Conference Proceedings

17th Australian Wine Industry Technical Conference
Adelaide, South Australia
21–24 July 2019

Edited by:
Kate Beames
Ella Robinson
Peter Dry
Dan Johnson

AUSTRALIAN Grape & Wine
OUTLOOK CONFERENCE

Click here to view full proceedings document >

The Australian Wine Industry Technical Conference Inc. Hartley Grove cnr Paratoo Road, Urrbrae, South Australia 5064, Australia

Introduction

Australian Wine Industry Technical Conference

The Australian Wine Industry Technical Conference is held every three years and is the premier technical conference for the Australian wine industry.

The first conference was held in 1970 in Mildura, Victoria. The conference structure and content are continually evolving to match the changing priorities of the Australian grape and wine sector. Feedback from delegates is gathered and assessed to improve subsequent conferences.

The 17th conference, held in July 2019 in Adelaide, South Australia, attracted more than 3,000 attendees across AWITC and the WineTech trade exhibition. The program included the Australian Grape & Wine Outlook Conference, which brought the latest business and technical content together in one forum. Key topics explored included: supply and demand outlook; the importance of diversity to continuing success; the wine sensory experience; AgTech – what's happening right now; protecting and building better vineyards; doing business in a changing climate; and opportunities from winemaking technology. A total of 11 formal sessions were presented over three days, with 9 international and 41 local speakers. The main program was complemented by 33 workshops, a display of over 160 technical posters and an extensive trade exhibition.

The Australian Wine Industry Technical Conference Inc. (AWITC) and the editors of this publication accept no responsibility or liability of any kind for any statement, opinion or other material contained in this publication. Articles published do not necessarily represent the opinion of the AWITC: articles and other comments represent the opinions of their respective authors and might contain mistakes of fact, hypotheses and other unsubstantiated material. Notwithstanding the mention of any products or services in this publication, the AWITC gives no warranty or endorsement in respect to them. The papers presented here have not been peer reviewed and represent the views of the authors as presented at the 17th conference.

The editors would like to thank Dr Markus Herderich, Anne Lord, Shiralee Dodd and Peter Godden for their assistance in the preparation of these proceedings.

ISBN 978-0-6485648-0-5
ISSN 0811-0743

Conference committees & staff

AWITC Inc. Executive Committee Members
Dr Dan Johnson (Chair)
The Australian Wine Research Institute

Kristy Bartrop
Casella Family Brands

Chris Day
The Australian Wine Research Institute

Dr Paul Grbin
The University of Adelaide

Dr Markus Herderich
The Australian Wine Research Institute

Dr Tony Robinson
Oakover Wines

Conference Planning Committee Members
Dr Dan Johnson
The Australian Wine Research Institute

Jason Amos
Lallemand Australia

Kristy Bartrop
Casella Family Brands

Tony Battaglene
Australian Grape & Wine Incorporated

Kate Beames
Australian Wine Industry Technical Conference

Colin Bell
AHA Viticulture

Wendy Cameron MW
The University of Melbourne

Chris Day
The Australian Wine Research Institute

Gary Fitz-Roy
Fair Events

Dr Paul Grbin
The University of Adelaide

Michael Hayes
Sirromet Wines

Dr Markus Herderich
The Australian Wine Research Institute

Ashley Keegan
Food and Beverage Australia Limited

Dr Fiona Kerslake
Tasmanian Institute of Agriculture

Dr Mark Krstic
The Australian Wine Research Institute

Dr Mardi Longbottom
The Australian Wine Research Institute

Matthew Moate
Wine Industry Suppliers Australia

Assoc. Prof. Paul Petrie
South Australian Research and Development Institute

Liz Riley
Vitibit Pty Ltd

Ella Robinson
The Australian Wine Research Institute

Dr Tony Robinson
Oakover Wines

Con Simos
The Australian Wine Research Institute

Andrew Weeks
Rivawine Collaboration Pty Ltd (until 5 March 2018)

Conference Manager
Kate Beames

Conference Secretariat
Sarah Ballantine

Media and Communications
Ella Robinson
The Australian Wine Research Institute

Poster Coordinators
Dr Mardi Longbottom
The Australian Wine Research Institute

Dr Paul Grbin
The University of Adelaide

Workshop Coordinators
Con Simos, Francesca Blefari
The Australian Wine Research Institute

Trade Exhibition Management
Fair Events

Program Sub-committee Members
Dr Dan Johnson (Chair)
The Australian Wine Research Institute

Dr Mark Krstic
The Australian Wine Research Institute

Tony Battaglene
Australian Grape & Wine Incorporated

Jason Amos
Lallemand Australia

Wendy Cameron MW
The University of Melbourne

Dr Markus Herderich
The Australian Wine Research Institute

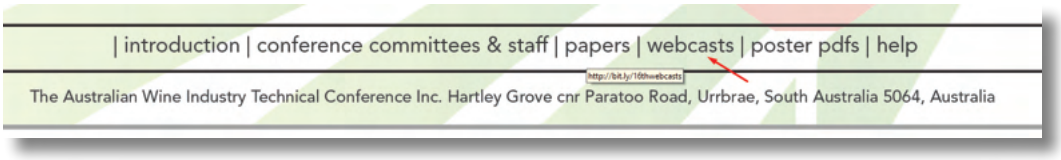
Assoc. Prof. Paul Petrie
South Australian Research and Development Institute

Dr Tony Robinson
Oakover Wines

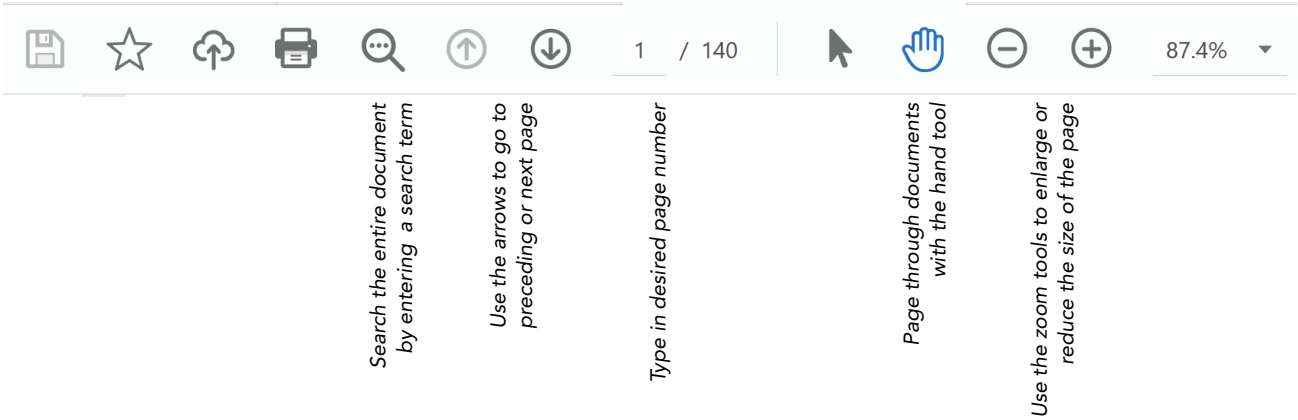
Help

To navigate through this document you may use the following methods:

Click on the buttons on the main navigation bar on the bottom of the introductory pages.



