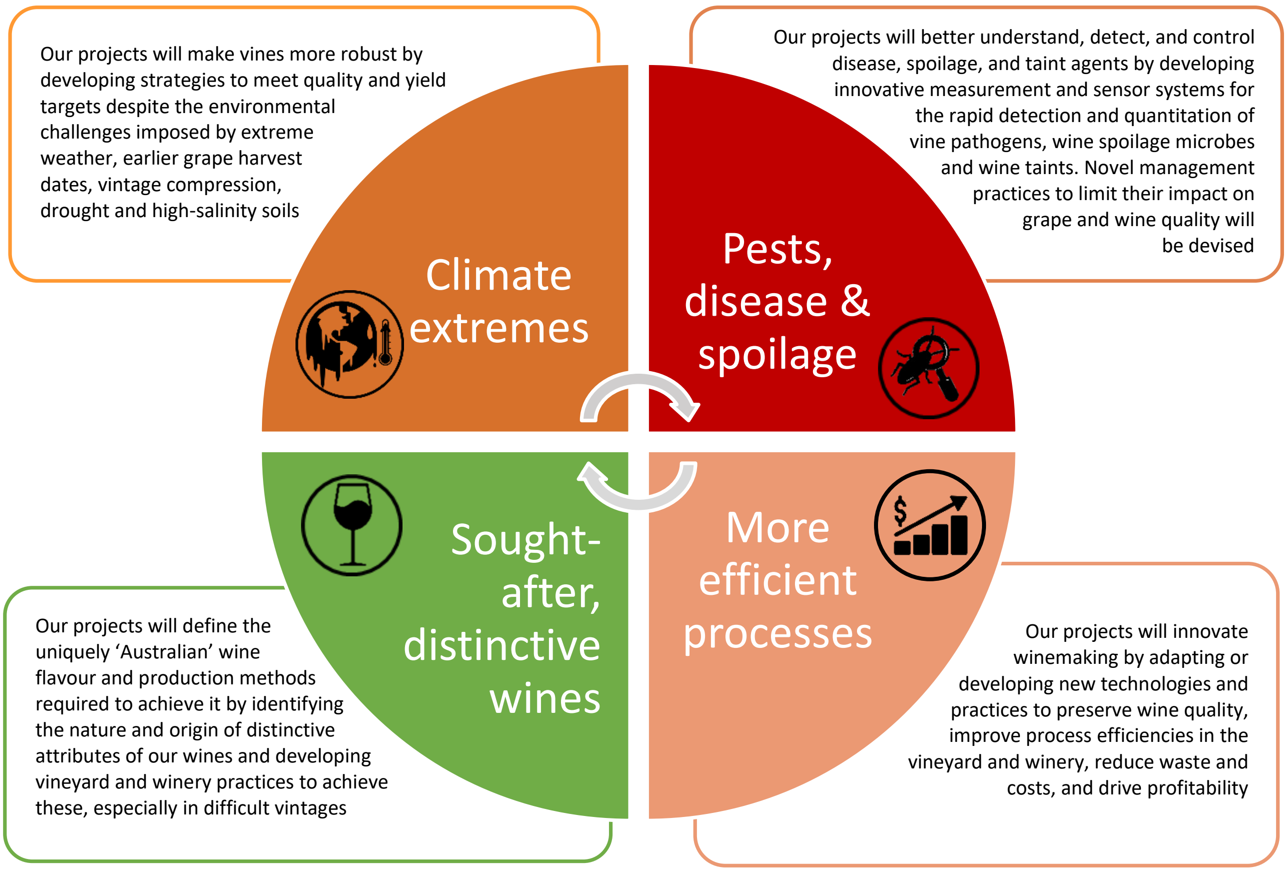
The ARC Training Centre for Innovative Wine Production will tackle new and age-old challenges to wine production, through innovative, multi-disciplinary research over five years. Focusing on responding to challenges and increasing profitability, the Centre's research projects fall into four broad themes: i) climate extremes, ii) pest, disease, and spoilage, iii) sought after distinctive wines and iv) more efficient processes. There are several projects that will tackle viticultural issues where several challenges lie including planting the right material, ensuring vine field survival and performance, and ensuring wine quality. Other projects will provide novel tools to tailor wine composition and increase efficiency in the winery.

Outcomes from research within the Centre will build Australia's competitive edge, by sustainably boosting the wine industry's profitability and resilience to challenges, while providing excellent research training and greater innovation capacity.

The specific aims of the Training Centre are to:

- Make grapevines more robust
- Better understand, detect, and control disease, spoilage, and taint agents
- Define the uniquely 'Australian' flavour proposition and methods to attain it
- Innovate winemaking by adapting and/or developing new technologies and practices
- Provide research training excellence for higher degree by research students and postdoctoral researchers
- Pursue commercialisation and/or adoption of project outputs

The Centre's multi-disciplinary team of researchers and industry partners have experience that spans the grape growing and wine processing value chain. Our research projects will be undertaken in conjunction with our industry partners, providing our student and post-doctoral researchers with valuable research training with a focus on end-user commercialisation.



ARC TRAINING CENTRE FOR INNOVATIVE WINE PRODUCTION

- Training Centre Director
- Researchers: 16 HDRs; 4 PDFs
- Chief Investigators
- Partner Investigators
- Associate Investigators
- Technical and Support Staff
- Research Staff (Funded by Wine Australia)

Current Advisory Board

Prof Vladimir Jiranek - UoA
Dr Eveline Bartowsky - Lallemand
A/Prof Paul Boss - CSIRO
Chris Brodie - Coonawarra Vignerons
Kim Chalmers - Chalmers Wines
Dr Nick Dokoozlian - E&J Gallo
Andrew Grant - Availer
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Dr Jean Macintyre - Pernod Ricard Winemakers
A/Prof Suzy Rogiers - NSW DPI
Prof Leigh Schmidtke - CSU
Dr John Lai - AGRF
Dr Liz Waters - Wine Australia
David Wollan - VA Filtration

Observer

Tammika Hutton – TC Manager

OUR PEOPLE

The ARC Training Centre for Innovative Wine Production brings together an exceptional team of researchers and collaborators:

Training Centre Director

Prof Vladimir Jiranek
Univeristy of Adelaide

Chief Investigators

Prof Kerry Wilkinson
University of Adelaide

A/Prof David Jeffery
University of Adelaide

Prof Steve Tyerman
University of Adelaide

A/Prof Susan Bastian
University of Adelaide

Dr Richard Muhlack
University of Adelaide

A/Prof Cassandra Collins
University of Adelaide

Dr Vinay Pagay
University of Adelaide

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University of Adelaide

A/Prof Christopher Ford
University of Adelaide

A/Prof Paul Grbin
University of Adelaide

Prof Leigh Schmidtke
Charles Sturt University, NWGIC

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University of Adelaide

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Training Centre Manager
University of Adelaide

Nick van Holst Pellekaan
Technical Officer
University of Adelaide

Carolyn Mitchell
Administration Officer
University of Adelaide

OUR PROJECTS

Climate Extremes

1 Inter-vine signalling via plant volatiles
2 The link between cell vitality and potassium in grape berries
3 Vascular transport into the berry – impact on fruit size and composition, yield and wine style
4 Breaking the sugar:flavour nexus – growing grapes with more flavour and less sugar
5 Genetic basis of salt exclusion in grapevine
6 Molecular genetic control of grapevine bud fruitfulness

Pests, Diseases and Spoilage

7 Rapid assessment of grapes prior to harvest to quantify fungal off-flavours and product composition
8 Alternatives to sulfur dioxide for controlling *Brettanomyces* spoilage in wine
9 Wine stability in bottle – impact of light
10 Overcoming taint from vineyard exposure to bushfire smoke

Sought-after, Distinct Wine

11 Characterising the distinctive flavours of Australian cabernet sauvignon wines
12 Managing berry heterogeneity
13 Defining and exploiting the indigenous microflora of grapes
14 Rootstock effects on bunch-stem composition

More Efficient Processes

15 Shaking up the microbiology of winemaking
16 Efficient red winemaking by monitoring extraction and evolution of colour and polyphenols
17 Membrane filtration technologies for efficient wine processing and recovering of valuable extracts

INTER-VINE SIGNALLING VIA PLANT VOLATILES

Project leader: Dr Vinay Pagay
Researcher: Joanah Midzi

Commenced: December 2019
Completing: June 2023
Industry partner:

NSW GOVERNMENT Department of Primary Industries

"Evidence of inter-vine VOC signalling could pave the way for priming grapevines for greater resilience to environmental stresses including extreme weather events such as heatwaves. This method offers growers an alternative to clonal selection or breeding."
Dr Suzy Rogiers, NSW DPI

Publications and conference presentations arising from this project:

- Poster and lightning talk: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022.
- Presentation: *Australian Society of Plant Scientists Adelaide Meeting*, November 2021.
- Presentation: *8th Annual EMBL Australia Postgraduate Symposium*, virtual, November 2021.
- Abiotic stress-induced inter-vine signalling via plant volatiles 'We are in this together': Thirsty vines warn neighbours of impending drought. Presentation: *11th International Symposium on Grapevine Physiology and Biotechnology*, Stellenbosch, November 2021.

Project Aims

The project hypothesises that water-stressed plants emit volatile organic compounds (VOCs) that induce defence responses against water stress in non-water stressed receiver grapevines.

We aimed to investigate plant-plant volatile-mediated signalling in grapevine under drought stress, by:

- Determining the differences in VOC emission from isolated well-watered and drought-stressed grapevines and co-located drought stressed emitter and well-watered receiver grapevines.
- Investigating whether receiver grapevines respond to VOCs from emitter vines with changes in expression of defence-related genes.
- Assessing whether water stress-induced VOCs can prime defence responses in well-watered receiver vines for effective responses to future stress challenges.

Experimentation and Findings

As the first step towards testing the afore-mentioned hypothesis, we set out to identify VOCs that may be involved in the priming process. We investigated emissions of five pre-selected grapevine VOCs: α-pinene, isoprene, methyl jasmonic acid, methyl salicylic acid and *cis*-3-hexen-1-ol, from isolated well-watered (WW) vines, isolated drought-stressed (DS) vines, co-located DS emitter and WW receiver vines in a controlled environment.

Using Teflon® sampling bags and solid phase microextraction (SPME) fibres, volatiles from the different treatments were collected and analysed on the gas chromatography mass spectrometer (GC-MS). We identified isoprenoids (α-pinene and isoprene) that may be potential signal chemicals involved in volatile-mediated communication in grapevine under drought stress.

Significant increase in isoprene concentration in emitter vines (purple) (Fig.1a) compared to isolated vines at peak drought (PD) (red) and maintained after recovery, indicating improved acclimation in emitters.

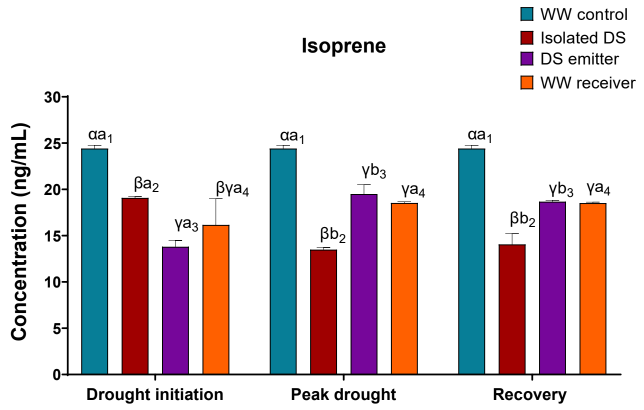

Figure 1a

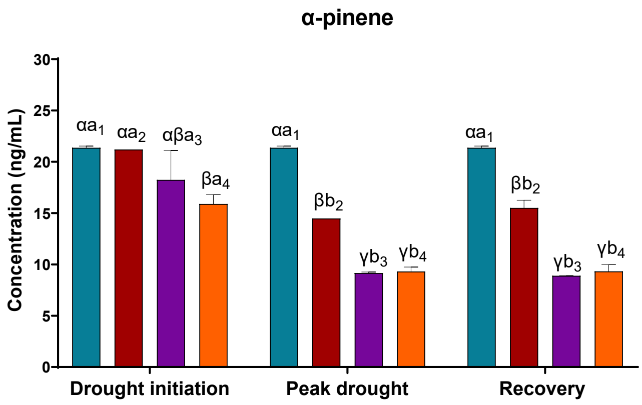

Figure 1b

Figure 1: VOC concentration of (a) isoprene and (b) α- pinene emission in isolated well-watered vines (WW control), isolated drought stressed (Isolated DS), co-located vines that were either drought stressed (DS emitter) or well-watered (WW receiver) during drought initiation, peak drought and recovery stages. Greek letters represent comparison of treatment within a time point, Latin alphabetical letters are comparisons of treatment response across all time points, with subscript number representing the treatment. (ANOVA, Tukey test $p < 0.05$).

Synchronised decrease in emission of α-pinene in both co-located treatments at PD (purple and orange) (Fig.1b), which remains low after recovery. This suggests possible entrainment behaviour.

These results suggest that α-pinene and isoprene may be possible signalling molecules involved in VOC-mediated plant-plant communication during drought stress.

Implications for Industry

The findings in this study provide insight into the underlying mechanisms and molecular basis of VOC-mediated defence priming, contributing towards the wider application of VOCs in viticulture as an eco-sustainable plant-protection strategy against environmental stresses, including extreme weather events such as heatwaves. In particular:

- VOC-mediated priming could potentially improve water use efficiency (WUE) of vines thereby directly saving on water and energy costs
- This method may potentially offer growers an alternative to clonal selection or breeding

References and Acknowledgements

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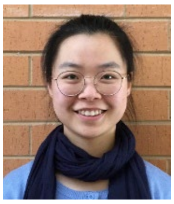
- Karban R, Baldwin IT, Baxter KJ, Laue G, Felton GW (2000) Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia* **125:**66-71.
- Kessler A, Halitschke R, Diezel C, Baldwin IT (2006) Priming of plant defence responses in nature by airborne signalling between *Artemisia tridentata* and *Nicotiana attenuata*. *Oecologia* **148:**280-292.
- Niinemets Ü, Kännaste A, Copolovici L (2013) Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Frontiers in Plant Science* **4:**262.

I would like to acknowledge Drs Vinay Pagay, David W. Jeffery, Ute Baumann, Suzy Rogiers and Stephen Tyerman for their contributions to the study. I also acknowledge the essential input of Dr Dimitra Liacopoulos Capone in the GC-MS work.

Wine Australia's supplementary PhD scholarship (WA Ph2104) is also acknowledged.

THE LINK BETWEEN CELL VITALITY AND POTASSIUM IN GRAPE BERRIES

Project leader: Dr Suzy Rogiers
Researcher: Yin Liu



Commenced: July 2019
Completing: January 2023
Industry partner:

Department of Primary Industries

"Better understanding of the internal factors impinging on berry ripening and ultimately senescence will help us devise practical strategies to maintain optimal quality."
Dr Suzy Rogiers, NSW DPI

Conference presentations arising from this project:

- Presentation: *Macrowine,* virtual, June 2021.
- Presentation: *Crush 2021 - The Grape and Wine Science Symposium*, Adelaide, July 2021.

Project Aims

High berry potassium (K^+) content is correlated with high sugar and low acidity in grape juice. K accumulates rapidly during ripening but eventually slows once berries reach their maximum size. The cessation of K^+ accumulation into berries may be associated with the loss of cell vitality in the hypoxic central region of mesocarp.[1,2,3] The expression patterns of several K^+ channels and transporters have previously been analysed in Shiraz berries during ripening.[4] However, the regulation of K^+ transport into and within the ripening grape berry is largely unclear.

This project aims to investigate K^+ transport in Shiraz berries at the cellular level via electric potentials, gene expression and chemical analysis.

Experimentation and Findings

The berry electric potential measurement was developed using a microelectrode applied to ripening Shiraz berries from véraison to the late-ripening stage. The developmental expression patterns were assessed in the pericarps of individual Shiraz berries in three ripening phases. The maturity of berries was determined by the total soluble solids (TSS).

Berry electric potential measurement

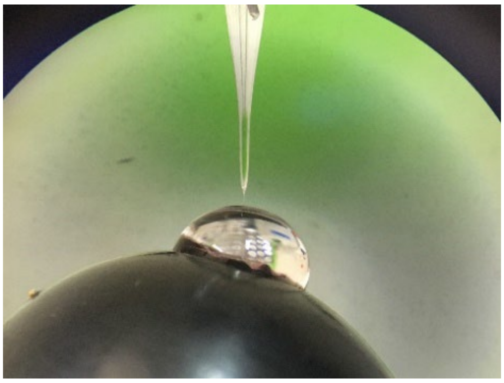


Figure 1. Microelectrode tip submerged into a drop of electrolyte solution placed on the exposed surface of berry flesh (after skin removal) to measure the trans-tissue voltage of a Shiraz berry.

Figure 2. Microelectrode inserted into the berry flesh to measure the trans-membrane voltage in the mesocarp of a Shiraz berry.

Gene expression analysis

The relative expression of three classes of genes (K^+ transport, plasma membrane and vacuolar membrane energisation, and cell death-associated genes) was analysed in the pericarp of individual Shiraz berries sampled at the véraison, ripening and over-mature stages.