To navigate while reading the various papers use the Acrobat toolbar:



For quick access to papers, use the bookmark bar.

Proceedings of the 17th Australian Wine Industry Technical Conference

Adelaide, South Australia
21–24 July 2019

edited by

K.S. Beames, E.M.C. Robinson, P.R. Dry and D.L. Johnson

The Australian Wine Industry Technical Conference Inc.
Hartley Grove cnr Paratoo Road,
Urrbrae, South Australia 5064
Australia

ISBN 978-0-6485648-0-5

ISSN 0811-0743

The papers presented here have not been peer reviewed and represent the views of the authors as presented at the 17th conference.

The editors would like to thank Dr Markus Herderich, Anne Lord, Shiralee Dodd and Peter Godden for their assistance in the preparation of these proceedings.

Produced by Michael Major Media

Webcasts of the AWITC presentations are available at https://awitc.com.au/webcast/

Contents

SESSION 6: Protecting and building better vineyards

SESSION 7: The wine sensory experience

SESSION 8: AgTech: the here and now

SESSION 9: Fresh science B

SESSION 10: Opportunities from new winemaking technologies

SESSION 11: The future of industry, technology and wine

The role of competition in fostering future wine industry growth

M. Keogh

Australian Competition and Consumer Commission, Canberra, ACT
emily.duff@accc.gov.au

Abstract

For the first 200 years after European settlement, the Australian agriculture sector was able to achieve continuous expansion through a combination of productivity growth and the use of additional resources – principally land and water. However, with resources now limited, growth depends primarily on productivity gains. Improved productivity in the winegrape sector will invariably require increased capital investment, but growers will underinvest unless they have confidence the market is fair and competition is working to deliver them an equitable share of supply chain value. The ACCC's market study of the winegrape sector has identified competition weaknesses that will need to be addressed to secure optimal future growth.

Introduction

For the first 200 years after European settlement, the Australian wine industry, like the broader agriculture sector, was able to grow because additional natural resources—principally water and land—were readily available for existing or new participants. However, over the last 20 years these natural resources have become increasingly limited, with Australian and state governments imposing a cap on additional water extractions in the Murray Darling Basin in 1995 and bans on the clearing of additional land for agriculture from 1995 onwards in most states.

Now you might be wondering why I would commence a talk about competition issues in the wine industry by talking about constraints governments have imposed on access to land and water in Australia. The reason is very simple. With access to land and water now limited, the ability of any sector of agriculture to grow in the future will depend very much on the rate of productivity growth businesses in the sector are able to achieve.

In the case of the wine industry, that means winegrape growers achieving increased yields while using water more efficiently. To achieve that will require widespread capital investment by growers in planting improved grape varieties, and in irrigation and water monitoring technology. That investment will only occur when winegrape growers have confidence:

- that the market operates fairly
- that they are receiving a fair share of industry revenue
- that any additional investment they make has a good chance of generating adequate returns in the future.

In the absence of this confidence, winegrape growers will find it increasingly attractive to sell their water, and to pull out their vines. This means it is in the interests of all involved in the sector, including winemakers, to make sure the market for winegrapes is efficient and fair, and that there is strong competition through every part of the wine industry supply chain.

As Australia's competition regulator, the ACCC promotes competition and fair trade in markets to benefit consumers, businesses and the community. In response to a significant number of complaints received over the years from winegrape growers, in September 2018 the ACCC launched a market study into the Australian winegrape sector, with the broad objective of identifying market failures or trade practices that hinder the functioning of competitive markets.

The winegrape industry is largely characterised by imbalances in bargaining power between a small number of major buyers and a much larger number of small-scale sellers. This is similar to many other agricultural industries, although the winegrape industry has some unique features which exacerbate these imbalances.

On occasions during the progress of our inquiry, we were disappointed with the responses, or lack of responses, received from some of the major businesses and organisations involved in the industry. This reticence to provide information or contribute seemed, on occasions, to be due to a fear of subsequent retribution from other industry participants. This is not something that would be expected in an industry where competition is working well, and markets are open and transparent.

We acknowledge that there are significant factors in the winegrape industry that make it different from other industries, including:

- the considerable variance in the quality and price of grapes and the wine produced from those grapes
- the broad diversity of business models adopted by the approximately 2500 winemakers in Australia
- the long lead times associated with grapegrowing and winemaking.

These unique factors were all taken into account in the ACCC's study which focused on the three largest grape-producing regions in Australia: the Riverland (SA), the Riverina (NSW); and the Murray Valley (which includes the Murray Darling and Swan Hill (NSW/Vic.) regions). While our study focused on the three warm climate growing regions, many of the observations and findings will also be relevant in the cool climate regions.

Findings

I will now discuss some of the findings of our inquiry, and the measures that the ACCC has proposed to address the problems we have identified. I stress that we are still consulting widely with stakeholders about our Interim Report, and that the Commission is open to changing its views before releasing its Final Report in September 2019.

There is one key challenge that the industry needs to address, which is the low level of competition between winemakers acquiring grapes from growers. The ACCC has proposed a number of measures we believe will improve the level of competition for winegrapes and enhance the future growth prospects of the sector. These involve three aspects of the current winegrape market: contracting practices, pricing and quality assessment.

Contracting practices

In our interim report we identified inequities in the contracts between winemakers and growers, particularly in warm climate grapegrowing regions. The inequities stem from a number of factors including the generic nature of warm climate grapes, the perishable nature of grapes, the small size of the growers' businesses compared to the major winemakers, the better access that winemakers have to market information, and a number of practices that have become ingrained from the period when there was an oversupply of grapes.

These factors result in an imbalance in bargaining power and in growers accepting contracts with suboptimal terms with limited ability to negotiate or to resolve disputes. One of the manifestations of the imbalance in bargaining power between growers and winemakers is the lengthy payment periods prevalent in grape supply contracts, sometimes up to nine months after delivery of the grapes. Such long-term payments are not consistent with any other industries and put growers at a significant financial disadvantage.

There is a view among winemakers that these arrangements are justified because the wine industry is special case, as it takes such a long time for the wine to reach the market. The ACCC is not convinced of this. There are many other industries, including in the agricultural sector, where the product takes a long time to reach the final consumer. In the wool industry, for example, it takes an average of two years for raw wool to be converted into finished consumer products. Despite this, growers in that industry are paid within seven days of the sale of their wool.

For those in the audience who still think that extended payment terms are justified, I have a proposal to put to you. As a wine consumer, I think I should be able to select a bottle of wine off the shelf and decide what it's worth. I pay one-third of that when I leave the store. I'll pay a second one-third payment in six months, and I'll pay the balance when I get around to drinking it, which could be years in the future. If you think this is a ridiculous proposal, then perhaps you need to reflect that this is essentially how the current contract and payment terms operate for winegrape growers.

The ACCC has recommended that lengthy payment terms should be phased out of most supply agreements between growers and large winemakers. A best practice standard of payment within 30 days of grape delivery should be adopted by all winemakers with processing capacity over 10,000 tonnes.

Our interim report identifies other contract clauses that we think may be unfair. These include winemakers reserving unilateral rights to modify contract terms, to change quality standards, to vary prices, and to restrict growers from supplying other winemakers. We note the business-to-business unfair contract laws that were legislated by the Australian Government in November 2016, which we believe are applicable to many terms in standard contracts between winemakers and winegrape growers. We will certainly consider taking enforcement action in the future against winemakers who have unfair contract terms, in the same way we have taken such action in the horticulture, dairy and many other industries.