Main findings

- At the veraison and ripening stages, living and purposely killed berries were significantly different in electric potentials. However, the voltages of living over-mature berries were similar to those that were killed.
- The change in electric potentials of ripening Shiraz berries theoretically indicates that the berry energy status is a function of the energy availability and energy requirement at various ripening stages.
- Gene expression analysis suggests that the energisation of the plasma membrane and the tonoplast were not consistent during ripening and senescence. This may have been a response to the energy limitation evident in very late ripening.
- K^+ channel and transporter genes were regulated differently during berry ripening.
- Genes potentially associated with cell death were upregulated during berry ripening.
- K^+ and H^+ transport regulation may be important to manage cell vitality and grape berry maturation.

Implications for Industry

1. Electric potentials across and in the grape mesocarp may be indicative of the driving force of solute transport, which may respond to external and internal factors.
2. The information provided by the voltage and gene expression in individual berries may be explored to better understand the physiology of grape berry development and senescence.
3. Future viticultural strategies for growing and harvesting premium fruit under a dynamic climate may be informed by the results of this project.

References and Acknowledgements

1. Rogiers SY, Coetzee ZA, Walker RR, Deloire A, Tyerman SD (2017) Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. *Frontiers in Plant Science* **8:**1629.
2. Tilbrook J, Tyerman SD (2008) Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. *Functional Plant Biology* **35(3):**173-184.
3. Xiao Z, Rogiers SY, Sadras VO, Tyerman SD. Hypoxia in grape berries: the role of seed respiration and lenticels on the berry pedicel and the possible link to cell death. *Journal of Experimental Botany* **69(8):**2071-83.
4. Coetzee ZA, Walker RR, Liao S, Barril C, Deloire AJ, Clarke SJ, Tyerman SD, Rogiers SY (2019) Expression patterns of genes encoding sugar and potassium transport proteins are simultaneously upregulated or downregulated when carbon and potassium availability is modified in Shiraz (*Vitis vinifera* L.) berries. *Plant and Cell Physiology* **60(10):**2331-2342.

I am thankful to Dr Suzy Rogiers, Prof Stephen Tyerman, Prof Leigh Schmidtke, and Dr Vinay Pagay. I would also like to acknowledge the support from Charles Sturt University, Gulbali Institute for Agriculture Water Environment, The University of Adelaide, NSW Department of Primary Industries, and ARC Training Centre for Invovative Wine Production.

VASCULAR TRANSPORT INTO THE BERRY – IMPACT ON FRUIT SIZE AND COMPOSITION, YIELD AND WINE STYLE

Project leader: Dr Suzy Rogiers

Researcher: Dr Zeyu Xiao

Commenced: March 2018

Completing: December 2022

Industry partner:

"The knowledge on the underlying mechanisms driving vascular flow into grape berries will better inform the grape and wine industries on fertigation and other management factors that can be implemented to optimise grape composition and yield."

Publications and conference presentations arising from this project:

- Xiao Z, Chin S, White RG, Gourieroux AM, Pagay V, Tyerman SD, Schmidtke LM and Rogiers SY (2021) Vascular connections into the grape berry: the link of structural investment to seededness. *Frontiers in Plant Science 12.*

- Xiao Z, Stait-Gardner T, Willis SA, Price WS, Moroni F, Pagay V, Tyerman SD, Schmidtke LM and Rogiers SY (2021) 3D visualisation of voids in grapevine flowers and berries using X-ray micro computed tomography. *Australian Journal of Grape and Wine Research* **27**:141-148.

- Xiao Z, DeGaris K, Baby T, McLoughlin S, Holzapfel B, Walker RR, Schmidtke LM and Rogiers SY (2020) Using rootstocks to lower berry potassium concentrations in 'Cabernet Sauvignon' grapevines. *Vitis: Journal of Grapevine Research*. **59 (3):**117-126.

- Poster display: *11th International Symposium on Grapevine Physiology and Biotechnology,* October 2021

Project Aims

The vascular system allows mass transport of essential materials and is important for whole plant communication and integration. A deeper understanding of vascular functioning in grapevines is important because berry size and composition are determined by the flavour and aroma precursors that are transported into the berry. This can ultimately impact grape quality, yield and wine style. However, due to the difficulties in accessing the vascular system in plants, little is known about the physiological factors affecting the dynamics of vascular flow in grapevines.

This project integrates advanced non-destructive imaging techniques such as micro-Computed Tomography (micro-CT) and Magnetic Resonance Imaging (MRI) to better characterise the vascular system in grapevines. The aim of this project is to provide new insights into the mechanisms driving xylem/ phloem flow and demonstrate how their close connection dictates water, carbohydrate, ion, and signal flow to the berry.

The key objectives were to:

- Characterise vascular anatomy of the pedicel and berry brush zone during berry development;
- Visualise internal structures of the grape berry and flower in 3D by micro-CT;
- Investigate tissue oxygen related morphology in other fruit (strawberry, blueberry) to better understand the uniqueness of grape berries;
- Investigate the potential of rootstocks to modify potassium uptake by Cabernet Sauvignon grapevines and their accumulation into grape berries;
- Visualise and measure vascular flow in living potted Shiraz by MRI.

Experimentation and Findings

Differential staining of the berry and receptacle was followed by fluorescent microscopy to examine the vascular continuity into the berry. Morphometric and vascular characteristic analyses within the brush zone were carried out in 5 commercial cultivars including seeded and seedless cultivars. Plant hydraulic modelling revealed that the variation in vascular growth between grape pedicels and berries was independent of seededness. The study also revealed that differences in receptacle xylem vessel size distribution could contribute to cultivar-dependent xylem backflow constraints, with ultimate impacts on berry water retention.

The internal structures of flowers and berries were captured through rapid micro-CT scanning and subsequently were recreated in 3D using image processing. The relative positions of the developing flower parts encased within the flower cap were visualised. The technique was successful at capturing the vascular traces connecting the receptacle to the seeds. Low density/porous tissue was identified within the pedicel and receptacle, connecting the lenticels with the interior of the berry. Voids were present in the proximal mesocarp of mature berries forming a 'detachment zone' in both seeded and seedless cultivars. Voids permeated the mesocarp of mature seedless grape cultivars, but not seeded grapes.

A study to compare tissue oxygen status in grape berry and other fruit, including strawberry and blueberry, was carried out. This study further confirmed the uniqueness of hypoxia developed in grape berries in comparison with other fruit during late ripening. Using a research protocol developed for grape berry research, we further investigated physiological features and discussed post-harvest quality implications of the different fruit.

Preliminary results of flow Magnetic Resonance Imaging (MRI) of potted Shiraz grapevines, a collaborative work with the National Imaging Facility and the Nanoscale Research Group at Western Sydney University, revealed slow xylem flow during winter dormancy followed with greater flow activity in individual xylem vessels during budburst. These preliminary results indicate that there is great potential to further explore MRI as a non-invasive technique to study vascular flow and grapevine physiology.

Rootstocks offer a practical means to manipulate nutrient acquisition from the soil and ultimately, once transferred into the vascular system, transport through the rest of the plant.

A field trial was carried out to investigate the feasibility of using rootstocks to lower berry potassium concentrations ([K]) in Cabernet Sauvignon grapevines. This study compared fruit and juice [K] and related compositions of grapevines grown on eight different rootstocks in the Limestone Coast of South Australia. It was found that juice titratable acidity was higher for the rootstocks 140 Ruggeri and 110 Richter, and juice pH tended to be lower for the rootstocks 110 Richter, 140 Ruggeri, Merbein 5512 and Merbein 5489.

Implications for the industry

This project combines several research avenues to explore vascular function and integrates advanced imaging techniques to explore physiological factors driving grape berry growth and development. These insights will help define management strategies to fine tune berry composition.

Key outcomes include:

- Understanding the vascular anatomy between the berry pedicel and brush zone is vital to understanding the movement of key precursors driving final berry composition
- Comparing the internal structure of grape berry and other perishable fruit allows us to better understand fruit oxygen status during ripening, which relates to fruit quality
- Accessing and integrating *in-vivo* imaging techniques (i.e., MRI) facilitates the understanding of the whole-plant movement of water, nutrients, carbohydrates, signal molecules and other metabolites important to plant growth, health and reproductive capacity
- A field trial on rootstock characterisation allows for more informed rootstock selection.

References and Acknowledgements

The researchers acknowledge guidance and support from TC supervisors, Prof Leigh Schmidtke, Emeritus Prof Stephen Tyerman and Dr Vinay Pagay. We thank Prof William Price, Dr Timothy Stait-Gardner, and Dr Scott Willis for expert technical assistance and acknowledge the facilities and assistance of the National Imaging Facility at the Biomedical Magnetic Resonance Facility, Western Sydney University. We thank the Wine Australia Incubator Initiative for additional funding.

BREAKING THE SUGAR FLAVOUR NEXUS: GROWING GRAPES WITH MORE FLAVOUR AND LESS SUGAR

Project leader: A/Prof Christopher Ford
Researcher: Pietro Previtali

Commenced: April 2018
Completed: March 2022
Industry partner: E&J Gallo Winery

"This work has shed some light on the importance of controlling sugar accumulation, especially in warmer and drier vintages, and provided tools to manipulate ripening to achieve the targeted yield and quality"
Nick Dokoozlian, E&J Gallo

Publications and conference presentations arising from this project:

- Previtali P, Dokoozlian NK, Pan BS, Wilkinson KL, Ford CM (2021). Crop load and plant water status influence the ripening rate and aroma development in berries of grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* **69(27):**7709-7724.
- Previtali P, Dokoozlian NK, Pan BS, Wilkinson KL, Ford CM (2022). The effect of ripening rates on the composition of Cabernet Sauvignon and Riesling wines: Further insights into the sugar/flavor nexus. Food Chemistry **373**:131406.
- Previtali P, Giorgini F, Mullen RS, Dookozlian NK, Wilkinson KL, Ford CM (2022). A systematic review and meta-analysis of vineyard techniques used to delay ripening. *Horticulture Research* **9**:uhac118

Project Aims

In this project, we focused on the relationship between sugars and specialised metabolites important for winemaking during the process of grape ripening. At harvest, growers seek to achieve a balance between sugars, organic acids, colour and mouthfeel (i.e. phenolic maturity) and aroma compounds (i.e. aromatic maturity), which are specific to the desired wine style. Achieving the sought-after grape composition is not easy for grape growers, this trait being controlled by the interaction of genotype, environment and management practices. In addition, changing climate conditions including more frequent and severe heat and water stress events, have been shown to accelerate sugar accumulation and 'decouple' sugars and organic acids or colour compounds[1,2]. The growers' dilemma becomes whether they should harvest grape at the targeted sugar level but with sub-optimal phenolic and aromatic maturity, or wait until colour and aroma compounds have fully developed, but at the cost of higher sugar levels and often berry weight losses due to late season shrivelling. To tackle this problem, our research implemented a series of field trials in which vineyard management practices were used to manipulate the rate of ripening and restore a balanced composition of grapes. Alongside this traditional approach, we adopted a data-analysis approach to pinpoint vineyard management practices that are best successful at delaying ripening, which was achieved by a longitudinal analysis across the results of studies published in the past two decades.

Experimentation and Findings

<u>Study 1</u> – The effect of sugar accumulation rates on grape composition

Cluster thinning and late season irrigation were used to manipulate rates of sugar accumulation in a vineyard of cv. Cabernet Sauvignon in Lodi (California). This region resembles several Australian ones which often experience heat and water stress extremes during the stages of grape development and ripening. The experimental treatments led to three kinetics of sugar accumulation: fast (cluster thinning), control and slow (cluster thinning and late season irrigation). The fast- and slow-ripening treatments were harvested at the same Total Soluble Solid (TSS) level than the control (26°Brix) which was reached one week in advance and two weeks later, respectively. The chemical composition was analysed at harvest for grapes ripening at different rates. The results showed that delayed ripening positively affected the grape aroma and phenolic, improving the overall quality of Cabernet Sauvignon. Specifically, a slower ripening led to a lower concentration of compounds responsible for green and grassy notes unwanted in red wine, namely C_6 compounds and IBMP. In turn, aroma precursors of some terpenes and norisoprenoids, especially β-damascenone, were increased in delayed ripening. Although bound to sugar and thus non-volatile in grapes, these compounds are freed by the action of yeasts and ageing releasing molecules that are known drivers for the fruity and floral character of Cabernet Sauvignon wines. Alongside a positive impact on grape aroma profile, delayed ripening also resulted in an increase in grape anthocyanins, with benefits for colour intensity and stability.

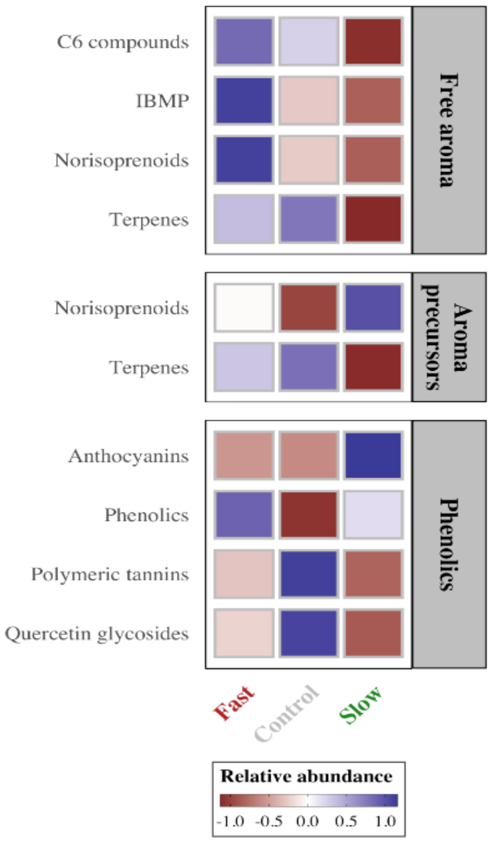


Figure 1. Effect of ripening rates on grape composition.

<u>Study 2</u> – Using a meta-analytic approach to unravel the most effective vineyard practices to delay ripening

We adopted a data-analysis approach called 'meta-analysis' to review vineyard practices that can be adopted to delay sugar accumulation, which also is beneficial to spread the harvest window and improve grape quality. Meta-analysis is the analysis of results of single studies on the same topic, the results of which are collated together in a new database. By re-analysing the results with powerful statistical techniques, this biometric approach allows to quantitatively assess the efficacy of treatments of interest.

In our meta-analysis we investigated the efficacy of three vineyard practices that have been proposed to delay ripening3[,4]:

- *Antitranspirants* – when sprayed on grapevines, these compounds limit the production of sugar or translocation thereof;
- *Delayed pruning* – when pruned after budburst, grapevine phenology is delayed and so is grape ripening;
- *Late source limitation* – reduction of the amount of photosynthetically-active leaf area limits sugar production and translocation.

The delaying power was quantified by creating a variable called "effect size", which was calculated as the difference between the TSS of the control and treated grapes on the day of harvest. Positive and negative values indicated a ripening delay or advancement respectively, while null effects indicated that the treatment did not affect ripening. In addition, the absolute value of the effect was a measure of the strength of the treatment to vary ripening, indicating how many Brix apart were the control and treated grapes.

It was confirmed that the three treatments investigated are effective tools to delay ripening. Average ripening delays were as follows: antitranspirants (0.74 °Brix); delayed pruning (1.5 °Brix); late source limitation (1.2 °Brix). In each treatment, it was also possible to model the effect size by other variables of interest such as environmental conditions or those indicating the timing and intensity of the treatment or vineyard characteristics. This important step allowed us to pinpoint what aspects growers should take into consideration in seeking to use the vineyard techniques investigated to delay ripening. (**Table 1)**.

Treatment	Significant factors
Antitranspirants	Active compound Number of applications Timing of application
Delayed pruning	Pruning stage Potential yield
Late source limitation	Targeted TSS for harvest Potential yield

Table 1. Factors affecting the efficacy of vineyard practices to delay ripening.

Implications for Industry

Our project has demonstrated the importance of minoring the rate of ripening (i.e. sugar accumulation) and adjusting it when necessary to align the technological, phenolic and aromatic maturity of grapes. Importantly, the efficacy of simple and low-cost techniques to delay ripening has been reported as well as key aspects that growers should control to calibrate their treatments.

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The support of Wine Australia's supplementary PhD scholarship to Pietro Previtali is greatly appreciated. We would like to thank all co-authors to the papers and E. & J. Gallo Winery for their kind contribution and financial support.

GENETIC BASIS OF SALT EXCLUSION IN GRAPEVINES

Project leaders: Prof Matthew Gilliham, Dr Anthony Borneman & Dr Mandy Walker
Researcher: Andres Zhou Tsang

Commenced: February 2020
Completing: August 2023
Industry partners:

"AGRF is delighted to have supported this study, which identified potential salt exclusion genes in grapevines that may ultimately lead to the selection of more resilient varieties."
Dr John Lai, AGRF

Publications and conference presentations arising from this project:

- Zhou-Tsang A, Wu Y, Henderson SW (2021) Grapevine salt tolerance. *Australian Journal of Grape and Wine Research* **27(2):**149-168.
- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022.
- Presentation: *14th International Terroir Congress,* Bordeaux, July 2022.

Project Aims

Environmental salinity is an expanding challenge for Australian viticulture, causing over 60 million dollars of yearly loss in the grape and wine industry. The implementation of salt tolerant rootstock varieties is the preferred solution to prevent the damage of salinity in grapevines, however the current diversity of these varieties is very limited.[1]

Our objective is to identify the genetic component associated with the trait of salt tolerance in grapevines and rootstocks. This will facilitate the breeding of future resilient varieties through the selection of genetic markers of salt tolerance.