Pricing practices

A second issue we identified in our report is the pricing practices employed in the industry. The pricing mechanisms used in winegrape supply agreements are varied, with some being essentially fixed and some being variable. Many agreements do not specify a fixed price, and instead refer to a 'fair market price' which is determined unilaterally by the winemaker close to harvest. Variable price supply agreements are often not benchmarked against any visible, objective or verifiable measures of grape prices, meaning growers do not have a sense of what is a fair market price.

In the winegrape market study we found that winemakers do not publicise the prices they pay to growers, and often have confidentiality terms in supply contracts intended to prevent growers from disclosing prices to other growers. Consequently, information about price offers being made by individual winemakers can be difficult for growers to access. This makes it hard for growers to assess the competitiveness of price offers which might be available for their grapes from different winemakers.

Pricing transparency is important because it helps markets to operate efficiently by encouraging buyers to make better offers, and provides clear signals for suppliers to assist them in their operating and longer term investment decisions. To improve pricing transparency, the ACCC has suggested that winemakers in warm climate regions be required to confidentially provide indicative winegrape prices for the coming harvest to an independent third-party body by 8 December each year. These prices could then be released simultaneously by the independent body by 15 December each year so they are available to all growers, while reducing the risk of the largest winemakers working together to use this process to price signal and therefore to inhibit competition.

The ACCC continues to consult with stakeholders on the detail and potential implementation of these recommendations, but it is clear that greater price transparency is needed in winegrape markets. Increased transparency of prices on offer will provide increased price certainty to the market, and not only improve growers' bargaining power but also improve competition between winemakers.

Quality assessment of winegrapes

A third matter of considerable contention in the industry is the assessment of quality of winegrapes delivered to a winemaker. Concerns about quality assessment arise because assessed quality has a big impact on the price growers receive for their grapes. We received intelligence through our survey and broader stakeholder engagement that there are significant differences in the ways winemakers assess the quality of grapes.

The growers we heard from raised concerns about the transparency, consistency, timing and subjectivity of quality assessment methods. In support of this, evidence was provided of different loads of the same grapes from the same vineyard being allocated very different quality grades by winemakers. These shortcomings contribute to mistrust about winery quality assessment processes and outcomes, with some growers claiming that these assessments are conducted arbitrarily or for ulterior motives.

Generally, grape supply agreements and grower manuals issued by winemakers clearly set out quality assessment specifications and associated penalties and bonuses. However, they do not always specify when testing will occur or the precise methods to be used. There is also limited standardisation of calibration of testing equipment. Some winemakers' contracts also contain clauses reserving a broad unilateral right for the winemaker to change quality specifications during the season, which creates uncertainty for growers and could be used to lower prices. There are further grower issues relating to tests for sugar content and the scientific reliability of colour assessment.

It is obviously important for growers to have certainty about the quality and hence value of their grapes, and trust in the grape assessment process, if they are to make any production decisions during a season to better meet quality requirements or consider investment plans for the future.

While we recognise the arguments challenging the value of objective tests to describe winegrape quality characteristics, I note that this very same argument prevailed in the wool industry back in the 1970s. Wool processors at that time argued it was impossible to

adequately describe the quality of wool using objective measurement. Fortunately, the industry invested heavily in research to develop objective tests which are now the mainstay of the wool trading system and critically important for breeding and production decisions.

The dramatic improvement in Australian wool quality that has occurred over the past 30 years is a direct result of the development of objective testing methods, and it is highly arguable that similar benefits would arise in the Australian winegrape sector if objective quality testing was introduced. We have recommended the National Measurement Institute and the Australian Wine Research Institute work with industry to develop uniform standards for testing and measuring grape sugar levels and colour.

A way forward: the Code

Our interim report made a number of recommendations aimed at improving competition in winegrape markets and ensuring the industry can optimise opportunities for future growth.

Where an industry is found to have widespread issues relating to both contracts and competition, an industry code of conduct can be an efficient mechanism to address these issues. The wine industry recognised some of these industry problems in 2008 and responded by developing the Australian Wine Industry Code of Conduct. Our inquiry has indicated that the adoption of this Code by many in the industry has been beneficial, but a number of shortcomings remain.

We heard from growers that there are important benefits to having a structured process for growers and winemakers to resolve their disputes. Noting that, we have made several recommendations to strengthen the Code including improving the dispute resolution mechanism. However, for the Code to be effective there must be a high degree of take-up by industry participants and sufficient disincentives to deter non-compliance. The fact that no major winemakers in the Riverina region have become signatories to the Code highlights its limitations, and means that none of the growers in that region—one of the most significant winegrape production areas in Australia—can access the benefits the Code provides.

The ACCC understands the preference of many that the winegrape industry continues to operate under a voluntary Code, with the industry control and flexibility that such a Code provides. However, the ACCC has concluded that if there is not an improved take-up of the Code by the large and medium-sized winemakers, it may be necessary to introduce a mandatory code in order to bring about the required industry reforms. The ACCC has undertaken to revisit this issue in one year, and if all major winemakers have not become signatories to the voluntary Code by that time, there will be a need to seriously consider a mandatory code.

You might think the issues I have raised today are simply about competitive business practices between winemakers and growers—with each side working hard for their own best interests. We believe these issues are much more significant, and at their core represent a very real threat to the future of the winegrape industry, especially in an era of scarce resources.

The winegrape industry will need capital investment from its growers if it wants to improve productivity and grow in the future. Only when growers have greater confidence and certainty in the market will they be prepared to make those investments.

Our recommendations are aimed at better balancing the bargaining power of growers and winemakers, and at providing more market transparency so that growers are better informed.

We are currently contacting growers and winemakers and speaking with stakeholders about their submissions. Our next step is to put together our report with our final recommendations. That final report is due in September. I encourage all of you to engage with this process.

In closing, I want to reinforce our key message: the lack of competition in the market for winegrapes will be a major impediment to the future growth of the industry, and unless this is addressed the industry faces a strong risk of long-term decline. It is in the interests of all industry participants to engage with us in the process of addressing this challenge.

Geopolitics and the impact on the Australian wine sector

A.N. Battaglene

Australian Grape & Wine Incorporated, Canberra, ACT
tony@agw.org.au

Abstract

Grape prices and demand for Australian wine are strongly dependent on international developments and the state of the global industry. Although international demand is strong and the China market in particular continues to show strong growth, the sector still faces a number of significant challenges. The Australian Government's $50 million Export and Regional Wine Support Package (the $50 million package) and the investment into the USA is also likely to see an increasing dependence on the three key markets of China, USA and the UK. There is a high degree of uncertainty around the global growth outlook amid a range of economic and geopolitical risks that continue to evolve. This uncertainty appears to be weighing on measures of global confidence, which have been falling in recent months. The uncertain geopolitical environment demonstrates the need to further diversify our markets to future-proof against the sovereign risk created by market specialisation. It also demonstrates the need for better use of soft power initiatives to develop relationships with our major trading partners. In this paper we look at some of the risks and implications for the Australian wine sector in this uncertain geopolitical environment.