The steps we are taking to achieve our objectives are:

- Record whole genome assemblies of salt tolerant and vulnerable rootstocks.
- Inspect the genomic and transcriptomic differences between rootstocks.
- Assess the functionality of potential salt exclusion mechanisms that differ across genotypes.

Experimentation and Findings

We produced high quality genome assemblies of salt tolerant rootstocks Ramsey and 140 Ruggeri, and the salt vulnerable rootstock K51-40[2]. These genomes were annotated for gene finding and predicted functions.

The rootstock genomes recorded in our project share a similar genome size, also compared to previously revealed grapevine genomes (i.e. chardonnay[3]). Allelic variation is observed in most of their genomic extent, which allows the selection of candidate alleles for each gene of interest.

Employing the genomes of Ramsey, 140 Ruggeri and K51-40 as references to match their specific transcriptomic data results in increased mapping rates compared to using grapevine reference genome, and in addition, shows the individual expression values for each ortholog allele between the three genotypes. This provides a faithful comparison of candidate genes across vulnerable and tolerant genotypes.

	Chardonnay	Ramsey	140 Ru	K51-40
Total size (Mb)	980	990	986	932
Heterozygous portion (Mb)	378	475	468	454
Genes	29,675	34,466	32,547	30, 406

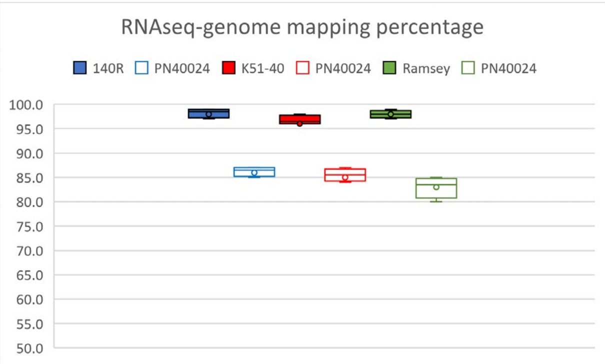

Figure 1. Higher mapping rates are achieved when RNA rootstocks 140R, Ramsey and K51-40 to the specific genomes assemblies that we produced, compared to the grapevine reference genome.

We have identified key differences in both gene expression and gene number in several sodium transport and chloride transport protein families, potentially involved in salt exclusion. These transporters are being functionally characterised in heterologous systems to find performance differences in performance between tolerant and vulnerable genotypes, establishing specific candidate alleles to be selected for the trait of salt tolerance.

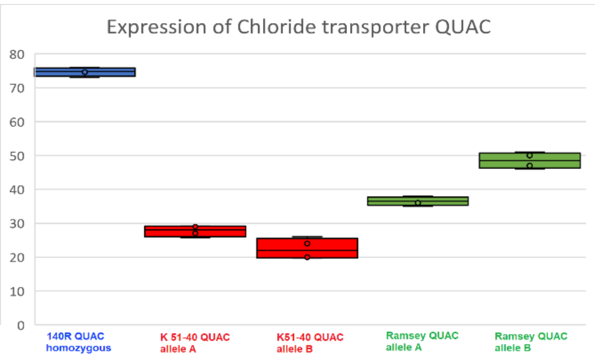

Figure 2. Salt excluder rootstock 140R shows higher expression of its own variant of the protein QUAC, a predicted chloride transporter. This could be a potential candidate marker for breeding future salt excluder rootstocks.

Implications for Industry

Candidate genes discovered in this project will help to produce new tolerant varieties to adapt grape production towards the increase of salinity driven by climate change.

The three genomes assembly that we produced for our project contain the entire genetic information for their respective genotypes. This means that they can guide the research for the genetic basis of other traits of interest, such as K51-40's disease tolerance, or Ramsey and 140 Ruggeri's increased vigour. Eventually, multiple traits of interest can be combined to produce a versatile of rootstocks, not only tolerant to salinity but also suited for different viticultural needs.

References and Acknowledgements

1. Zhou-Tsang A, Wu Y, Henderson SW, Walker AR, Borneman AR, Walker RR, Gilliham M (2021). Grapevine salt tolerance. *Australian Journal of Grape and Wine Research* **27(2):**149-168.

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We acknowledge our colleagues from AWRI, AGRF, CSIRO, University of Adelaide and ARC-CoE in Plant Energy Biology for their invaluable assistance through different steps of our project. This research was conducted as part of the Australian Research Council Training Centre for Innovative Wine Production (www.ARCwinecentre.org.au; project number IC170100008), funded by the Australian Government with additional support from Wine Australia, Waite Research Institute and industry partners. The University of Adelaide, CSIRO and The Australian Wine Research Institute are members of the Wine Innovation Cluster.

MOLECULAR GENETIC CONTROL OF GRAPEVINE BUD FRUITFULNESS

Project leader: Prof Matthew Gilliham
Researcher: Xiaoyi (Eva) Wang

Commenced: December 2018
Completing: August 2022
Industry partner:

"These findings add to our knowledge and understanding of reproductive development in grapevines which we can use to manipulate yield in vineyards."
A/Prof Cassandra Collins

Publications and conference presentations arising from this project:

- Presentation: *17th Australian Wine Industry Technical Conference,* Adelaide, July 2019.
- Presentation: *XIIIth International Terroir Congress*, Adelaide, November 2020.
- Presentation: *11th International Symposium on Grapevine Physiology and Biotechnology,* Stellenbosch, November 2021.

Project Aims

Bud fruitfulness is a key reproductive parameter of grapevine and an important determinant for seasonal yield fluctuation. To date the molecular control of grapevine bud fruitfulness in response to environmental signals is largely unknown.

This study aims to investigate the effects of temperature and light intensity on:

- Vegetative growth in Semillon vines
- Bud fruitfulness, including number and size of inflorescence primordia in compound buds
- The bud transcriptome at three different stages: pre-flowering, veraison and harvest.

Experimentation and Findings

Semillon cuttings were propagated in controlled environment. Six treatments were applied on the vines including two levels of temperature (day/night 30/25°C and 20/15°C) and three levels of light intensity (90, 200 and 600 PAR).

Shoot vigour measures were taken at E-L Stage 17 including shoot length and weight, shoot leaf area, shoot internode number and average internode length.

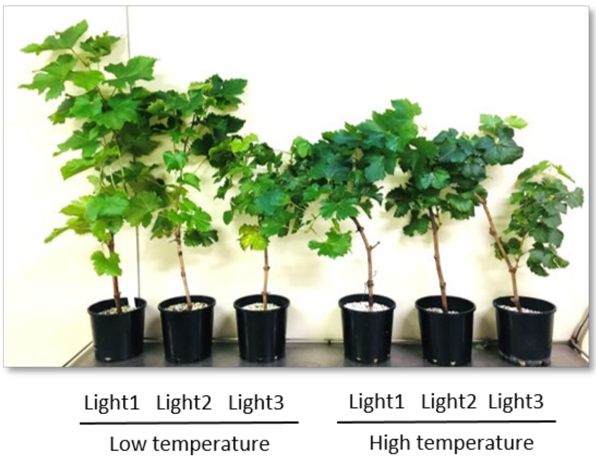

Figure 1. Representative vines from each treatment group. (Light 1, 2 and 3 indicates low, middle, and high level of light intensity, respectively.)

Results showed a clear disparity of vine size and shoot growth between each growth temperature and each level of light. In general, vine size has a negative correlation with temperature and light. High temperature and higher light promoted shoot growth with more internodes, but low temperature and lower light led to greater vine vigour with longer shoots and larger leaves.

For bud fruitfulness, numbers of inflorescence primordia were recorded at E-L Stage 35, 38, and 43 in order to compare bud fruitfulness in all six groups and keep track of the development of inflorescence primordia with time.

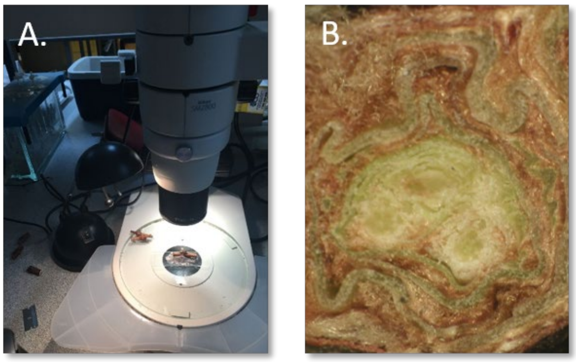

Figure 2. Bud dissection analysis. A, a compound bud under a light microscope; B, a transverse cut of a primary bud containing two inflorescence primordia.

Results showed that bud fruitfulness was significantly improved by high temperature and higher light, with higher number and larger size of inflorescence primordia.

RNA-seq analysis was performed for bud samples from all six groups and results revealed that temperature had a greater influence at early development (pre-flowering, E-L Stage 17) with 8530 differentially expressed genes (DEGs). Light, however, was most important later (veraison, E-L Stage 35) with 5716 DEGs.

Gene ontology enrichment analysis showed that the DEGs were mainly involved in biological functions of stress management under the temperature treatment and active cellular development under the light treatment.

Implications for Industry

Producing a consistently yielding and high-quality crop is of central importance to vignerons but has been challenging due to seasonal environmental changes[1].

This study showed that the development of inflorescence primordia in latent buds can be significantly affected by temperature and light.

The results suggest that:

1. Altering bud microclimate can be effective to manipulate yield potential in commercial production with a wide time window in the growing season from early spring to veraison.

2. It is possible to further explore the bud transcription data to identify genes as potential molecular markers for high fruitfulness via breeding or management.

References and Acknowledgements

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This project is co-supervised by A/Professor Cassandra Collins and Professor Dabing Zhang from the University of Adelaide.

RAPID ASSESSMENT OF GRAPES PRIOR TO HARVEST TO QUANTIFY FUNGAL OFF-FLAVOURS AND PRODUCT COMPOSITION

Project leader: Prof Leigh Schmidtke
Researchers: Dr Morphy Dumlao, Liang Jiang


Commenced: March 2019
Completing: December 2023
Industry partner:

NSW GOVERNMENT Department of Primary Industries

"This new innovation will hopefully aid growers and winemakers in ensuring quality and objective assessment, thereby offering better wine to consumers, but could also be applied more broadly to other horticultural crops."

Conference presentations arising from this project:

- Presentation: *Crush 2021 - The Grape and Wine Science Symposium,* Adelaide, July 2021
- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022
- Presentation: *Agilent Canberra Mass-Spec Day*, September 2022

Project Aims

Fungal contamination and smoke exposure have significant economic impacts on the grape and wine industries as they produced off-flavours and led to changes in colour. To assist in timely vineyard management that minimises yield and quality losses, decision support tools that correlate quality and quantification of potential off-flavour development are desirable.

Our project aims to develop an innovative tool for a rapid and direct assessment of wine and grape. There are different proposed strategies (Fig. 1) that may be helpful to achieve this overarching objective, including:

a) Measure potential *Botrytis cinerea*-related volatile biomarkers and establish prediction models for infection levels
b) Develop a method that can selectively capture through adsorbent materials with ultrahigh surface areas and/or isolate quick extraction important compounds such as biomarkers
c) Develop a non-destructive air sampling method in field for the detection of fungal infection biomarkers

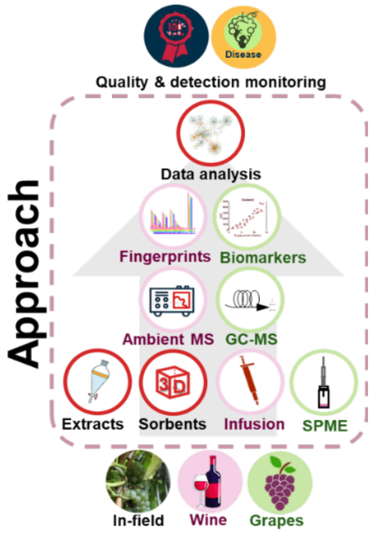


Figure 1. The overall approach in developing a rapid and direct quality assessment.

d) Develop a direct method acquiring volatile fingerprint profiles to determine product attributes and indicators (*e.g.*, smoke taint, fungal infection and contamination);
e) Integrate new sampling and an ambient ionization source for mass spectrometry for rapid detection and identification.

Key Experimental Findings

a) Volatile markers, measured by SPME GC-MS, have been identified as predictors of *Botrytis cinerea* fungal infections of grape s (Fig. 2). These biomarkers may enable early detection leading to early quality management of grapes.

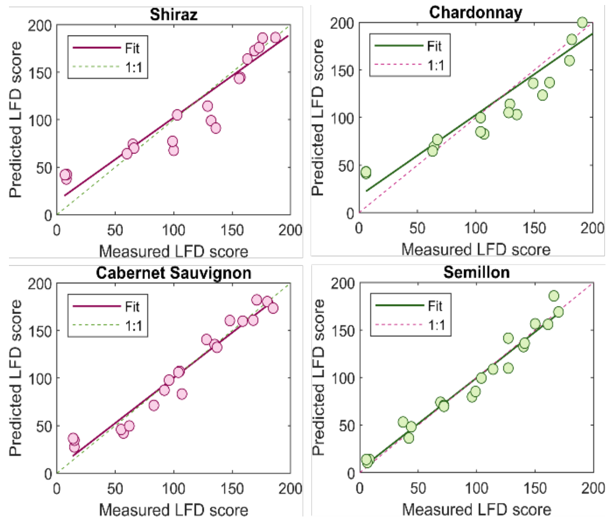


Figure 2. Prediction models developed using whole bunch inoculated SHZ, CAB, CHA and SEM using 1,5-dimethyl tetralin, 1,5-dimethyl naphthalene, phenylethyl alcohol and 3-octanol as biomarkers.

b) Testing of novel materials to capture volatile compounds from in-field grape samples (Fig. 3, left) and from direct coupling to ambient MS (Fig. 3, right) is showing promising results. Increased sensitivity and specificity for detection of volatile markers associated with grape or wine contamination and faults will enable more rapid non-destructive testing for management decision support during the wine making process.

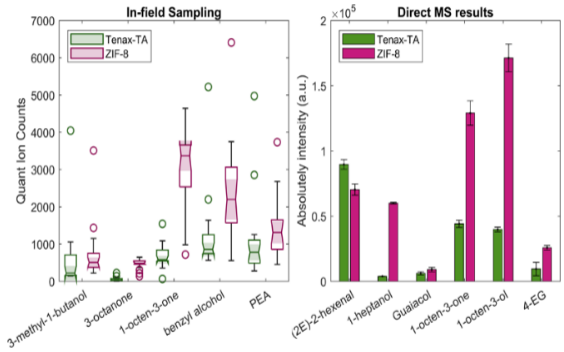


Figure 3. Efficient trapping of low level volatiles using ZIF-8 for in-field sampling (*left*) and the direct coupling to ambient MS (right) compared to Tenax-TA.

c) Developing a quick wine extraction method using a common solvent (Fig. 4) to rapidly isolate important analytes before direct infusion to ambient MS has progressed. This approach requires on only small µL volumes of wine to rapidly (< 2 mins) detect wine taints and faults including smoke taints and *Brettanomyces* yeast taint.

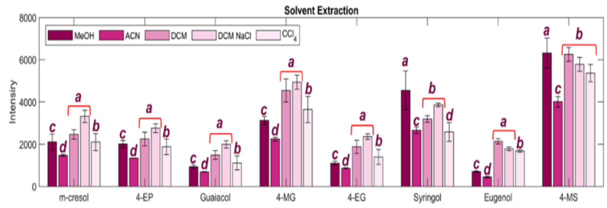


Figure 4. Extraction performance of common solvents using spiked wine.

Implications for Industry

The development of rapid and direct techniques provides support in management decision. It will significantly reduce analysis time, reduce complexity of instrumentation and lower maintenance costs, which are often challenging in many industries. In the end, the industry will benefit from the dramatic economic impact as they continually maintain product quality.

References and Acknowledgements

The research team gratefully acknowledge the valuable contributions of colleagues, industry partners and collaborators to this work.

ALTERNATIVES TO SULFUR DIOXIDE FOR CONTROLLING *BRETTANOMYCES* SPOILAGE IN WINE

Project leader: A/Professor Paul Grbin
Researcher: Yanina Giordano

Commenced: February 2021
Completing: August 2024
Industry partners:

AWRI Pernod Ricard Winemakers

"Yanina's project will provide options for controlling* Brettanomyces *in wine while relieving pressure around potential adaptation to sulfur."
Dr Anthony Borneman, AWRI

"Yanina's project will lead to new ways to prevent* Brettanomyces *spoilage that are more consumer-friendly than the existing preservative options, giving winemakers more options to manage their wines."
Dr Jean Macintyre, Pernod Ricard Winemakers

Forthcoming conference presentations arising from this project:

- *17th International Conference on Industrial Microbiology,* Paris, May 2023.
- *ISSY 2023 Yeast Biotech Conference,* Adelaide, November 2023
- *II International Congress on Grapevine and Wine Sciences,* Spain, November 2023

Project Aims

Brettanomyces is a world-renowned yeast that detrimentally affects the organoleptic properties wine by producing various undesirable metabolites. This yeast may survive the challenging winemaking process, into the wine aging stage. Current control techniques are focused on the addition of sulfur dioxide (SO_2) to control *Brettanomyces* development in wines. However, recent research has determined that some *Brettanomyces* strains can develop resistance to this preservative agent. Thus, it is critical to develop new strategies to manage this spoilage yeast.