The Australian wine sector

The Australian wine sector is a driver of jobs, economic growth and prosperity across rural and regional Australia, contributing $40 billion annually to Australia's economy from winegrape growing, winemaking and wine tourism. Australian Grape & Wine, the national association of winegrape and wine producers, focuses on providing leadership, strategy, advocacy and support that serves Australian wine businesses now and into the future.

Australian wine businesses are expressing a renewed sense of optimism following a challenging period. The $50 million package is helping to drive growth in the sector. We are beginning to see improved market sentiment and sales in key export markets, particularly China and the USA, along with increased wine-related tourism in Australia. While these early signals are positive, we are conscious that current funding arrangements will end on 30 June 2020.

Australian Grape & Wine is working to make sure these early gains are protected and built upon, but we need to partner with the Australian Government to future-proof our sector. This is particularly important in the face of an increasingly uncertain trading environment, impacted by increasing trade tensions and geopolitical instability.

Building demand

The future profitability of the Australian wine sector depends on exports. Growth in the domestic market is slow, and the biggest gains for Australian producers in 2018 came from exports to high-growth markets overseas. In total, 63% of Australia's wine production was exported to 125 markets in 2018.

The $50 million package has a very strong focus on marketing and promotion in export markets, assisting existing exporters and developing capabilities for new exporters in China and/or other free trade agreement (FTA) markets. This funding is vital in assisting industry SMEs to capitalise on their existing export success or enter the export market. However, the $50 million package and the investment into the USA is also likely to see an increasing dependence on the three key markets of China, USA and the UK. The uncertain geopolitical environment demonstrates the need to further diversify our markets to future-proof against the sovereign risk created by market specialisation.

The international economy

The International Monetary Fund (IMF 2019) forecast in April a decline in growth in 2019 for 70% of the global economy. In summary, the IMF economists state the following:

> The global recovery is expected to strengthen, led by advanced economies. Growth in emerging market and developing economies is expected to pick up only modestly. Global growth was projected to grow by 3.6% this year, compared with 3% in 2018. For the advanced economies the outlook is for 2.2% growth versus 1.3% last year. For the emerging and developing economies, the quickening is slight, 4.9% versus 4.7%, but the rate is over twice that for the advanced economies.

The IMF qualifies this outlook by noting, 'however, important downside risks remain – notably a yet-greater general slowdown in emerging market economies; risks to activity from lower-than-expected inflation rates in advanced economies; incomplete reforms; and rising geopolitical tensions'. In April, the IMF cut its forecast for global growth this year to 3.3%, warning that a sharp escalation in trade tensions could wreak havoc on supply chains and disrupt industries, leading to the weakest rate of expansion since the great recession of 2009.

China, the world's second-largest economy, is the most important by far to Australia's wine trade. While it has slowed from last year, the slowdown is not as great as many had feared. The current size of this economy means its >7% growth continues to contribute greatly to global economic growth. However, recent economic data suggests the slowdown is more pronounced than expected, with annualised quarterly GDP growth slowing from 6.4% in the first three months of the year to just 6.2% in the June quarter. This suggests that the Trump trade war and continuing slow economic activity in much of the rest of the world are 'hitting the brakes' in China. China's growth is still impressive compared to much of the rest of the industrialised world, although the slowing trend is of concern when you compare it to the 6.6% last year and the 6.8% in 2017.

It is true that the Chinese economy is much larger now than it was a couple of years ago, so slower growth still provides plenty of momentum for Australia's exports. However, with Australia stuck in a slowing growth trend of its own, the trend is concerning. Australia

is very closely linked to China's fortunes given that it is our largest trading partner and we will certainly feel the pain if Chinese growth were to slide closer towards 6% over the rest of the year. What makes the slowdown more worrying for Australia is that China is now saddled with significant debt from previous measures to stimulate its economy, providing less scope for spending measures in future downturns.

Heightened geopolitical tensions are the most worrying risk to global economic growth. The Ukraine crisis appears to be worsening as the Geneva agreement between the USA, Europe, Ukraine and Russia fails to bring the hoped-for de-escalation. Putin, pursuing his vision of a greater Russia, appears ready to accept the costs of isolation and a likely deep and prolonged recession. There are rising tensions between China and Japan, the world's second and third largest economies. While neither country is as likely as Russia to act rashly, accidents could escalate tensions quickly as a consequence of the strong feelings on both sides. Global equity markets would react sharply if either of these situations should boil over. The Middle East crisis and the continued drama being played out with North Korea add to the complex puzzle of international trade tensions.

Geopolitics – the major risks

Internationally there is a high degree of uncertainty, which appears to be weighing on measures of global confidence amid a range of economic and geopolitical risks. The key ones discussed in the context of this paper are China and the ongoing trade dispute with the USA, Brexit, Free Trade Agreements and the future of the World Trade Organization.

Trumpian economics

The central issue is what will continue to happen with the US/China trade talks, which are delicately poised at the moment. The Trump administration has already raised tariffs on US$250 billion of China's exports to the USA and came close to adding tariffs to another US$300 billion. That has been stalled for now after President Trump deferred the extra tariffs at the G20 leaders' meeting. That has allowed negotiations to continue between China and the USA but there is no sign yet of a breakthrough in the talks so a large expansion in the tariff program is still a likely result. Adding to the pressure is the chance that President Trump could still widen his trade wars to Europe and elsewhere. Already the tariffs are hurting China and both exports and imports have fallen, with the 7.3% fall in imports in the last month particularly severe. Australia can really only watch and hope as the world's biggest economy—the US—does battle with the second biggest—China—with every chance that the third major economic group of the Eurozone could well be sucked into the trade war as well.

As an open trading country we are particularly suited to the previous relatively open world trading regime, and the rise of protectionist measures leaves us exposed. We are price takers rather than price makers and any further slowing in world economic activity will be felt keenly in Australia, particularly given our current growth is already sluggish and slowing. Significantly, there has been no breakthrough on the fundamental issues that led to conflict between the world's two largest economies. Analysts think the lack of progress means that existing tariffs will not be lifted anytime soon, keeping pressure on supply chains and a lid on global growth.

Free trade agreements

The lack of confidence in the World Trade Organization and the slow pace of reforms initiated there have led to a great deal of free trade agreement activity.

FTAs are generally positive for trade but can create distortions. For example, the European Union recently announced that it had agreed its biggest ever trade agreement with the four-nation group of South American countries made up of Argentina, Brazil, Paraguay and Uruguay, known as Mercosur. The agreement was reached after two decades of negotiations and is expected to remove more than 90% of agricultural and industrial tariffs on both sides.

Under the agreement, Mercosur has pledged to eliminate taxes on wine, chocolate, spirits, biscuits, tinned peaches, carbonated drinks and olives.

However, the European Union will continue to protect its highly subsidised agriculture sector through quotas. Importantly, it is unclear at this time what agreement was made on geographical indications (GIs). Developments in this area could have serious ramifications for Australian wine exporters.

The agreement still needs to be ratified by both the European Parliament and the four Mercosur countries—something which could take years.

Closer to home, the European Union is negotiating free trade agreements with both New Zealand and Australia. Both of these agreements potentially have important consequences for Australian wine trade, with the European Union making no secret of its wish to remove the rights of Australian producers to use a range of grape variety names when they sell wine. Most obvious is 'prosecco' but this is by no means the only grape variety targeted by the European Union.

World Trade Organization (WTO)

All stakeholders in agrifood value chains, and particularly smaller export-focused economies like Australia, stand to benefit from fully participating in a strengthened and dynamic multilateral trading system. The global trading system – with the WTO at its heart – is facing a 'make or break' moment. All three of the WTO's functions are under pressure and in need of reform: administering multilateral trade rules, serving as a forum for trade negotiations and providing a mechanism to settle trade disputes.

The most immediate flashpoint is addressing the shortcomings of the dispute settlement system. Though President Donald Trump's repeated threats to pull the USA out of the organisation are a cause for concern, this is unlikely to happen given the powerful role of Congress and the economic costs involved.

Instead, the real danger lies in the current administration hollowing out the rules-based international trading system from within through such actions as raising tariffs in the name of US national security and blocking the appointments of members to the WTO's Appellate Body. If the latter practice continues, the Appellate Body will not have enough members to hear cases come December when the terms of two members end, thereby risking that the WTO dispute settlement system effectively ceases to function.

Many of the current concerns raised by the USA pre-date the Trump administration and are shared by other WTO members – especially regarding procedural aspects (for example, a disregard for the 90-day deadline for issuing rulings, or the continued service by Appellate Body members on cases that continue after their terms have expired). The Trump administration has also voiced substantive concerns about 'judicial overreach' by the Appellate Body, which is a more controversial issue and will be difficult to resolve.

Moreover, without the traditional US leadership, other WTO members are beginning to take on a more central role in advocating for the global rules-based international trading system. While it is important that increased leadership comes from major trade powers (such as the EU and Japan), smaller and medium-sized players should take on larger roles as well. For them, safeguarding the WTO is especially important because the organisation provides the main path to participate in setting the rules for new trade policy areas.

Efforts to resurrect the WTO's relevance as a forum for trade liberalisation is another area where green shoots are sprouting. The effective collapse of the Doha Round in 2008, which was launched in 2001 but then stalled over agricultural subsidies as a major controversial issue, raised questions about the viability of conducting trade talks involving more than 160 members based on the principles of consensus (meaning that all members must agree) and a single undertaking (whereby nothing is agreed until everything is agreed).

The failure to conclude the Doha Round also impeded WTO members from focusing efforts on updating the rules of the global trading system in order to address the changes that have occurred since the WTO was established in 1995. In particular, the WTO is not currently fit for purpose to deal with the increased role of state-owned enterprises or digital trade.

To tackle some of these new trade issues in the wake of the Doha Round impasse, trading partners turned to bilateral free trade agreements or larger regional ones—such as the Comprehensive and Progressive Agreement for Trans-Pacific Partnership (CPTPP). A subset of WTO members has pursued plurilateral negotiations by focusing on narrower issues. Most recently, 76 WTO members – including the USA, China, Japan and the EU—agreed to start negotiating rules on e-commerce. Plurilateral efforts are no panacea, but they can fill important gaps.

Finally, the seeds of reform have been sown for improving the ability of the WTO to administer and monitor member states' trade policies. The failure by many countries (including China) to comply with the WTO's notification requirements—for instance to notify WTO of government subsidy programs—has been a topic of concern for years. WTO members are now seeking to address the notification issue, with the USA, EU and others suggesting penalties for non-compliance.

Another area for reform concerns the lack of an agreed definition as to what constitutes a developed or developing country at the WTO and that members self-designate their status.

WTO members that use the latter designation benefit from so-called 'special and differential treatment'. The fact that 10 of the G20 countries—including China, India, and South Korea—currently claim developing country status at the WTO is a major point of contention. Brazil's recent decision to forego its developing country designation is a potential milestone and could inject momentum into the discussion about setting quantifiable criteria to clarify a country's development status.

To be successful, reforming the WTO will have to reform all three of its functions. However, because decisions at the WTO are based on consensus, the chances for a fundamental overhaul are slim. Therefore, WTO reform should cover broader institutional issues and members should revisit some of the organisation's principles and system of decision-making.

In the short term, efforts to reform the WTO dispute settlement system should be prioritised to avert an acute Appellate Body crisis that looms in late 2019. This narrower reform endeavour has a greater chance of success.

The G20 met recently in Osaka and gave guarded support to international trade. According to the G20 Osaka Leaders' Declaration (2019):

> Global growth appears to be stabilizing, and is generally projected to pick up moderately later this year and into 2020. This recovery is supported by the continuation of accommodative financial conditions and stimulus measures taking effect in some countries. However, growth remains low and risks remain tilted to the downside. Most importantly, trade and geopolitical tensions have intensified.

The G20 Osaka Declaration pledged, among other things, leaders' commitment to support for the necessary reform of the World Trade Organization to improve its functions:

> We will work constructively with other WTO members, including in the lead up to the 12th WTO Ministerial Conference. We agree that action is necessary regarding the functioning of the dispute settlement system consistent with the rules as negotiated by WTO members. Furthermore, we recognize the complementary roles of bilateral and regional free trade agreements that are WTO-consistent. We will work to ensure a level playing field to foster an enabling business environment. (G20 Osaka Leaders' Declaration 2019)

Notably, amid mounting trade tensions between the USA and China, the joint statement does not include words such as 'fight against protectionism', continuing the theme from last year's meeting.

Brexit

I am not going to forecast in this paper the form of the eventual Brexit outcome. Suffice to say, our efforts have concentrated on trying to mitigate risk from whatever shape, form or timing of Brexit, at or around (or some time other than) the revised withdrawal date set for 31 October 2019.

Following his appointment as Prime Minister on Wednesday, 24 July, Boris Johnson has been quick to name his new Cabinet. Johnson has appointed a Cabinet that is widely seen as one that will assist him deliver on his promise to have the UK leave the EU on 31 October 2019. The UK Parliament has thus far demonstrated its lack of appetite for permitting a 'no deal' Brexit. On the other hand, the EU has thus far shown no willingness to renegotiate Johnson's predecessor's (Theresa May) withdrawal agreement which includes the backstop element which Johnson himself has described as dead. The prospect of the UK withdrawing Article 50 and remaining in the EU seems as unlikely as ever, and there is no guarantee that the EU will grant the UK a further extension beyond 31 October, even if the UK were to request it. As has been the case for many months, the UK's future remains in a state of uncertainty.

On 18 January 2019, the Australian and UK governments finalised the Agreement on Trade in Wine between the Government of Australia and the Government of the United Kingdom of Great Britain and Northern Ireland (AU-UK Wine Agreement). This document for the most part mirrors the conditions of trade currently outlined in the Australia-EU Agreement on Trade in Wine and will allow for greater certainty over the allowable practices of wine trade between the two. On 13 February 2019, the AU-UK Wine Agreement was introduced into the Australian Parliament and subsequently passed into law. Concurrently, the Wine Australia Amendment (Trade with United Kingdom) Bill 2019 was introduced and was passed by the Senate unamended on 15 February. This Bill allows for the minor yet necessary changes to the Wine Australia Act 2013 to account for the Brexit changes.

However there will still be some outstanding issues following Brexit, particularly with respect to wine that is exported from the UK into Europe.