This project focuses on:

- Understanding *Brettanomyces* growth, nutrition, metabolism, and physiology in oenological environments
- Developing efficient and innovative alternatives to manage *Brettanomyces* in the winemaking process

Furthermore, the production of wines with lower SO_2 concentrations needs to be explored and supported by research without compromising wine quality in terms of organoleptic characteristics and microbiological stability. As a result, it is important to find alternatives to SO_2, therefore producing healthy and novel wines that match current wine consumer needs.

Experimentation and Findings

The roles of minerals (Mg, Zn, Fe) in *Brettanomyces* metabolism are yet to be elucidated. For this reason, the effect of mineral limitation and omission on the metabolic process of this spoilage yeast will be studied.

Experiments are currently investigating cell aging in *Brettanomyces* under mineral stress conditions. A serial sequence of fermentations is being undertaken, whereby batch cultures of mineral-stressed *Brettanomyces* are grown to a defined cell number and then re-inoculated into a fresh media to determine the long-term effect on growth and development.

A parallel study is also being carried out on Mg requirements of *Brettanomyces*. Once these studies are complete, a physiological characterization of *Brettanomyces* will be undertaken to determine an indicator of the stress response (mineral stress) at the genetic level. The research will continue to gather substantial knowledge about the nutrition, metabolism, and physiology of *Brettanomyces* yeast development in winemaking conditions, leading to developing innovative strategies to manage *Brettanomyces* spoilage in wine.

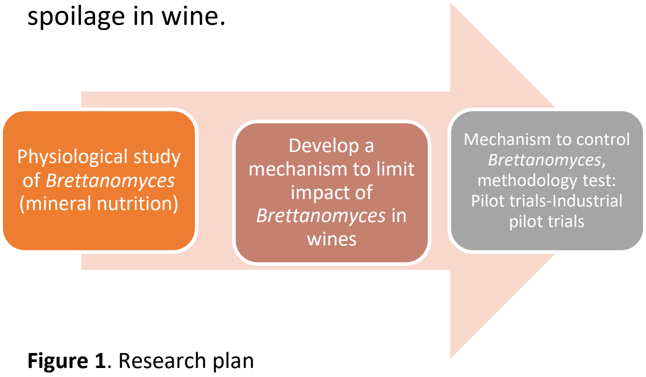


Figure 1. Research plan

Implications for Industry

Winemakers will benefit from the outcomes of this project by making more informed decisions about techniques to control *Brettanomyces* based on information that will be disseminated at the end of this project. Furthermore, this project seeks to unlock the full potential for premium wine production, making the Australian wine industry more competitive across the globe.

References and Acknowledgements

The research team gratefully acknowledge the valuable contributions of colleagues, industry partners and collaborators to this work.

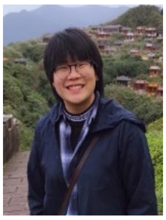
Financial support for this work is provided by:

- The ARC Training Centre for Innovative Wine Production
- Wine Australia Supplementary Scholarship

Figure 2: Microscopic analysis of *Brettanomyces* yeasts

THE IMPACT OF LIGHT ON THE OXIDATIVE AND REDUCTIVE AGING OF WINE

Project leader: Dr Andrew Clark
Researcher: Isara Vongluanngam



Commenced: January 2022
Completing: January 2025
Industry Partner:

Department of Primary Industries

"My focus is to limit the occurance of light strike effects on wine and explore potential benefits of light during wine production."

Project Aims

PART 1. Controlling the impacts of light on wine

- Determine the impact of light on the binding of Cu (II) during the aging of wine in bottle
- Determine the impact of Cu forms in delaying the onset of light strike aroma in bottle aged wine
- Determine the impact of light on the efficiency of the main preservative in wine (sulfur dioxide) to remove oxygen

PART 2. Assess the potential to utilise the exposure of wine to light during production

- Accelerate the formation of stable pigment in red and rosé wines

Experimentation and Findings

Given that the project has recently commenced, there are no key project findings to report at this current time. However, progress has certainly been made on identification of key scientific literature, definition of knowledge gaps and the commencement of experimental design. The PhD researcher has also been trained on the analytical instrumentation that will be critical for the project.

Implications for Industry

This project commenced in early 2022 and consequently has no findings to report at this time. However, in terms of focus, the project will concentrate on two key aspects related to the impact of light on wine.

The first main topic of research will investigate approaches to minimise light-induced spoilage of wine. Many sparkling and/or white wines are bottled in transparent glass and may be stored under fluorescent light in retail shops/ supermarkets. The storage of the wine under these conditions can promote undesirable aroma attributes and discolouration (i.e., browning). The off-flavour linked to the exposure of wine to light is termed 'light strike' (e.g., cooked cabbage) and is related to volatile sulfur compounds (particularly methanethiol and dimethyl disulfide). Copper fining is a commonly used agent in wineries to remediate against reductive aromas in wine. This study will provide improved insight into the capability of copper usage to inhibit light strike flavours.

The second key topic of research will be to utilise light to aid wine production. More specifically, the feasibility of using light to accelerate the colour maturation of red and rosé wine will be investigated. Light is already known to have the ability to act as a catalyst for phenolic polymerisation reactions but utilising this capability while limiting detrimental impacts on wine flavour will be a key aim of this project.

Acknowledgements

I appreciate the kind support and guidance from my supervisory team – Dr Andrew Clark, Dr Xinyi Zhang, Dr John Blackman, Prof Leigh Schmidtke and Prof Kerry Wilkinson.

OVERCOMING TAINT FROM VINEYARD EXPOSURE TO BUSHFIRE SMOKE

Project leader: Professor Kerry Wilkinson
Researcher: Colleen Szeto

Commenced: May 2018
Completed: September 2022
Industry partner: E & J Gallo Winery

"Modelling forecasts bushfires will increase in frequency and severity with future climate change. As such, there is a clear, strategic imperative to implement strategies that enable the wine sector to better predict and mitigate smoke taint."

Publications and conference presentations arising from this project:

- Szeto C, Ristic R, Capone D, Puglisi C, Pagay V, Culbert J, Jiang W, Herderich M, Tuke J, Wilkinson K (2020). Uptake and glycosylation of smoke-derived volatile phenols by Cabernet Sauvignon grapes and their subsequent fate during winemaking. *Molecules* **25**:3720.
- Szeto C, Ristic R, Wilkinson K (2022) Thinking inside the box: a novel approach to smoke taint mitigation trials. *Molecules* **27**:1667.
- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022

Project Aims

Bushfire frequency and severity have increased due to climate warming. Taint from vineyard exposure to smoke therefore remains an issue of major concern for grape and wine producers worldwide and an ongoing threat to long-term economic viability, despite significant scientific progress.

In this project, field trials were undertaken in Australian and US vineyards to:

- Evaluate environmental sensors for monitoring vineyard exposure to smoke;
- Identify factors (e.g., variety, vintage) that influence smoke uptake by grapevines;
- Develop strategies for mitigating the sensory perception of smoke taint

The resolution of smoke taint diagnostics was improved through investigations of the chemical markers most indicative of smoke exposure in grapes, analysis of their accumulation over time, and their subsequent fate in wine. Additionally, a novel approach was developed for small-scale mitigation trials, enabling evaluation of several vineyard-based smoke taint mitigation strategies.

Experimentation and Findings

The uptake and glycosylation of volatile phenols by Cabernet Sauvignon grapes was monitored following exposure of grapevines to smoke (for 1 hour) under experimental conditions. Volatile phenols 'disappeared' soon after smoke exposure (due to metabolism), but the accumulation of volatile phenol glycoconjugates was delayed, with a significant increase in volatile phenol glycoconjugates occurring between 1 week (t = 3) and 4 weeks (t = 4) post-smoke exposure (Figure 1). This suggests sequestration, the presence of intermediates within the glycosylation pathway and/or other volatile phenol storage forms.

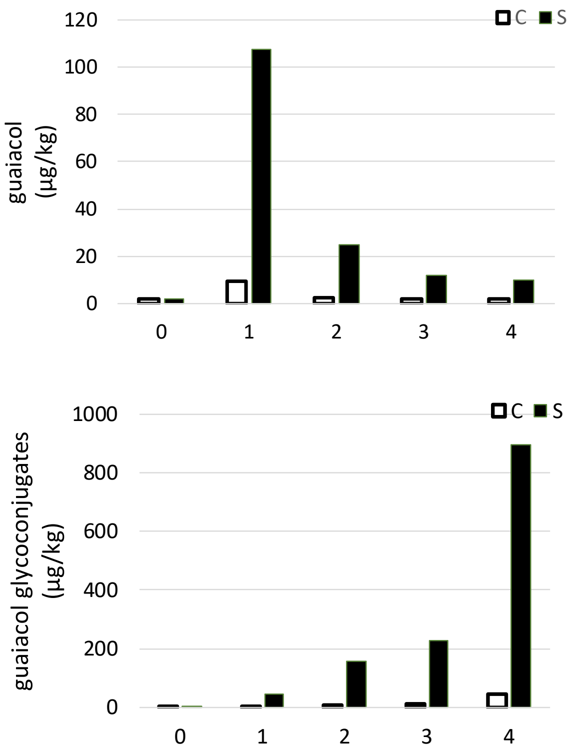


Figure 1. Concentration of free and glycosylated guaiacol in grapes before (t=0), and 1 hour (t=1), 1 day (t=2), 1 week (t=3) and 4 weeks (t=4) after grapevine smoke exposure.

This finding has implications for industry in terms of detecting smoke-affected grapes following vineyard smoke exposure. It suggests sampling grapes several days after smoke exposure might result in under-estimation of the extent to which grapes have been affected by smoke.

The smoke treatments applied to Cabernet Sauvignon grapevines were of sufficient density (PM_{10} >2000–2500 µg/m^3) that detector saturation occurred. However, the sensors were successfully used to monitor smoke emissions during prescribed burning of pea stubble.[1]

In a separate study, a purpose-built smoke box was constructed and evaluated as a convenient approach to reproducibly applying smoke to grape bunches, post-harvest. Some variation was observed between replicate smoke treatments, but this was overcome by implementing appropriate controls and replication. The smoke box was subsequently employed to evaluate the efficacy of agrochemical sprays (kaolin and an anti-transpirant) and activated carbon fabric as novel methods for mitigating smoke contamination of grapes (Table 1).

The agrochemical sprays did not provide effective protection from smoke, but enclosing grape bunches in activated carbon fabric prevented the uptake of smoke-derived volatile phenols, thereby demonstrating a promising new strategy for preventing smoke taint.

Table 1. Concentration of guaiacol in juice from control grapes, smoke-exposed grapes, and grapes treated with kaolin or an anti-transpirant, or enclosed in activated carbon fabric, during smoke exposure.

Treatment	Guaiacol (µg/L)
Control	not detected
Smoke	231 ± 16
Kaolin	183 ± 19
Anti-transpirant	239 ± 24
Activated carbon fabric	4.5 ± 1

Of the mitigation strategies that were evaluated, the activated carbon fabric was by far the most effective. A subsequent study, taken through to a wine outcome, demonstrated activated carbon fabric mitigated the uptake of volatile phenols by grapes, preventing the perception of smoke taint in finished wine.[2] Ongoing research seeks to address shortcomings associated with the use of activated carbon fabric for mitigation of smoke contamination in commercial vineyards.

Implications for Industry

This research improves the wine industry's capacity to predict, mitigate and respond to risk associated with vineyard exposure to smoke, and to support timely decision-making during the vintage (harvest and winemaking) period.

References and Acknowledgements

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2. Wilkinson, K.L., Ristic, R., Szeto, C., Capone, D.L., Yu, L., Losic, D. (2022) Novel use of activated caron fabric to mitigate smoke taint in grapes and wine. *Australian Journal of Grape and Wine Research* **28**:500–507.

The research team gratefully acknowledge the valuable contributions of colleagues, industry partners and collaborators to this work.

CHARACTERISING THE DISTINCTIVE FLAVOURS OF AUSTRALIAN CABERNET SAUVIGNON WINES

Project leader: A/Professor David Jeffery
Researchers: Dr Dimitra Capone and
Dr Lira Souza Gonzaga

Commenced: Lira (Jul 2018) & Dimi (Mar 2018)
Completed: Lira (Sep 2021) & Dimi (Aug 2023)
Industry partners:

CSIRO COONAWARRA

"This research has provided insights into the chemical and sensory traits underpinning regional typicity and places us in a better position to be able to maintain our wines' unique attributes in the future."

Ockert Le Roux, Coonawarra Vigneron

Project Aims

To define the uniqueness of Australian Cabernet Sauvignon wines by:

- Identifying the sensory attributes that characterise Australian Cabernet Sauvignon wines
- Evaluating the chemical composition that underpins regional typicity
- Correlating the chemical and sensory data to define the most important compounds related to relevant sensory attributes
- Defining how viticultural and/or winemaking practices can effect chemical composition

Publications and conference presentations arising from this project:

- Souza Gonzaga L, Capone DL, Bastian SE, Danner L, Jeffery DW. Using content analysis to characterise the sensory typicity and quality judgements of Australian Cabernet Sauvignon wines. Foods (2019) 8**(12):**691.
- Gonzaga LS, Capone DL, Bastian SE, Danner L, Jeffery DW 2020. Sensory typicity of regional Australian Cabernet Sauvignon wines according to expert evaluations and descriptive analysis. Food Research International **138**:109760.
- Gonzaga LS, Bastian SE, Capone DL, Ranaweera RK, Jeffery DW 2021. Modelling Cabernet-Sauvignon wine sensory traits from spectrofluorometric data. OENO One 55**(4):**19-33.
- Gonzaga LS, Bastian SE, Capone DL, Danner L, Jeffery DW 2022. Consumer perspectives of wine typicity and impact of region information on the sensory perception of Cabernet Sauvignon wines. Food Research International **152**:110719.
- Presentation: *Crush - The Grape and Wine Science Symposium,* Adelaide, September 2018 & July 2021
- Poster displayed at: *17th Australian Wine Industry Technical Conference,* Adelaide, June 2019
- Presentation: *Waite Research Institute AgriFood & Wine Showcase,* Adelaide, November 2019
- Presentation: *Unified Wine and Grape Symposium,* California, 2020
- Presentation: *XIIIth International Terroir Congress*, November 2020
- Presentation: *72nd ASEV National Conference,* June 2021
- Presentation: *Macrowine,* June 2021

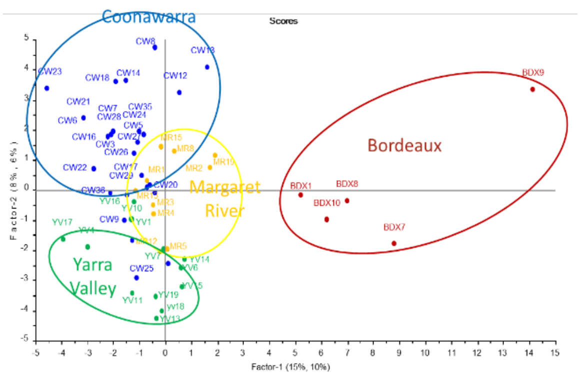
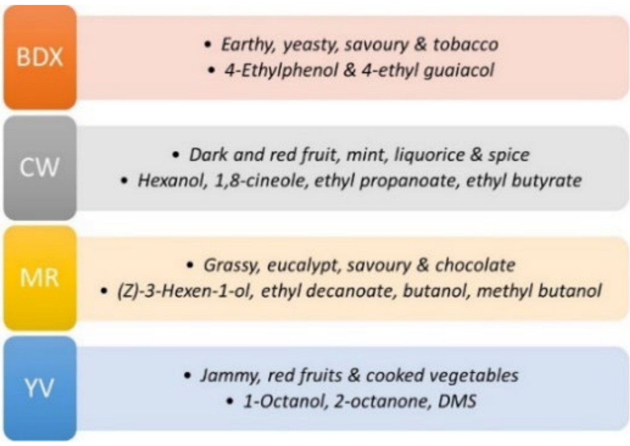


Figure 1. Scores from preliminary correlations of chemical and sensory data using PLSR for 52 Cabernet Sauvignon wines from different regions

Experimentation and Findings

Commercial Cabernet Sauvignon wines from 2015 (n = 86) were selected from Coonawarra (SA), Margaret River (WA), Yarra Valley (VIC), and Bordeaux (France) for comparative purposes. An expert tasting was carried out to profile the wines and a subset (n = 52) underwent comprehensive sensory and chemical analysis. Volatiles selected for chemical analysis spanned across grape, fermentation, oak, and exogenous sources. The combined data underwent statistical analysis using partial least squares regression (PLSR) to examine relationships between the studied regions (Figure 1) and determine which chemical components were able to explain particular sensory attributes.

The following sensory characters and chemical compounds were linked to Bordeaux (BDX), Coonawarra (CW), Margaret River (MR) and Yarra Valley (YV):



Consumer preference

Two wines deemed most representative of each region were selected for a consumer preference study with novice wine consumers (n = 112). Half carried out the tasting with information on sample origin (informed group) whereas the other half tasted blind (uninformed group), to assess their preferences regarding the regional wines.