One significant labelling change concerns the obligation to include the details of the importer. One way to comply post-Brexit would be to include details of **both** the person responsible for bringing the wine into the UK **and** the one responsible for bringing it into the remaining 27 EU member states. Wine Australia discussions with UK authorities have confirmed that this would be an acceptable approach in that market. Unfortunately the European Commission does not have the same flexibility and only the name and address of the relevant EU importer can be displayed in the remaining 27 markets. Including the details of the UK importer would only be permitted if clearly

separated from the word 'importer' or 'imported by' and does not mislead consumers over the entity responsible for bringing the wine into the EU.

Wine Australia will continue to discuss this matter with the European Commission in the hope that their position may change prior to the UK's withdrawal. In the interim, the safest option is to modify labels in order to comply with the position of the European Commission.

A further consideration is the impact of the UK's withdrawal on the required import document, the VI1 certificate.

The suite of analyses will be the same, post-Brexit, regardless of whether a shipment is bound for the UK or the EU.

The UK has drafted a revised format for the document intended for use on shipments entering the UK once Brexit is complete. The changes are purely cosmetic, and Wine Australia's new export approval system has been designed to distinguish between the two documents.

Uncertainty remains, however, in the situation where wine is exported in bulk to the UK prior to packaging for subsequent shipment to the EU.

In these circumstances, the EU VI1 form would need to be drawn up by UK authorities, not by Wine Australia. It is not yet certain they have the resources necessary to cope with what would appear to be an enormous number of transactions.

Future-proofing the Australian grape and wine sector

We can mitigate risk for the Australian grape and wine sector in three ways. This requires an understanding that maintaining our export focus and increasing profitability in international trade is the only way to improve grape prices and maintain profitability for domestic-focused producers.

First, we require investment in marketing and brand building activities in new and emerging markets. The $50 million package has shown us how this can be achieved and a further investment within these markets would build on this and allow the Australian sector to gain a permanent foothold in the key economic regions of Asia and Africa. A further investment of $5-10 million per annum would enable the sector to replicate our China growth in these new markets, diversifying risk and growing profitability.

Second, the current government and the previous Labor government have shown a deep and abiding commitment to lower trade barriers and improved market access for exports through a network of international trade negotiations and sustained efforts to reduce non-tariff barriers. At a time when farmers around the country are in a crippling drought, free trade agreements give hope for a long-term sustainable future and recognise the importance of agriculture and, more importantly, exports of agricultural produce as vital to Australia's economic well-being and rural and regional Australia's prosperity. FTAs have real and immediate benefits for the wine sector. Australia gains a competitive advantage in new and developing markets. In addition, there is now a real effort to address non-tariff barriers. Non-tariff barriers are the biggest problem for exporters and add cost and complexity in navigating key markets.

Third, we must recognise that the ongoing trade tensions that exist between major world powers, political tensions in the Middle East and Asia, and the ramifications of Brexit all have the potential to impact on global trade and consumer confidence. Although Australia cannot influence these directly, there are measures we can take to mitigate risk and future-proof our sector so that we can ride out external shocks.

The China market

China is worth special mention as it is Australia's most significant wine export market. The market has grown from $27 million in 2006 to $1.1 billion in 2019, and Australia has now overtaken France as China's biggest wine supplier by value—an incredible development in less than 15 years.

While we recognise this high level of growth will not last forever, it is clear Australia has established its reputation as a provider of high-quality wines with consumers. China represents a long-term commercial opportunity for Australian winemakers and exporters, and these benefits flow through the supply chain to grapegrowers. However, we are not the only producers of quality wine and other exporting countries are working hard to capture market share. We are working to cement our position and enable future growth through the $50 million package and individual company efforts.

We also understand that our focus on China carries risk. Geopolitical tensions, trade and security issues and other irritants in the bilateral relationship mean that our position in the Chinese market is not guaranteed. While bilateral tensions can arise from time to time in any relationship, Australian Grape & Wine remains alert to the fact that in recent years these tensions have become more frequent and high-profile, creating potential commercial problems for Australian wine businesses. Mitigating against these government-to-government tensions is critical, and we hope the establishment of a firm business foundation can help achieve this aim.

As well as building intergovernmental relationships, building relationships outside of government and harnessing Australia's soft-power credentials should be an important focus. Australia's wine sector is a soft-power asset. Australia's world-class food and wine tourism offering is part of what makes Australia attractive as a destination for people, investment and ideas, and each bottle of Australian wine sold in China can tell a story, providing a unique perspective of Australia. Increasing the number of Chinese tourist visitors to Australia enables us to harness our natural assets, culture and people to shape positive perceptions of Australia which are brought home to China and shared. Enabling better links between Australian and Chinese business people, scientists and researchers should be another focus for the country-to-country partnership.

To future-proof the Chinese market for Australian wine businesses, and strengthen Australia's bilateral relationship with China, we must do more to build relationships and reduce sovereign risk. By building robust foundations to expand new business opportunities in the China market, we can maintain a competitive edge and continue to ensure Australia is viewed as an attractive and dynamic tourist destination.

The $50 million package has bolstered the sector's engagement in promotional activities in China, including through Wine Australia's coordination of a China Influencer Media & Trade visit, a China Sommelier visit and a China Key Opinion Leader (KOL) Influencer visit. Further to this, the fortnight-long China roadshow, which took 80 Australian wineries to cities across China, helped promote our wine offering and educate Chinese consumers about our wines, regions and people.

These events have shown great promise for helping to expand the Chinese market, however with the $50 million package coming to an end by June 2020, further support will be needed to maintain this momentum and cement our gains. While promotional events are good at raising awareness at a commodity/industry level, practical cooperation is best done though collaborative events across multiple stakeholders. A good example of such an opportunity is the annual AFL game in Shanghai aimed at promoting AFL and more broadly promoting business, people-to-people and cultural exchange. Events such as this provide a centrepiece around which other events can be built and promoting this as a 'Festival of Australia' has significant potential to promote greater cooperation.

The greatest opportunity for promoting practical cooperation with China is to support improved cooperation between regulatory

authorities. We know that both Chinese and Australian regulators are interested in improving regulatory cooperation to improve food safety and product integrity. Previous work that we have undertaken in plurilateral forums, including the Asia Pacific Economic Community (APEC) Wine Regulatory Forum (WRF) and the APEC Food Safety Cooperation Forum have resulted in strong participation by Chinese regulators and have proven to be highly effective in addressing regulatory issues and barriers to trade through education and knowledge exchange. This indirect approach can encourage the removal of barriers to trade in a non-confrontational setting, without the need for more formal direct approaches which can often be met with resistance or even embarrassment.

To fully utilise the benefits of a 'soft-power approach', Australian regulators must be able to work collaboratively with Chinese regulators to build a system that provides consumers on both sides with surety about food safety and integrity. A good building block for this will be Australia's development of the Australian Wine Label Intellectual Property Directory (the Directory). The Directory will be a searchable intellectual property register of wine labels to allow businesses to monitor export wine labels and ensure copycat products are identified and acted upon. The next step will be to build a cooperative network with Chinese regulators to exchange information and, eventually, develop a joint regulatory system with checks and balances of the wine supply chain in China. Using a staged process will build trust and relationships. Such a system will increase Australia's competitiveness, build trust with consumers and mitigate sovereign risk when political tensions arise.

Conclusion

The Australian wine sector is well placed to grow into the future. However, the international environment provides significant risks and challenges. It is imperative that the wine sector collectively engages in strategies to mitigate the sovereign risk that arises from the volatile international environment. It is also important to realise that these risks are shared by many sectors and economies, thus solutions require cross-sectoral and international collaboration.