Interestingly, knowing the region of origin appeared to increase panellists' liking over the uninformed group for both wines from Coonawarra, and one of the wines from each of Bordeaux and Margaret River (Figure 2). The greater liking for the Coonawarra samples in particular was ascribed to the South Australian panellists' familiarity with this region.

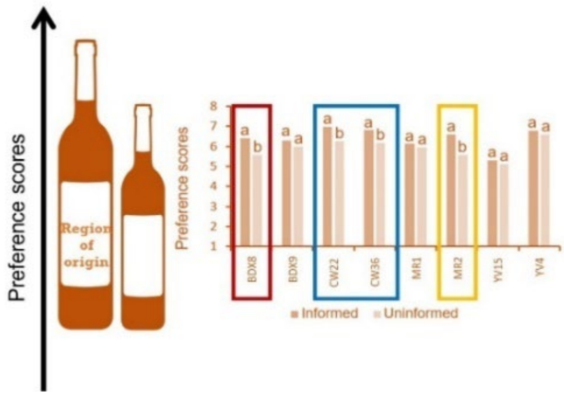


Figure 2. The following sensory characters and chemical compounds were linked to Bordeaux (BDX), Coonawarra

Among a range of questions, the consumers were asked what they considered to be associated with wine 'typicity'. Their responses were assessed in terms of whether they were defined as wine enthusiasts, aspirants, or no-frills consumers. The majority thought that typicity was related to grape variety, country of origin, or regionality. However, a sizeable proportion were unsure what the term meant, thereby revealing the potential need for raising awareness in relation to regionality and uniqueness of wines.

Nonetheless, consumers showed familiarity for certain sensory traits such as minty and herbaceous, but preferred wines that were fuller bodied with fruit-driven characters. They could also differentiate wines from distinct regions.

GC-MS-olfactometry

A selection of representative wines underwent analysis by gas chromatography-mass spectrometry combined with olfactometry (GC-sniff), to determine the potential sensory importance or uniqueness of characteristic compounds for a region. Modified frequencies were calculated and a series of volatiles were identified. Work is ongoing, but as an example, an odorant displaying aromas of coconut, vanilla, and sweet oak with a high modified frequency was identified as cis-oak lactone, which is of relevance to oak obtained from different sources.

Implications for Industry

We have identified various sensory descriptors and compounds being associated with the uniqueness of Cabernet Sauvignon wines from different regions from the 2015 vintage. Consumer studies revealed that knowing the region of origin could influence a consumer's liking of that wine. This can be beneficial for promoting regional wines, with tailor-made marketing campaigns that highlight and educate consumers about regionality and uniqueness.

Beyond consumers, the research has provided information to make informed decisions that can help preserve or enhance regional characters. Identifying the attributes and compounds that define wine origin is one thing but being able to maintain or modulate the desired sensory attributes through viticultural or winemaking practices is the desired outcome. For instance, factors influencing the concentration of aroma compounds from grapes that are attributed to green characters can relate to ripeness and vineyard management approaches rather than fermentation techniques.

Yet other factors being deciphered will relate to compounds arising from different origins (vineyard or winemaking). This information will ultimately provide tools that the industry can use to control grape and wine composition to meet consumer demands.

Acknowledgements

Project team: A/Prof Sue Bastian and A/Prof Cassandra Collins. Partner investigators: A/Prof Paul Boss and Mr Chris Brodie. Partners: Coonawarra Vignerons and CSIRO. Industry contributors and sensory panellists.

MANAGING BERRY HETEROGENEITY

Project leader: A/Professor David Jeffery
Researcher: Claire Armstrong

Commenced: October 2018
Completion: November 2022
Industry Partner:

CSIRO

"Understanding variability trends and evaluating grape heterogeneity levels in the lead up to harvest provides valuable information to winemakers to achieve their desired wine style and quality."

Publications and conference presentations arising from this project:

- Armstrong CE, Ristic R, Boss PK, Pagay V, Jeffery DW. Effect of grape heterogeneity on wine chemical composition and sensory attributes for *Vitis vinifera* cv. Cabernet Sauvignon. *Australian Journal of Grape and Wine Research (2021)* **27**:206-218.
- Presentation: *XIIIth International Terroir Congress*, November 2020
- Presentation: *Macrowine Congress*, June 2021
- Presentation: *Crush - The Grape and Wine Science Symposium,* Adelaide, July 2021
- Presentation: *Pacifichem Congress,* December 2021

Project Aims

The aims of this project were multifaceted and included 1) to determine a method to rapidly measure the extent of grape maturity variability throughout berry maturation in a given vineyard, 2) to address the lack of understanding on the effect of grape heterogeneity on red wine sensory and chemical characteristics, and 3) to investigate the use of conventional vineyard management techniques to control grape heterogeneity by the time of harvest.

Experimentation and Findings

One aspect of the project involved a vineyard trial in Coonawarra with sustained deficit irrigation (approx. 50 % decrease) and crop load manipulation (Figure 1) implemented during the 2019/2020 vintage. Treatments were set up as a 2 × 2 factorial design (Table 1) in triplicate Cabernet Sauvignon blocks within a vineyard.

Irrigation	Crop load	Abbreviation
Deficit	Low	DL
Deficit	Normal	DN
Full	Low	FL
Full	Normal	FN

Table 1. Vineyard management treatments and abbreviations.

Fruit was sampled every 10 days from véraison through to harvest and taken back to the University laboratory to be sorted into maturity classes using 14 salt density baths. The berries of each maturity class were homogenised and analysed for eight parameters: total soluble solids (TSS), average fresh weight (FW), pH, malic and tartaric acids, colour (CIELAB), total tannins, and 3-isobutyl-2-methoxypyrazine (IBMP). These parameters permit an estimate of technological, flavour, and phenolic maturities.

Vines were analysed on each sample date for rate of photosynthesis, leaf water potential, leaf area index, and bunch-zone temperature, to assess the influence of treatments on vine function. At the time of harvest, yield components were measured, and 30 kg of fruit was harvested from each treatment for small-lot winemaking. Rate-all-that-applies sensory analysis was performed on wines 3 months after bottling and comprehensive chemical analyses were completed. The trial was repeated in 2021, with the addition of analysing vines from across the vineyard block to investigate the relationship between spatial variability and grape heterogeneity.

Figure 1. Fruit dropped for low crop load treatment (top) and the Coonawarra vineyard at harvest (bottom).

In the first step of data analysis, the main source of grape maturity variability within a sample date was identified as cluster-to-cluster variability intra vine, which aligned with the literature.[1]

Treatment effects were inconsistent between vintages, sample dates and grape maturity measures, highlighting the complexity and difficulty of managing grape heterogeneity.[1] Interestingly, at the time of harvest, Control treatment (FN) had significantly lower berry fresh weight and pH variability in 2020 and 2021.

A classification system was formulated for four grape heterogeneity levels (Very High, High, Low, Very Low), incorporating the variability of each grape maturity measure (TSS, pH, FW, etc.) into a single index. At the time of harvest, treatments DN, FN, and DL were classified as High heterogeneity in 2020 and Very Low in 2021. Treatment FL was classed as Low heterogeneity in 2020 and High heterogeneity in 2021. To accelerate the process of determining grape heterogeneity, a rapid analytical method was developed to determine TSS, pH, malic and tartaric acids, IBMP, and total tannin simultaneously from grape homogenates using variable-fused absorbance and fluorescence data obtained by spectroscopy with an Aqualog. The proposed workflow for the determination of grape heterogeneity is shown in Figure 2. To accelerate the process of determining grape heterogeneity, a rapid analytical method was developed to determine TSS, pH, malic and tartaric acids, IBMP, and total tannin simultaneously from grape homogenates using variable-fused absorbance and fluorescence data obtained by spectroscopy with an Aqualog. The proposed workflow for the determination of grape heterogeneity is shown in Figure 2.

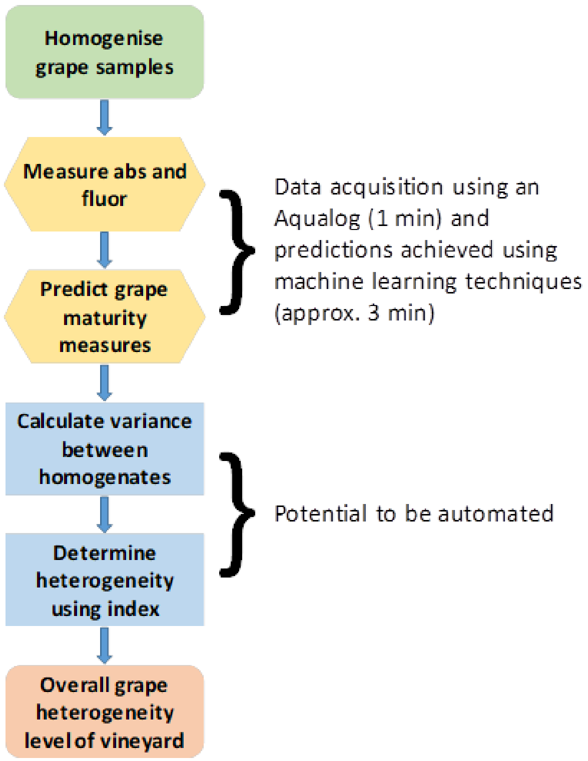


Figure 2: Proposed workflow for rapid determination of grape maturity measures and heterogeneity.

Implications for Industry

Managing grape heterogeneity is challenging due to many abiotic and biotic factors that contribute to heterogeneity that cannot be controlled. It is apparent that cluster-to-cluster variability is a key source of grape maturity variabiltiy. Therefore, vineyard management systems could be applied with the aim of creating uniform micro-climates within vines (e.g., shading, etc.).

With use of the formulated variability index, grape heterogeneity level at the time of harvest provides some explanation of wine sensory traits and differences in wine chemistry. Knowing the grape heterogeneity level prior to winemaking could aid winemakers in their decision making to produce their targeted wine style.

References and Acknowledgements

Calderon-Orellana A, Mercenaro L, Shackel KA, Willits N, Matthews MA 2014. Responses of fruit uniformity to deficit irrigation and cluster thinning in commercial winegrape production. *American Journal of Enology and Viticulture* 65 **(3):**354-62.

We thank Drs Paul Boss, Vinay Pagay, Adam Gilmore, and Rob Bramley and University of Adelaide colleagues and students, especially Xingchen Wang, Pietro Previtali, Ross Sanders and Nick van Holst for their support. We acknowledge the input and advice received from staff at Balnaves of Coonawarra, particularly Pete Balnaves and Pete Bissell, who provided fruit and access to vineyards. Wine Australia is thanked for providing a supplementary PhD scholarship to Claire Armstrong (WA Ph1802).

DEFINING AND EXPLOITING THE INDIGENOUS MICROFLORA OF GRAPES (A)

Project leader: Professor Vladimir Jiranek
Researcher: Dr Krista Sumby

Commenced: October 2018
Completion: November 2022
Industry Partner:

THE UNIVERSITY of ADELAIDE
LALLEMAND
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Original by culture
AWRI
CHALMERS
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"At Chalmers we make wine by spontaneous fermentation so having a comprehensive understanding of the diversity of population of microflora in our winery and vineyards is an amazing tool for understanding spoilage risks or beneficial yeasts or malo bacteria we can encourage and exploit."
Kim Chalmers

Publications and conference presentations arising from this project:

- Sumby K, Caliani N, Jiranek V (2021) Yeast diversity in the vineyard: how it is defined, measured, and influenced by fungicides. *Australian Journal of Grape and Wine Research*, **27**:169-193.
- Presentation: *Crush - The Grape and Wine Science Symposium,* Adelaide, July 2021
- Presentation: *18th Australian Wine Industry Technical Conference*, Adelaide, June 2022

Project Aims

Vineyards contain a community of microbes and several factors can influence the makeup of this community including: grape cultivar, insect activity, berry physiology, species-species interactions, geographical location, climate, soil, terrain and farming and harvesting practices.

Yeast present on harvested grapes, which are often different types of *non-Saccharomyces* yeast, can undertake alcoholic fermentation (un-inoculated fermentations). This method is used in at least 3% of red and 6% of premium white wines in Australia. However, issues of fermentation reliability, due to the *non-Saccharomyces* yeast not being as tolerant to winemaking stresses (e.g., ethanol) and the production of undesirable traits/characteristics are not uncommon.

The key aims of this project are to;

- Identify yeast from different grapes grown in the same soil
- Investigate vine and grape attributes favouring particular yeast species
- Isolate and identify novel winemaking yeast

Experimentation and Findings

This project had the unique resource of a large number of grape varieties all grown in the same soil (Fig. 1A). Loose and tight bunches (Fig. 1B) were sampled for yeast species from nine different varieties.

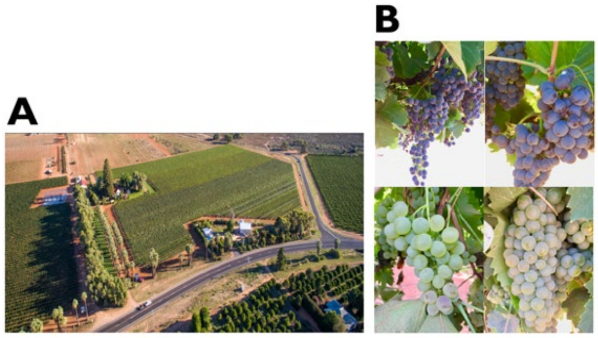


Figure 1: Aerial view of part of the Chalmers Merbein field site in Victoria (A) and different grape architecture of the sampled grapes (B).

Implications for Industry

Yeast present on the grapes were identified using two different genetic methods. Method one involved isolation on selective media and identification using ITS-PCR and sequencing (Fig. 2A). This method is advantageous as isolated yeast can be used for further testing, however, it does not give information about the overall yeast population. Method two, known as diversity profiling, involved DNA extraction direct from the grape samples then ITS-PCR and sequencing of the pooled samples (Fig. 2B). This method enabled a complete overview of all fungal species present and their relative abundance.

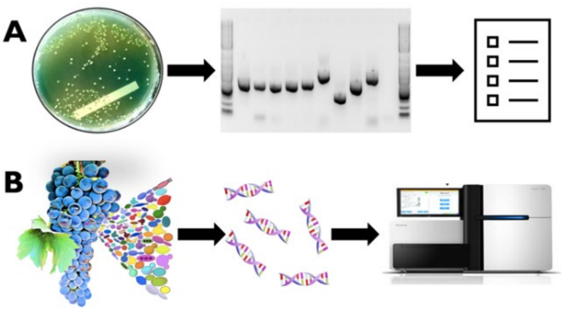


Figure 2. Yeast identification using an isolation and genetic identification method (A) versus a more direct DNA based approach that does not require culturing (isolation) (B).

During vintage 2019 a total of 480 yeast isolates were recovered and identified using Method A (Fig. 2). A wide range of non-*Saccharomyces* and *Saccharomyces* yeast were isolated and identified from the Chalmers vineyard (Fig. 3).

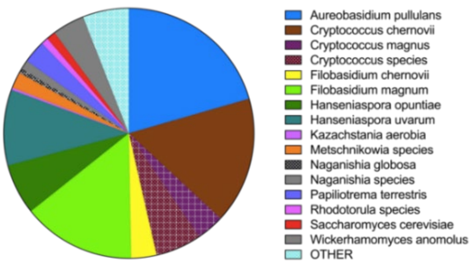


Figure 3. Yeast isolated from the Chalmers Merbein vineyard during vintage 2019.

Twenty of these yeasts were chosen, based on their species identification and isolation source, for downstream experiments to screen for useful winemaking attributes including ethanol and SO_2 tolerance. This was performed using solid media spiked with increasing concentrations of either ethanol or SO_2. Thirteen isolates showed some tolerance and were tested further in a chemically defined grape juice medium prior to being transferred to PhD student, Natalia Caliani, for final evaluation.

Direct diversity profiling using Method B (Fig. 2) showed *Aureobasidium* sp. & *Alternaria* sp. were the most abundant fungal genera present in the vineyard over seasons 2019 and 2020 (Fig. 4). Fungal diversity on grapes was highly variable between vintages. In brief:

- Vintage 2019 had low rainfall (2.8 mm, Feb) and showed higher abundance of *Aureobasidium* sp., which is considered an indicator of good plant health
- Vintage 2020 had higher rainfall (11 mm, Feb) and dust storms. *Alternaria* sp. was the most abundant fungi present with only low numbers of non-*Saccharomyces* species.
- Year to year differences were observed in the same grape variety and in the vineyard
- Non-*Saccharomyces* yeast had low relative abundance
- No correlation was identified between bunch architecture and fungal diversity

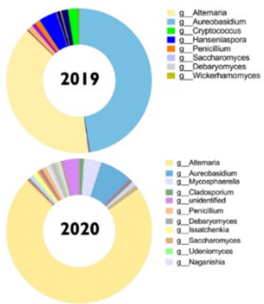


Figure 4: Fungal diversity during vintage 2019 (top) and vintage 2020 (bottom).

Implications for Industry

Seasonal variation was the biggest driver of fungal diversity and will in turn affect uninoculated fermentation reliability. The isolated non-*Saccharomyces* yeast will be tested for their suitability as starter cultures will allow for more reliable diverse fermentations.

Acknowledgements

This research would not be possible without the support of Chalmers Wine, Lallemand Australia and The ARC Training Centre for Innovative Wine Production.

DEFINING AND EXPLOITING THE INDIGENOUS MICROFLORA OF GRAPES (B)

Project leader: Professor Vladimir Jiranek
Researchers: Dr Krista Sumby, Natalia Caliani

Commenced: April 2020
Completing: November 2023
Industry partners:

"Lallemand Australia is excited to be part of Natalia's PhD project – it will help us to develop appropriate strategies and products for non-Saccharomyces + Saccharomyces co-fermentation permutations in Australian winemaking."
Eveline Bartowsky, Lallemand Australia

Conference presentations arising from this project:

- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022
- Presentation: *ISSY 2022*, Canada, July 2022 Broadening your winemaking yeast portfolio: Identification of an SO2 tolerant non-Saccharomyces.
- Presentation: *Australian Society for Microbiology (ASM Hour)*: Food microbiology, July 2022.

Project Aims

The indigenous microbial consortia (yeast and bacteria) of grapes, also referred to as the microbial '*terroir*', is gaining winemaker interest for its potential contribution to wine quality. Before it can be exploited, we first need to define the microbes in this terroir and what their impact might be. It is also essential to further our understanding of their metabolism and how different stressors affect it and strain their performance. Stressors are not only encountered in the vineyard, but the winery also. Two of the most common winery stressors during winemaking are ethanol and sulfur dioxide (SO_2). Ethanol is produced during the fermentation and SO_2 is a common antimicrobial and antioxidant agent added during winemaking. Both can be toxic to yeast at different levels, depending on the species and the strain. As this project focuses on the yeast component of the microbial terroir, its main aims are to:

1. Discover and assess novel non-*Saccharomyces* yeast strains useful for winemaking (e.g., ethanol and SO_2 tolerant, low nitrogen requirements and enhanced desired volatile compound production)

2. Define the impact of fungicides on indigenous yeast

Experimentation and Findings

The first aim of this project was to discover and assess novel non-*Saccharomyces* yeast strains. I am working with a subset of thirteen strains pre-screened by Dr Sumby. These strains were chosen based on their species and because they showed some degree of tolerance to ethanol and SO_2. Their capacity to ferment sugar and withstand the ethanol produced was evaluated in Chemically Defined Grape Juice Medium (CDGJM), using 4 Lallemand commercial non-*Saccharomyces* and *Saccharomyces cerevisiae* (EC1118) as a benchmark. Amongst the tested strains, one *Hanseniaspora* sp. showed the highest sugar consumption (113 g/L), producing 6% v/v of ethanol.

The SO_2 tolerance of the same strains was evaluated using CDGJM spiked with 20, 35, 50 and 65 mg/L of total SO_2 (as potassium metabisulfite). Results showed the same *Hanseniaspora* sp. withstood 65 ppm of total SO_2, similar to the *Saccharomyces cerevisiae* (EC1118) control (Figure 1). Even though it could not finish fermentation, the *Hanseniaspora* sp. consumed around 150 g/L of sugar (Figure 2). We will next assess the performance of these strains in Riesling and Shiraz grape juice.

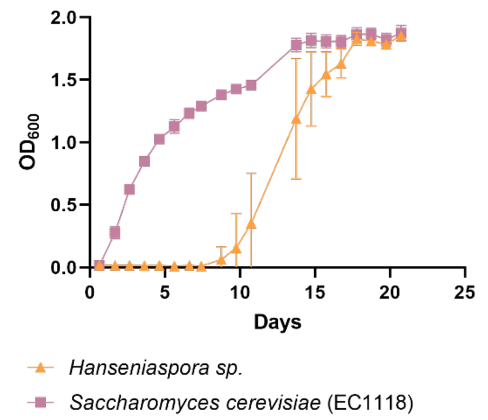


Figure 1. Growth curve of *Hanseniaspora* sp. and *Saccharomyces cerevisiae* (EC1118) in CDGJM spiked with 65 mg/L of total SO_2.

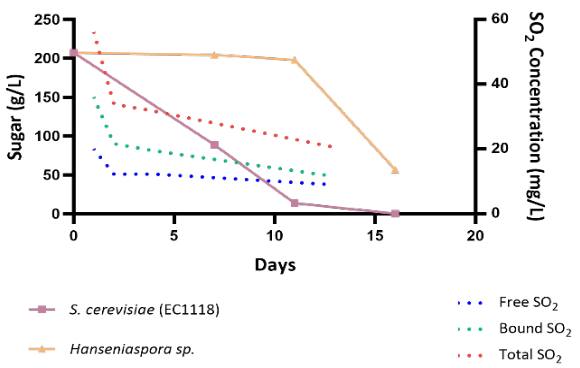


Figure 2. Sugar consumption of *Hanseniaspora* sp. and *Saccharomyces cerevisiae* (EC1118) (left) and free, bound, and total SO_2 losses (mg/L) over time at 21 °C (right).

The second aim was to assess whether different disease management practices (fungicide spray regimes) impact the indigenous yeast communities on grapes. This is particularly relevant in wineries running spontaneous or uninoculated fermentations (i.e., fermentations performed by only the indigenous yeast found on the grapes, without the inoculation of a commercial strain). We are defining yeast communities from a Riesling vineyard (Yalumba – Pewsey Vale; Figure 3) where diseases are managed conventionally or biodynamically, running uninoculated fermentations during two consecutive vintages. Yeast communities are being defined at different time points during these fermentations using genome sequencing. Individual yeast strains were also isolated and are being assessed for their potential use as new non-*Saccharomyces* starter strains.

Implications for Industry

This project aims to identify novel non-*Saccharomyces* yeast that may be useful for winemakers because of their robust fermentative capacity (ethanol and SO_2 tolerance) and as a tool to enhance wine aroma, flavour, and mouthfeel properties due to their secondary metabolite production.

Figure 3. Pewsey Vale Riesling vineyard, biodynamic (front) and conventional (back).

In addition, it will shed light on how indigenous yeast communities evolve in a vineyard where fungal diseases are managed either biodynamically or conventionally.

References and Acknowledgements

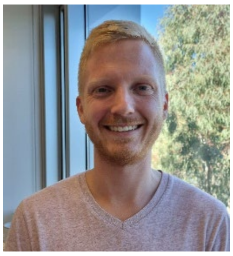
Sumby K, Caliani N, Jiranek V (2021) Yeast diversity in the vineyard: how it is defined, measured, and influenced by fungicides. *Australian Journal of Grape and Wine Research* **27**:169-193

Financial support for this work was provided by The ARC Training Centre for Innovative Wine Production.

HOW DOES ROOTSTOCK CHOICE EFFECT METHOXYPYRAZINE CONCENTRATIONS IN CABERNET SAUVIGNON AND SHIRAZ RACHIS?

Project leader: A/Prof David Jeffery

Researcher: Ross Sanders



Commenced: February 2019

Completing: February 2023

Industry Partners:

CSIRO

TREASURY WINE ESTATES

"Ross was meticulous in his approach to the field trials and willingness to push into an area somewhat out of the subject matter required for his PhD. He was always highly organised when it came to field data collection, interested, and enquiring, and easy to work with."

Dr Catherine Kidman, Wynns Coonawarra Estate

Publications and conference presentations arising from this project:

- Sanders RD, Boss PK, Capone DL, Kidman CM, Bramley RGV, Nicholson EL, Jeffery DW (2022) Rootstock, vine vigour, and light mediate methoxypyrazines in the grape bunch rachis of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* **70**:5417-5426.
- Presentation: *XIIIth International Terroir Congress,* November 2020.
- Presentation: *Crush - The Grape and Wine Science Symposium,* Adelaide, July 2021
- Presentation: *Macrowine Congress,* June 2021.
- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022
- *In Vino Analytica Scientia 2022*, Neustadt an der Weunstraβe, July 2022.

Project Aims

To investigate aspects related to the presence of methoxypyrazines (MPs) in rachis by:

- Determining how rootstock choice affects the concentration of MPs in the rachis of Cabernet Sauvignon (CS) and Shiraz (Sh) grape bunches.
- Understanding how MPs are distributed across the different components of CS and Sh rachis and how this changes throughout the growing season.

Experimentation and Findings

The effect of rootstock on the concentration of MP in rachis was explored in several South Australian Geographical Indications (GIs) across multiple growing seasons by sampling rachis material at harvest and quantifying their MP concentrations with gas chromatography-tandem mass spectrometry (GC-MS/MS) coupled with stable isotope dilution assay (SIDA).

A key research finding is that rootstock significantly impacted the concentration of MPs at harvest, particularly 3-isobutyl-2-methoxypyrazine (IBMP), in the rachis of CS1 grown in Coonawarra and Sh2 grown in Barossa Valley, Mount Lofty Ranges, Murray Darling, and Padthaway.

Changes in IBMP concentration were attributed to differences in rootstock-mediated vigour characteristics as a function of canopy density. For CS, however, any effect of rootstock-mediated vine vigour was outweighed by the spatial distribution of vine vigour across a vineyard, as evidenced with similar patterns in the spatial distribution of vine vigour and IBMP (Figure 1).

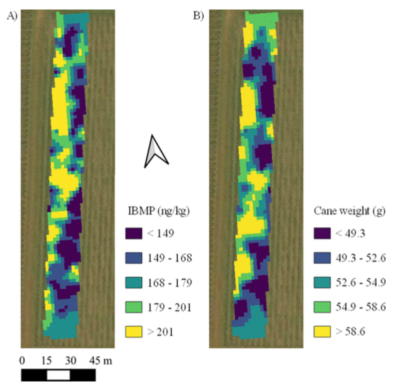


Figure 1: Patterns of spatial variation in A) IBMP concentration (ng/kg) in rachis and B) vine vigour (cane weight in g) across the Coonawarra rootstock trial at harvest in 2020. Reprinted with permission from the authors (Copyright 2022 American Chemical Society).[1]

As MP concentrations in berry are known to significantly increase in the absence of ambient light,[3] it was postulated that the positive relationship between vine vigour and IBMP concentration may be due to increased canopy density changing the rachis light environment. This was explored through the application of light exclusion boxes to CS and Sh bunches throughout grape development, which revealed an increase in the concentrations of MPs for both varieties by up to 10-fold.[1-2]

A segmentation trial of Sh from Barossa Valley and CS from Coonawarra highlighted that the concentration of IBMP was not uniform across rachis components (peduncle, top rachis, bottom rachis, and pedicel), with significantly higher concentrations in the pedicel at harvest.4 Interestingly, the concentration of IBMP decreased in all vine organs throughout ripening, reaching their lowest levels at harvest.

Implications for Industry

Beyond MPs in grape berry, the presence of rachis during fermentation as a component of matter other than grape (MOG) has implications for red wine style and flavour. This research aims to inform producers about the potential effect of rootstock on the concentration of IBMP in rachis at harvest. If total IBMP concentration in Sh and CS bunches (i.e., rachis plus berries) is a key consideration, growers could contemplate planting low vigour rootstocks that moderate the accumulation of IBMP in rachis. Furthermore, as vine vigour can significantly impact IBMP in rachis independent of rootstock, growers could choose to establish management practices that decrease vigour and canopy coverage.

Importantly, the high levels of IBMP in pedicel at harvest and the likely greater presence of pedicel in MOG compared to other rachis components has relevance during winemaking. The results suggest that wineries could better control IBMP in red wine through the implementation of techniques that minimise the amount of rachis components (and especially pedicel) making their way into a fermenter.

References and Acknowledgements

1. Sanders RD, Boss PK, Capone DL, Kidman CM, Bramley RG, Nicholson EL, Jeffery DW (2022). Rootstock, vine vigor, and light mediate methoxypyrazine concentrations in the grape bunch rachis of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* **70**:5417-542626.

2. Sanders RD, Boss PK, Capone DL, Kidman CM, Maffei SM, Jeffery DW. Methoxypyrazine concentrations in the grape bunch rachis of *Vitis vinifera* L. cv Shiraz: Influence of rootstock, region and light. *Food Chemistry - under review 2022.*

3. Dunlevy JD, Soole KL, Perkins MV, Nicholson EL, Maffei SM, Boss PK. Determining the methoxypyrazine biosynthesis variables affected by light exposure and crop level in Cabernet Sauvignon. American journal of enology and viticulture (2013) **64**:450-8.

4. Sanders RD, Boss PK, Capone DL., Kidman CM, Nicholson EL, Jeffery DW. Distribution of 3-isobutyl-2-methoxypyrazine across rachis components of *Vitis vinifera* Shiraz and Cabernet Sauvignon. *Australian Journal of Grape and Wine Research - under review 2023.*

Ross Sanders acknowledges financial support provided by UA-CSIRO iPhD funding in conjunction with Treasury Wine Estates and by Wine Australia for a supplementary PhD scholarship (WA Ph1901).

We would like to thank Dr Paul Boss, Dr Dimitra Capone, Dr Catherine Kidman, Mrs Sue Maffei, and Ms Emily Nicholson for their contributions to this research and the publications arising from it. Wynns Coonawarra Estate, Nuriootpa Research Centre, Treasury Wine Estates, and Vinehealth Australia are acknowledged for providing access to vineyards and grape samples required for this study.

SHAKING UP THE MICROBIOLOGY OF WINEMAKING

Project leader: Professor Vladimir Jiranek
Researcher: Dr Krista Sumby

Commenced: February 2018
Completed: September 2022
Industry partner:

LALLEMAND OENOLOGY
Original by culture

"Krista's project is using directed evolution, a non-GMO approach, to generate more stress-tolerant non-**Saccharomyces** ***and bacteria strains for the wine industry. Lallemand Australia is excited to be part of the Training Centre and collaborate with Krista.***
Her project is directly aligned with addressing fermentation reliability, an on-going challenge for the wine industry with climate unpredictability. The prospect to evaluate a few promising isolates is a great outcome from the project."
Eveline Bartowsky, Lallemand Australia

Conference presentations arising from this project:

- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022

Project Aims

We have been working with industry partner Lallemand to increase the number of commercially available yeast and lactic acid bacteria with improved or novel winemaking properties.

Winemaking involves complex interactions between microbes and the juice that they are fermenting. Primary fermentation requires yeast to carry out alcoholic fermentation, which may be followed by a secondary fermentation where lactic acid bacteria (LAB) undertake malolactic fermentation (MLF). Most winemakers add (inoculate) commercially available *Saccharomyces cerevisiae* yeast to grape must as these have been shown to have good reliability and timely completion of alcoholic fermentation. However, wines made in this way are sometimes regarded as lacking complexity in aroma and flavour.

A further challenge for winemakers occurs during MLF, which is desired during red wine production. MLF is often conducted at the end of vintage as temperatures decrease through autumn and winter. Lower temperatures can cause slower and drawn-out MLF, which can be detrimental to wine quality and/or lead to increased production costs in maintaining optimal temperatures (e.g., energy costs associated with heating).

Experimentation and Findings

With these challenges in mind, Dr Sumby has been working to;

1) adapt a commercial Lallemand non-*Saccharomyces* yeast (*Metschnikowia pulcherrima*) to improve its ethanol tolerance enabling longer survival in alcoholic fermentations whilst producing more of its desirable aromas; and

2) identify new LAB strains with the ability to complete MLF at low temperatures (< 16°C).

3) Increased ethanol tolerance of *Metschnikowia pulcherrima*.

Progress has been positive to date with several ethanol tolerant *M. pulcherrima* isolates identified. Data on these isolates was shared with industry partner Lallemand and a sub-set of 60 isolates were tested for improved fermentation performance compared to the original commercial parent (Fig. 1). The top five isolates, i.e., those that fermented more sugar, will be tested in non-sterile juice fermentations. This will assess how well they will perform in an industrial setting, which features competition with indigenous microbes.

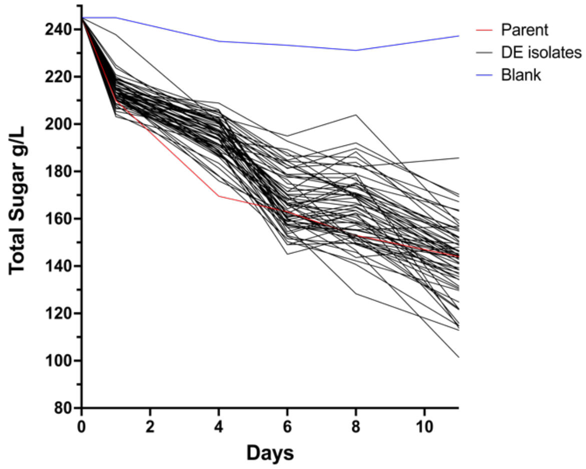


Figure 1. Total sugar consumption of ethanol tolerant *Metschnikowia pulcherrima* isolates and the parent. Results are the average of triplicate fermentations in Riesling juice up to Day 11. Isolates of interest are below the red line (parent = starting *M. pulcherrima* isolate).

4) Cold tolerant lactic acid bacteria

In the search for cold tolerant LAB, isolates sourced from un-inoculated fermentations and directed evolution experiments were inoculated into red wine (Shiraz) and incubated at 16 and 10 °C. MLF speed and ability to complete MLF were measured over a 4-month period.

- At 16 °C the commercial reference strain finished MLF in 12 days, with eleven isolates taking between 10 to 33 days (Fig. 2A)
- At 10 °C the commercial reference strain took >100 days, and while only nine of the isolates completed MLF, the fastest took as little as 26 days or less than 45 days for the top six isolates (Fig. 2B).