References

G20 Osaka Leaders' Declaration (2019) 28–29 June: https://g20.org/en/g20/Documents/2019-Japan-G20%20Osaka%20Leaders%20Declaration.pdf

International Monetary Fund (2019) World Economic Outlook, April 2019, Growth Slowdown, Precarious Recovery: https://www.imf.org/en/Publications/WEO/Issues/2019/03/28/world-economic-outlook-april-2019

What the $50m is delivering for the sector: a company perspective

M. Taylor

Taylors Wines, Auburn, SA
mitchell.taylor@taylorswines.com.au

Abstract

The wine sector is on the cusp of transformational change. The activities and engagement funded by the Australian Government's $50 million Export and Regional Wine Support Package (the $50 million package) are underpinning improving market sentiment and sales in key export markets, particularly the USA and China, and increased wine-related tourism. The Australian wine community thrives on the success of our regional and rural communities where most of our operations and tourism experiences are based. The $50 million package has provided crucial funding in ensuring that success, both in bringing international tourists to regions like the Clare Valley, or telling the rich stories of our great producers all around the world. The financial stimulus from this package for Australia's First Families of Wine has provided ample opportunities and capabilities to drive growth in overseas markets. A further investment within new and emerging markets would build on this success and provide the Australian sector the ability to gain a permanent foothold in key economic regions. Additionally, it would enable the sector to replicate the growth seen in China in these new markets, diversifying risk and growing profitability.

Introduction

In May 2016 the Australian Government announced it would invest $50 million over four years (2017–2020) to support wine industry initiatives to grow demand for Australia's wine exports and showcase the nation's wine tourism offering.

The package targeted three areas for funding:

- Wine Export Grants
- International Wine Tourism State Grants
- International Wine Tourism Competitive Grants.

For the Australian wine industry this investment has been truly transformative, allowing a step change in the messaging, visibility, promotion and competitiveness of Australian wines in the key export markets of the USA and China. The activity has focused on building Australia's quality credentials and challenging stereotypes of Australian wines held among media and gatekeepers. In addition, it sought to build the capability of Australian wineries to grow wine exports and wine tourism through skills development workshops and focused training.

A challenging global environment for winemakers

The $50 million funding package came at a critical time for the Australian wine industry. Trade wars, Brexit, drought, changing consumer preferences and geopolitical tensions have all created an uncertain environment for wine producers.

Despite the promise of the China Australia Free Trade Agreement, which saw tariffs on wine eliminated fully in 2019, the Chinese economy is facing headwinds as it grows at its slowest pace in nearly 30 years due to the impact of a bitter trade war with the USA. This uncertainty around the US-China trade relationship, along with fading consumer confidence and a weakening of the renminbi has contributed to the overall decline in imported wine. Yet the China market, which is valued at $1.28 billion, remains critical for premium Australian wine exporters. The backing from the $50m package sought to consolidate Australia's newly earned position as the number one source for imported wine, ahead of France, by giving China's wine trade, media and consumers a better understanding of the uniqueness and diversity of Australian wines.

The challenge in the USA, the world's largest wine market, is very different but equally important. In the USA, Australian wine producers have been faced with year-on-year declines since our export peak in 2007.

For small to medium Australian wineries the challenge of competing in these markets is further amplified by the large subsidies available to European producers creating an uneven playing field. The European Union dedicates a budget of over €1.1 billion per year to support its wine sector, an average of nearly €220 million per year. As detailed in the EUCAM 2019 report: 'In the period 2014-2018 almost 20% of this funding was allocated to promotion measures, or an average of nearly €220 million per year. For the period 2019-2023 a similar share of the budget is previewed for promotion'.

A producer's perspective – Taylors Wines

Taylors Wines has been present in the China and US market for more than 20 years. Over this time Taylors has been active in marketing its brand and has spent significant time in-market supporting trade partners. However, as any producer knows, as a single winery it is often difficult to capture the attention of busy gatekeepers and media. The support package, however, created significant opportunities for Australian wineries to act as a collective and be heard above the competitive noise of rival wine-producing nations.

Events like Hong Kong Vinexpo—which saw 17,500 key trade visitors including importers, wholesaler/distributors, off-trade, on-trade, media and e-commerce representatives—offered Australian brands excellent opportunities to make new business contacts. Australia's place as the 'Country of Honour' served to increase our standing and prominence and injected a new energy into the way Australia was communicating its wine story.

Our winery has aligned its strategic push behind the Wine Australia focus markets. In China we have a new team in place to capitalise on the opportunity created by the package and growth in Australian wines. In the USA, similarly, we have placed additional human resources to capture opportunities that have been created. Certainly, at a marketing level the industry investment has allowed a greater scale in brand marketing activities.

A rising tide lifts all boats

The results for our winery and industry overall have seen an improvement across a range of brand and economic measures. Our China

sales have grown 87% over the past three years. While at an industry level, in December 2019 total exports were valued at $2.91 billion – ahead of the 2017 benchmark of $2.3 billion (see Figure 1).

China exports including Hong Kong and Macau also surpassed their 2017 benchmark of $721 billion, reaching $1.28 billion. The industry also saw an increase of 8% in average winegrape prices in 2018, moving from $565 per tonne to $664 per tonne. At the same time there was an improvement in average bottled wine moving from $5.46 per litre (FOB) to a near-record value of $7.04 per litre (see Figure 2)

However, signifying the size of the challenge in the US market, exports of Australian wine continued to ease from $464 million to $419 million. There are some encouraging signs for Australia in the USA, with the value of exports at A$10 or more per litre increasing by 4 per cent to $43 million.

The programs have also lifted trade and consumer quality perceptions of Australian wine, increased the number of wine media mentions and buyers with Australian wines in their portfolio (see Figure 3).

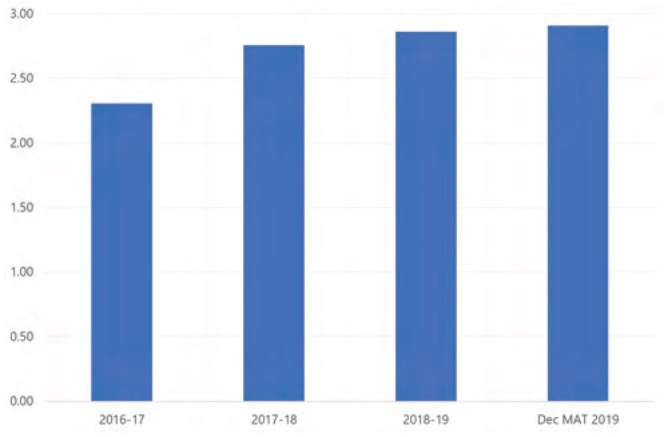


Figure 1. Total value of Australian wine exports (A$ billion free on board (FOB)). MAT = moving average total. Source: Wine Australia

Figure 2. Average value of bottled exports and average winegrape price over time. Source: Wine Australia

Figure 3. Key performance indicators of Program 1 of the Australian Government's Export and Regional Wine Support Package administered by Wine Australia

China Roadshow and multi-channel consumer campaign

The China Roadshow—supported by the $50 million package—showcased the rich diversity of world-class Australian wine (21–29 May 2019). The Roadshow visited Tianjin, Hangzhou, Kunming and Shenzen, bringing an Australian wine tasting exhibition to each city, along with masterclasses run by leading winemakers and experts. It was the largest travelling Australian wine tasting in China.