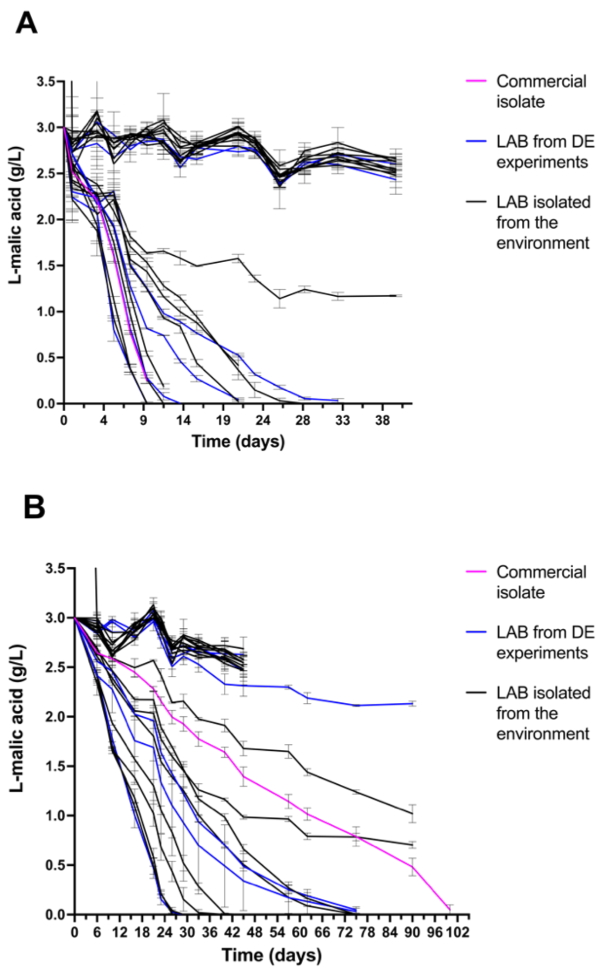


Figure 2. L-malic acid reduction by lactic acid bacteria isolates at two temperatures A) 16 °C and B) 10 °C. Results are the average of triplicate fermentations undertaken in Shiraz wine: 12% ethanol, pH 3.48, 3 g/L L-malic acid. Promising isolates finished MLF at 10 °C before the commercial reference (pink line).

Implications for Industry

This project has identified; ethanol tolerant *Metschnikowia pulcherrima* isolates and six LAB isolates able to complete MLF at 10 °C in a timely manner (< 2 months). PDF Sumby continues to discuss these promising results with representatives from Lallemand and they are working together to assess their suitability for commercialisation so that one day they may be made available to the wine industry.

Acknowledgements

This research would not be possible without the support of Lallemand Australia, including industry partner Dr Eveline Bartowsky, and The ARC Training Centre for Innovative Wine Production.

EFFICIENT RED WINEMAKING BY MONITORING EXTRACTION AND EVOLUTION OF COLOUR AND POLYPHENOLS

Project leader: Dr Richard Muhlack
Researcher: Judith Unterkofler

Commenced: November 2018
Completing: April 2025
Industry partner:

Pernod Ricard Winemakers

"This new process will give more visibility on what's happening in our ferments in real time, and help our winemakers make smarter and quicker decisions about ferment management, from pump overs through to timing of pressing off skins, and help achieve the right tannin and colour profiles for our wines."
Dr Jean Macintyre, Pernod Ricard

Publications and conference presentations arising from this project:

- Unterkofler J, Muhlack RA, Jeffery DW (2020). Processes and purposes of extraction of grape components during winemaking: Current state and perspectives. *Applied microbiology and biotechnology* **104**:4737-4755.
- Presentation: *18th Australian Wine Industry Technical Conference*, Adelaide, June 2019.

Project Aims

Process control and automation have become more and more important in all production industries as they provide an optimisation of plant efficiency, plus increased and consistent product quality. The quality of red wine is highly dependent on its compounds, which among other things, influence the colour, mouthfeel, bitterness and astringency of the wine. Yet there are no automated process controls used in the industry to monitor phenolic extraction, which could guarantee a certain quality, as this extraction is dependent on grape characteristics. However, these are not only different for each batch, but can also change yearly. Therefore, no set standardised production and monitoring procedure can be put in place.

This project intends to further develop dynamic mathematical simulation models for the extraction and reaction of phenolics in red wine ferments. These models will lead to predictive tools that together with real-time data acquisition can be implemented into automatic process control systems.

Figure 1. Vials with different wine solutions are used to test the sensors

Experimentation and Findings

Based on industry fermentation samples taken in 2019, Judith was able to show promising results of an anthocyanin extraction model[1] for Shiraz and Merlot. However, as berry size and skin thickness play an important factor to the extraction additional ferment samples have been taken and will be analysed this year. With this additional data, Judith hopes to further the mathematical model by variety dependant coefficients.

The acquired data will hopefully also help validate a second model predicting tannin extraction during fermentation which was developed in 2021.This mathematical model is based on a small-scale experiment in which catechin was extracted from grape seeds and skins at various temperatures in different model wine solutions.

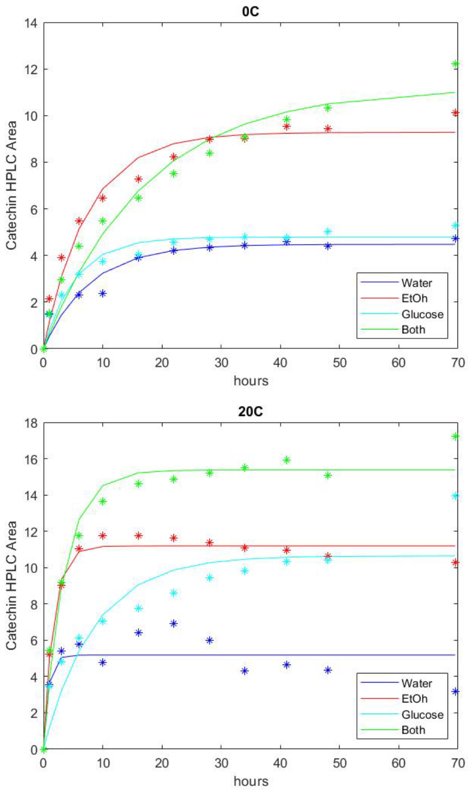


Figure 2. Experimental and fitted models for catechin extraction at 0 °C and 20 °C in different solutions

Since the end of 2021 a range of RGB and spectral sensors are being tested for their applicability as an inline measurement in a winery setting. To do this the sensors are set up with a microcontroller in a box to shield them from external light sources. The aim is to find a suitable sensor that can be set up on sight glass and measure the colour of the ferment during pump overs. [2, 3]

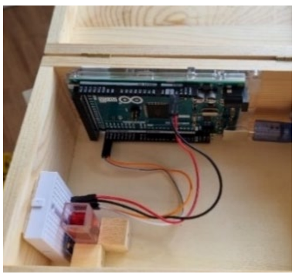


Figure 3. Sensor testing setup, the sensor is connected to a microcontroller to measure the colour of the sample in the vial.

Figure 4. Different RGB and spectral sensors that are being tested for their applicability

Implications for Industry

Extraction models will lead to predictive tools that together with real-time data acquisition can be implemented into automatic process control systems. These will regulate temperature and cap management to optimize extraction to increase the efficiency in red wine production and its plant availability, and optimize logistics in terms of plant use, thus decreasing production costs. Additionally, the process control system can improve wine quality, consistency of colour and tannin profile as well as improved documentation for quality control and management.

References and Acknowledgements

1. Setford PC, Jeffery DW, Grbin PR, Muhlack RA (2018). Modelling the mass transfer process of malvidin-3-glucoside during simulated extraction from fresh grape solids under wine-like conditions. *Molecules* **23(9):**2159.

2. Terrades S, Wagner S, Ros-Lis JV, Ibáñez J, Andrés A (2019). Optical system for automatic color monitoring in heterogeneous media during vinification processes. *Sensors and Actuators B: Chemical* **285**:513-518

3. Shrake NL, Amirtharajah R, Brenneman C, Boulton R, Knoesen A (2014). In-line measurement of color and total phenolics during red wine fermentations using a light-emitting diode sensor. *American Journal of Enology and Viticulture* **65(4):**463‐470.

I'd like to thank my supervisors, industry partner and the Training Centre, especially Nick van Holst Pelekaan, for their help and support throughout this project.

MATHEMATICAL MODELLING OF RED WINE COLOUR AND POLYPHENOL EXTRACTION AND EVOLUTION TO ENHANCE WINEMAKING EFFICIENCY

Project leaders: Dr Richard Muhlack & A/Professor David Jeffery
Researcher: Rachael Tindal

Commenced: August 2019
Completing: May 2025
Industry partner:

Pernod Ricard Winemakers

"Having a more accurate method to predict colour extraction and stability will be a valuable tool to guide winemaking decisions, both through fermentation and blending."
Dr Jean Macintyre, Pernod Ricard

Publications and conference presentations arising from this project:

- Tindal RA, Jeffery DW, Muhlack RA (2021). Mathematical modelling to enhance winemaking efficiency: A review of red wine colour and polyphenol extraction and evolution. *Australian Journal of Grape and Wine Research* **27**:219-233.
- Presentation: *Crush - The Grape and Wine Science Symposium,* Adelaide, July 2021
- Presentation: *(SIAM) Society for Industrial and Applied Mathematics Conference* on the Life Sciences, September 2022

Project Aims

- Development of a kinetic mathematical model to describe the behaviours of monomeric and self-associated anthocyanin species within physical anthocyanin-containing systems
- Development of a Fourier spectral deconvolution model to provide greater sensitivity for anthocyanin spectral data than is available experimentally
- Development of a fluid dynamics model to describe the spatial variability of anthocyanin species throughout tanks during red wine fermentation
- Experimental validation under controlled laboratory conditions for each developed mathematical model

Experimentation and Findings

Our project investigated the behaviour of pigmented phenolic compounds called anthocyanins that contribute red, purple and blue colours to flowers, fruit, and young red wines. A kinetic mathematical model was developed to describe the evolution in concentration of monomeric (chemically unstable) and self-associated (temporally stable) anthocyanin species over time, and model results were validated experimentally using HPLC analysis. Results indicated that pigmented anthocyanin monomers decrease in concentration over time, while the colourless monomeric species (i.e., the hemiketal) increases in concentration. As such, a physical system containing solely anthocyanin monomers would experience colour loss over time. Results further indicated that self-associated species exhibit nonlinear kinetics such that the concentrations of all self-associated species evolve to be greater than the concentrations of the monomeric species, including the colourless hemiketal. Self-association was therefore shown to increase colour enhancement and stability within anthocyanin-containing systems. Model simulations were then run with conditions corresponding to the vacuolar conditions of anthocyanin-containing plant organs (namely, *Vitis vinifera* grape berries and *Geranium sylvaticum* flowers). Modelled results indicated that anthocyanin self-association largely contributes to colour expression and stability within these real-world systems. Furthermore, the simulated anthocyanin species concentrations corresponded with the colour profiles observed within these plant organs.

Figure 1. Our modelling describes important chemical processes that generate red, purple and blue colours in anthocyanin-containing plant organs such as grape berries (left) and geranium flowers (right).

Our projected also developed a Fourier spectral deconvolution model to enhance the sensitivity of anthocyanin spectral data available within laboratory settings. The model transforms experimentally generated spectra from HPLC-DAD analysis that only represents one monomeric anthocyanin species (i.e., the red flavylium cation), into a comprehensive system of data that characterises the spectral behaviour of all existing monomeric and self-associated anthocyanin species. This model is currently being applied to the analysis of experimentally generated anthocyanin spectra for a collection of young red wine samples provided by Pernod Ricard winemakers. Thus far, results indicate that the red flavylium cation, purple quinonoidal base, and dark red self-associated flavylium cation species are significant contributors for young red wine colour profiles and degrees of colour stability.

In the future, modelling work will also be conducted with the aim of describing the more long-term effects of the anthocyanin chemical system on wine quality characteristics such as colour and shelf life.

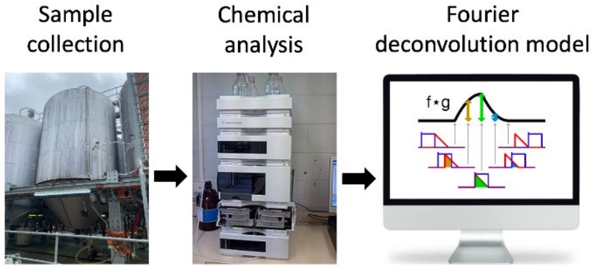


Figure 2. A schematic illustrating the steps of our deconvolution process, where red wine samples are collected during fermentation (left), samples are chemically analysed using HPLC-DAD methods (middle), and resulting chemical data are transformed computationally using our Fourier deconvolution model (right).

Implications for Industry

By providing predictive information about anthocyanin behaviour with respect to solution parameters, our project aims to increase the control and commercialisation of anthocyanin-based products. In particular, the ability to reduce anthocyanin colour loss may lead to the increased use of anthocyanins as pH-indicators in food packaging systems and as natural products such as food colourants and clothing dyes. For the wine industry, our predictive modelling aims to provide quantitative information about the effects of winemaking parameters (e.g., wine pH, anthocyanin concentration, and maceration time) on overall wine quality. Our project is currently investigating the behaviour of this system in the presence of other winemaking factors such as ethanol, oxygen and heat, which could allow for winemaking processes to be controlled with greater accuracy.

The modelling described above is incorporated with our Fourier modelling work, with the goal that this information will then be more accessible to winemakers and scientists in laboratory and winery settings. Having predictive information about red wine colour profiles and shelf-lives may provide winemakers with a quantitative guideline for what interventions could be taken throughout the winemaking process to generate wines with specific desired conditions. Such model-informed winemaking may therefore lead to the optimisation of time and physical materials within wineries, while providing winemakers with the ability to more easily control the quality of their finished red wines.

MEMBRANE ULTRAFILTRATION TECHNOLOGIES FOR EFFICIENT WINE PROCESSING AND RECOVERY OF VALUABLE EXTRACTS (A)

Project leader: Prof. Kerry Wilkinson
Researcher: Yihe (Eva) Sui

Commenced: April 2018
Completed: May 2022
Industry partner:

VAF MEMSTAR

"Ultrafiltration: a novel approach to managing phenolics and protein in white wine."

Publications and conference presentations arising from this project:

- Sui Y, McRae JM, Wollan D, Muhlack RA, Godden P, Wilkinson KL (2021). Use of ultrafiltration and proteolytic enzymes as alternative approaches for protein stabilisation of white wine. *Australian Journal of Grape and Wine Research* **27**:234-245.
- Sui Y, Wollan D, McRae J, Muhlack R, Tuke J, Wilkinson K (2022). Impact of commercial scale ultrafiltration on the composition of white and rosé wine. *Separation and Purification Technology* **284**:120227.
- Presentation: *Macrowine Congress*, June 2021.
- Presentation: *18th Australian Wine Industry Technical Conference,* Adelaide, June 2022

Project Aims

This project aims to evaluate the use of membrane ultrafiltration (UF) in combination with selective treatment of the retentate fraction as an alternative to bentonite fining to achieve protein stability in white wine. Specifically, the ability of heat and/or protease treatment to reduce protein concentrations will be assess as well as the impact of retentate heating on sensory and chemical profiles as heating is necessary to facilitate protease protein degradation. Heating wine is not a generally accepted practice by winemakers due to concerns over the loss of fresh fruit characters. However, UF can potentially overcome this issue by concentrating wine proteins in a smaller fraction followed by selective heat and protease treatment instead of heating the whole wine. It is critical to learn the efficacy and selectivity of protein removal in the overall UF and heat/protease treatment to evaluate to what extent the treatment modifies wine chemical and sensory profiles compared with traditional bentonite fining. The potential for UF to be applied in commercial scale wine treatment will also be investigated to assess the practical use of UF as a bentonite alternative.

This project seeks to provide alternative options for winemakers to stabilise wine during white wine production and to equip winemakers with understanding and confidence to adopt UF technology that can potentially improve wine quality and longevity.

Key aims:

- To evaluate the use of UF technology to facilitate protein stability in wine as an alternative method to traditional bentonite fining
- To optimise heat and protease treatment conditions to remove haze-forming proteins in retentate obtained after UF of wine
- To assess the chemical and sensory consequences of the application of UF combined with heat and/or protease treatment
- To validate the application of UF on commercial wine treatment.

Experimentation and Findings

Pilot scale UF treatments were performed on two wine samples using a 10 kDa nominal molecular weight cut-off membrane, and gave protein stable permeate and protein concentrated retentate. Targeted protein removal from retentate following UF enrichment using heat or heat and protease treatment was subsequently optimised. Heating retentate at 62 °C for 10 min (with or without protease addition) achieved significant protein removal (30 – 96%, depending on the initial wine protein composition). Semi-commercial scale trials were therefore conducted on a third wine to confirm the performance of UF and heat and/or protease treatments. Recombination (blending) of treated retentate with permeate delivered wine that was almost heat stable, such that significantly less bentonite addition (~50 – 60%) was required to achieve complete heat stabilisation of the wine.

The impact of the combined UF/heat/protease treatments on the heat stability, volatile composition, sensory profiles and quality of white wine formed the basis of a second study. Treatment effects on wine composition were determined by analysis of varietal, fermentation-derived and oxidative volatiles using gas chromatography-mass spectrometry, while sensory profiles were determined using the Rate-All-That-Apply analysis and wine quality scores by an expert panel. Wine treated by a combination of UF/heat/protease, with or without bentonite addition, was compared against traditionally bentonite fined wine. Heating retentate (with and without protease) removed significant quantities of haze-forming proteins, thereby improving the heat stability of recombined wine. As a consequence of protein removal, the bentonite required to fully stabilise recombined wine was also substantially lower. The results from volatile and sensory analyses suggested that the combined UF/heat/protease treatments retained wine aroma and flavour without imparting any oxidative characters to the wine, compared with bentonite fining.

The application of UF treatment to remove excessive phenolics from wine was performed at commercial scale. Dynamic filtration parameters (i.e. filtration flux and transmembrane pressure) were monitored during treatment to illustrate membrane fouling issues. Physico-chemical analyses of samples before and after commercial scale UF were studied to determine the compositional consequences of treatment, especially changes in macromolecule composition. UF gave clarified and stable permeate, while proteins and phenolics were concentrated in the retentate. Both the initial wine matrix and the membrane batch contributed to variation in UF performance. Overall, the results generated from this study contribute to a better understanding of the impacts of UF treatment of wine.

Implications for Industry

Results from this project provide evidence for the efficacy of combined UF/heat/protease treatments removing haze-forming proteins and improving wine protein stability without sacrificing wine quality. The significantly lower phenolic concentration achieved in permeate generated from UF are likely to enable winemakers to achieve a targeted wine style, thereby eliminating proteinaceous fining product to modulate wine organoleptic qualities. UF achieved protein stabilisation for all permeate therefore eliminating the need for bentonite fining. The permeate volume (> 90% of wine volume) was comparable to the volumes typically recovered after the removal of lees from fining processes. UF could potentially improve production volumes and profitability since UF generated clarified, protein-stable permeate and potentially eliminate the need for subsequent fining processes. Therefore UF offers an innovative approach to management of phenolics and protein in white wine.

Acknowledgements

Dr Jacqui McRae and Dr Richard Muhlack as co-supervisors and Peter Godden as independent advisor. David Wollan, Matthew Hooper and Steven Clarkson from VAF Memstar for technical support and access to UF equipment and expertise. WIC Winemaking Services for cellar assistance. Pernod Ricard Winemakers for providing wine samples.

MEMBRANE ULTRAFILTRATION TECHNOLOGY FOR EFFICIENT WINE PROCESSING AND RECOVERY OF VALUABLE EXTRACTS (B)

Project leader: Prof. Kerry Wilkinson
Researcher: Stephanie Angela

Commenced: March 2020
Completing: March 2023
Industry partner:

VAF MEMSTAR

"This project aims to understand and validate the ultrafiltration process, with a special emphasis on quantifying the chemical and sensory effects."
David Wollan, VAF Memstar

Publications and conference presentations arising from this project:

- Presentation: *18th Australian Wine Industry Technical Conference*, Adelaide, June 2022

Project Aims

Phenolic management is an important aspect of winemaking, affecting wine chemical and sensory properties. Ultrafiltration (Fig. 1) can fractionate wine using semipermeable membranes and this technology offers an innovative approach for fine-tuning wine composition, to enhance quality. This study established the compositional consequences of UF treatment of red and white wines, to identify novel winemaking applications, including phenolic management.

The specific project aims were:

- Investigation of the compositional consequences of ultrafiltration of red and white wines
- Evaluation of the suitability of wine concentrate / retentate as an additive to enhance the chemical and sensory quality of various low alcohol wines.
- Investigation of the suitability of white wine concentrate / retentate to stabilize colour and enhance mouthfeel properties in winemaking (red ferments).
- Investigation of membrane fouling mechanisms at the lab scale and what drives them.

Evaluation and Findings

Red and white wines were fractionated by UF using membranes, with different molecular weight cut-off specifications (10 and 20 kDa) and different degree of permeation (50 and 95%). The result acknowledges that interactions between membrane surface - organic feed and physiochemical reactions between solutes contributes to concentration polarisation that leads to fouling. The chemical analysis result (Fig 2) of wine, permeate and retentate demonstrated some parameters (e.g. pH and viscosity) were not affected by UF, whereas organic acids, anthocyanins (for UF of red wine), phenolic compounds, polysaccharides, and proteins (for UF of white wine), were progressively concentrated in the retentate as the degree of permeation increased.

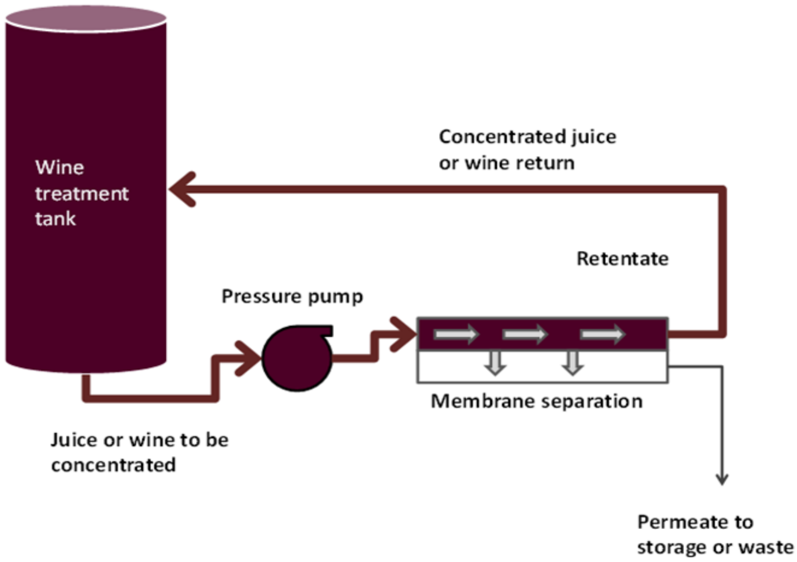


Figure 1. Systematic of ultrafiltration system for winemaking.

Implications for Industry

UF treatment of white wines with excessive phenolics (including heavy pressings) could recover commercially-acceptable wine (permeate) for consumption or blending, thereby increasing product yield / profitability.

The addition of phenolic-rich retentate to commodity wine and/or no/low alcohol wine could enhance mouthfeel properties, which are often considered to be lacking in these wine styles.

A better understanding on the membrane fouling mechanisms for wine, including the behaviour or phenolic compounds and macromolecules around membrane filtration leads to a better membrane filtration decision making.

References and Acknowledgements

The research project was conducted by the Australian Research Council Training Centre for Innovative Wine Production (project number IC17100008), financially supported by the Australian Government and industry partners. The authors would like to thank Matthew Hooper, David Wollan, and Katheryn Cordova from VAF Memstar (Nuriootpa, South Australia) for the filtration equipment and wine samples provided, WIC Winemaking Service (Urrbrae, South Australia) for wine logistics support, and AWRI (Australia Wine Research Institute, South Australia) for their technical support.

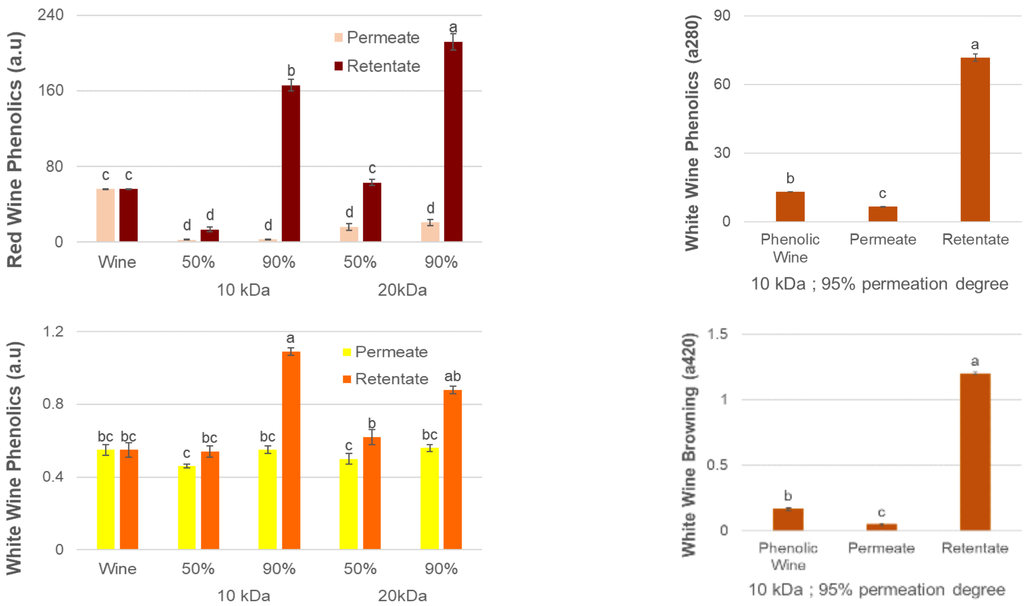


Figure 2. (top to bottom, left to right): Compositional changes following UF fractionation of (a) red wine, (b) white wine, and (c,d) a highly phenolic / oxidised white wine.

TELLING OUR STORIES

We communicate our research to academic and scientific audiences, the wine industry, our industry partners, funding bodies and research peers.

- We are eager to use every opportunity to talk about our exciting research to various audiences
- We travel to different wine regions and participate in industry seminars
- We conduct scientific and conference workshops twice per year
- We use our website to inform peers and the public about new publications and Training Centre activities

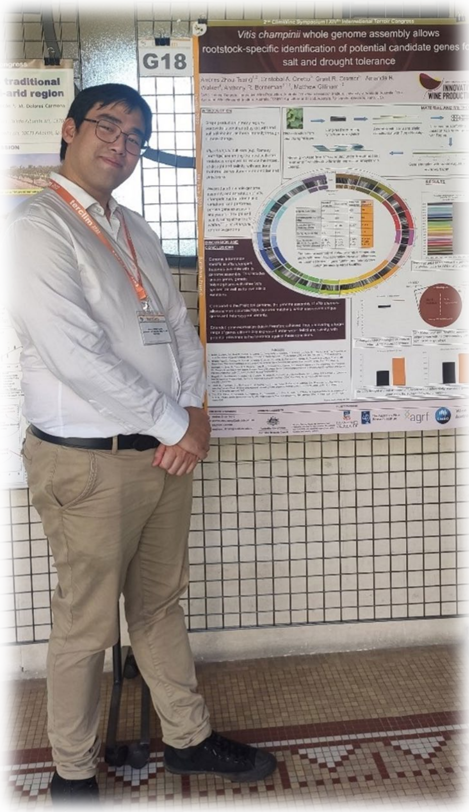
https://www.arcwinecentre.org.au/

CONFERENCES AND INDUSTRY TALKS

Our Training Centre Students actively participate in conferences world-wide, and industry talks to engage with the industry and showcase our research.

Below are a few of our 2020 – 2022 highlights:

- *Pacifichem 2021* – **Colleen Szeto** presented to the *Advances in Wine and Beer Chemistry Symposium* within the *Chemistry for Sustainability* subject area
- *Crush 2021* – Most TC students presented and participated. **Dimi** was a chair and Crush conference organiser & **Ross** won 'Best Innovation in Science' award
- **Dimi, Pietro, Eva Sui, Claire & Ross** online presentations for *MacroWine 2021*
- **Pietro, Zeyu, Joanah** presented virtually at XIth International Symposium of Grapevine Physiology and Biotechnology 2021
- **Ross Sanders** presented his poster at *Vino Analytica Scientia, Germany,* 2022
- **Rachael Tindal** presented at *Nonlinearity and Anthocyanin Colour: A Mathematical Analysis of Anthocyanin Association Kinetics and Equilibria* 2021
- Training Centre Director, **Vladimir Jiranek**, was a Session Chair and Invited Speaker at *the 36th International Specialised Symposium on Yeast* in Vancouver (ISSY 2022)
- **Andres Zhou Tsang** presented his poster at *Terclim* Bordeaux – July 2022 and won the 'Young Scientist Award'
- **Joanah Midzi** was chosen to present at the *Combio* conference at the Convention Centre in Melbourne – Sept 2022




18th AUSTRALIAN WINE INDUSTRY TECHNICAL CONFERENCE & TRADE EXHIBITION

The 18th Australian Wine Industry Technical Conference & Trade Exhibition was held at the Adelaide Convention Centre in June 2022. It is the premier technical event for the Australian wine industry. Held every three years since 1970, it combines an extensive program of plenary sessions, workshops, posters, student forum and social events with the industry's most respected and extensive trade exhibition.

Our Training Centre students presented many posters during this event, and we had nominated speakers representing the TC IWP, including:

- **Leigh Schmidtke (Chair)**
- **Ross Sanders (Fresh Science)**
- **Natalia Caliani (In the Wine Light)**
- **Colleen Szeto (In the Wine Light)**
- **Joanah Midzi (In the Wine Light)**

The Student Forum: *In the Wine Light* is an entertaining introduction to the future people and projects in grape and wine research; from the poster abstracts received, students were selected to present one slide each to answer the age-old questions:

- "What are you researching?"
- "Why does it matter?"

The 'Wine Light' event is a highlight at the conference for many!

The ARC Training Centre of Innovative Wine Production would like to thank the organising committee of AWITC and any event sponsors for another successful conference. We were delighted to have a high presence at this event, highlight our TC student's innovative research and the opportunity to connect face-to-face with the wine industry.

TRAINING CENTRE WORKSHOPS

Our Training Centre hosts up to two multiday workshops each year for students, industry partners, chief investigators, and the advisory committee to all come together to share progress on our research projects.

Whilst Covid-19 hindered our plans to host all of these in person, we were still fortunate enough to be able to successfully host a combination of Zoom events and in-person events throughout South Australia; one being in the Coonawarra Wine Region in 2021.

Students **Yanina Giordano** and **Natalia Caliani** share part of their experience of the TC Workshop in Coonawarra below:

"In the picturesque charm of Wynns Coonawarra Estate, Centre researchers presented their latest research to industry partners, Coonawarra Vignerons and Winemakers. It is essential to highlight that three Centre projects are primarily focused on the Coonawarra wine region.

1. ***Typicity of Coonawarra Cabernet***
2. ***Is it bad being different, understanding grape maturity in the Coonawarra***
3. ***Rootstocks, vine vigour and sunlight change the amount of methoxypyrazines in Cabernet Sauvignon rachis***

The Coonawarra visit was an excellent opportunity to see a uniquely beautiful Australian wine region and meet its leading players. This interaction between research and industry was a fruitful experience. We received constructive feedback on our projects and discussed potential collaboration strategies. We also discovered a distinctive 'terroir' that produces full-bodied and, at the same time, delicate Cabernet Sauvignon and Shiraz wines.

As a result of the Coonawarra immersion workshop, we could exploit the Centre's connections, expanding our own networks. We could also enjoy a unique opportunity for experiential, accelerated and deeper learning. Finally, this experience was a reciprocal exchange for the Centre students to see where their research projects will positively contribute to the wine industry globally.

ARC TC IWP would like to thank the following for their generous hospitality: Coonawarra Vignerons, Wynns Coonawarra Estate, Dr Catherine Kidman, Balnaves of Coonawarra and Bellwether Wines."

WHERE ARE THEY NOW?

As some of our TC projects start to wrap up, our students have started to submit their theses, started to graduate, and/or continued to work for our Industry Partners. Congratulations and thank you to our finished students, whom we wish all the best in their future endeavours!

Eva Wang

Thesis Submission Date:
2 August 2022
Graduated:
April 2023
Current role:
Postdoctoral Researcher at UoA with A/Prof Cassandra Collins

Lira Gonzaga

Thesis Submission Date:
14 May 2021
Graduated:
April 2022
Current role:
Lecturer for the Food Science and Nutrition Bachelor's Degree at UoA

Eva Sui

Thesis Submission Date:
28 October 2021
Graduated:
May 2022
Current role:
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Pietro Previtali

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Colleen Szeto

Thesis Submission Date:
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Graduated:
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CENTRE PUBLICATIONS

YEAR 2022

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In the Media

- 2021 South Australian Country Hour, Mon 5 July 2021 ABC South Australia Country Hour (beginning at 15.45 mins)
- 2020 Test to end wine fraud - 2GB radio interview
- 2020 Rural News Country Breakfast - ABC Radio National, radio interview (beginning at 3.20 mins)
- 2020 SA researchers put spotlight on wine fraud Stock Journal
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Industry articles

- 2022 Ultrafiltration: a novel approach to managing phenolics in white wine. Y Sui, J McRae, R Muhlack, D Wollan, K Wilkinson - Wine and Viticulture Journal
- 2022 Does delaying the rate of ripening alter aroma compounds? P Previtali, N Dokoozlian, B Pan, K Wilkinson, C Ford - Wine and Viticulture Journal
- 2021 A review of the techniques for mitigating the effects of smoke taint in wine production YA Mirabelli-Montan, M Marangon, A Graça, CM, Mayr Marangon, KL Wilkinson International Viticulture & Enology Society (IVES) Technical Reviews, Vine and Wine
- 2021 Slow the ripening for a better outcome - Wine Australia
- 2020 Less K = less acid = less money - Wine Australia

Book Chapters

- 2020 Authenticity and traceability in the wine industry: From analytical chemistry to consumer perceptions. In Reference Module in Food Science (pp. 1 – 29) RKR Ranaweera, L Souza Gonzaga, DL Capone, SEP Bastian, DW Jeffery - Publisher: Elsevier

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