The four-city roadshow, in its eighth year, featured more than 170 Australian wine brands, giving thousands of Chinese trade and media an opportunity to experience Australia's regional and fine wines.

As part of this, Australia's First Families of Wine (AFFW) hit the road, joining the annual China Roadshow. This was the second time that AFFW had taken part in the Roadshow with Wine Australia and the third time travelling across China as a group. The group hosted a classic Shiraz masterclass on the opening day in Tianjin and a mature icons masterclass in Shenzhen to finish.

In addition to the China Roadshow, Wine Australia's multi-channel consumer campaign—Australian Wine Month—was featured in China for the first time in May. The campaign was spearheaded by a consumer-facing retail promotion in 80 high-end supermarkets including Ole' and BLT. Another promotional activity was run on Wine Australia's flagship Tmall store. The flagship store provided an opportunity to highlight Australian wines to the younger generation of Chinese wine consumers and offered Taylors and other Australian wineries a chance to feature in the '9.9 Promotion'. To aid with promoting the flagship store, an influencer event with the Chufei Churan Twins was organised to share their experiences of Australian wine with their one million plus followers.

Communicating Australian wine – 'Far From Ordinary' US campaign

The US market campaign made up the largest proportion of the funding. Here the approach was also multi-tiered with initiatives designed to engage media and influencers, trade customers and consumers. In 2019 the US market saw an $8 million campaign (held from 17 September to 10 October) that was a key milestone and integrated more than three years of targeted marketing strategy into a single campaign.

With more than 300 attendees, the 'Far From Ordinary' Roadshow allowed more than 100 Australian exhibitors to show their fine wines to the American wine trade in six cities: New York City, Chicago, Miami, Dallas, Los Angeles and San Francisco. Each city immersed itself in Australia's dynamic winemaking scene – learning about the people, the places and the grape varieties that make Australian wine unique. The Roadshow incorporated educational seminars, awards, media events, importer/distributor networking opportunities, in-store and online retail promotions and consumer activation.

At a retail level, an Australian category promotion was targeted to major off-premise retailers including ABC Fine Wines & Spirits, Total Wine & More, Wine.com, Binny's Beverage Depot, Raley's Supermarkets, Bottle King, Sigel's Beverages, Wally's Wine & Spirits, The Wine House, Draeger's Market and Harris Teeter. Distributors included Republic National Distributing Company (RNDC), Winebow (25 states), Young's Market Company (California), Johnson Brothers, Frederick Wildman and Skurnik. On-premise activations were also held at 47 Vino Volo wine bars at 34 airports across the USA. Landry's Mastro's Restaurants ran consumer events in New York and Chicago across multiple venues.

The 'Far From Ordinary' campaign culminated with 100 American wine influencers, including sommeliers, wine writers and other industry members, gathering at Lake Tahoe, California for the second 'Australia Decanted' event. This exclusive event featured 16

pre-eminent Australian winemaker ambassadors sharing stories about our fine wines over four days.

Growing regional tourism

Tourism and cellar door businesses play an important role for Australia's regional economy. According to Tourism Research Australia, 8.4 million people visited Australian wineries in the last financial year, spending a total of $9.6 billion on their trips overall.

For wineries, tourism and these cellar door visits are an important source of sales with direct-to-consumer business contributing 17% of all wine sales by value – an estimated $1 billion (Wine Australia Cellar Door and Direct-to-Consumer report 2019). Smaller wineries have an even greater reliance on this channel with wineries under 5,000 cases deriving more than half their income this way.

Under the package, wine regions benefited from a $7.4 million investment boost for 21 international wine tourism projects (see Figure 4). For the Clare Valley this meant a $175,000 grant towards a $411,000 project to create a Wine Exchange series for Chinese television and digital channels, and a multi-lingual regional website.

Figure 4. Key performance indicators for Programs 2b and 3 of the Australian Government's Export and Regional Wine Support Package administered by Wine Australia

Figure 5. Key performance indicators for Program 2 of the Australian Government's Export and Regional Wine Support Package administered by Wine Australia

Capability development

Of equal importance to the market-facing programs were the skill development and capability workshops (Figure 5). Regional 'export ready' and 'export plan' sessions over one to two days were run for wine exporters looking to capture export opportunities in target markets.

Additionally, the Clare Valley region participated in Wine Australia's Growing Wine Tourism program, a two-day workshop to develop wine tourism experiences for the inbound tourism market.

Conclusion

The Export and Regional Support Package has been administered superbly by the team at Wine Australia. It has added real value in our key export markets for the Australian wine community, with our value increasing for six consecutive years. Most importantly, the value per litre has risen to record levels at $7.04 per litre for bottled wine. This builds a profitable and sustainable sector for the future. We need to continue this investment so that the Australian wine industry continues to grow with value creation to all stakeholders as we compete in the very competitive and unpredictable global markets in the future.

References

EUCAM (European Centre for Monitoring Alcohol Marketing) (2019) EPHA: Stop EU-money for wine promotion. 1 April: https://eucam.info/2019/04/01/epha-stop-cap-money-for-wine-promotion/

Wine Australia. Cellar Door and Direct-to-Consumer survey report 2019: https://www.wineaustralia.com/market-insights/cellar-door-and-direct-to-consumer-survey-report

Where to next? What does the future look like in terms of products and markets?

S. Tremble

Endeavour Drinks Group, Bella Vista, NSW
shane.tremble@edg.com.au

Abstract

Across the broader retail sector, we can expect to see more change in the next five years than we have seen in the last 50. This is especially true for drinks retailing, which has for various reasons been slower to adapt to the digital world than some other forms of retail. In our business the percentage of sales taking place online is rapidly approaching double digits, and in the wine category specifically it's already greater than that. The total value of all digital transactions in Australia now exceeds $25 billion and it's growing at over 10% per annum. New product development in both the beer and spirits categories outpaced that of wine.

Digital disruptors, direct-to-consumer models by brand owners and more specialised bricks and mortar retailers are all competing for an ever more demanding consumer—a consumer that is more time poor than ever, more interested in making healthier choices, and drinking less alcohol by pure volume than either their parents or their grandparents.

Our challenge, if our industry is to continue to grow and prosper, is to create strategies that will ensure that this reduction in consumption does not necessarily translate to a drop in sales revenue. We know that drinking less doesn't mean spending less on what we are drinking, that terrible portmanteau word 'premiumisation' is actually a thing. Consumers are seeking quality, discovery, authenticity and provenance, and they're prepared to pay for it. If we are to stay at the leading edge of this 'flight to quality' we need to gain a much more intimate understanding of the consumer. We need to use the data they share with us to personalise offers for them and to connect every customer with a drinks experience that they will love.

Rapid change and the increasing complexity of the entire industry brings the need for usable data and actionable insights into sharp focus. But businesses need to understand that the sharing of data is a transaction like any other. Customers know that their data has value, they hand it over in the expectation that we will give them something in return, that the payback for them will be to help them navigate through the complex buying process as seamlessly as possible, and that we will curate and offer a range that is relevant to their lifestyle and their ever-evolving tastes. In the new world of retail, the easiest way to turn your customers toward your competitors is to waste their time.

To paraphrase the financial advisers, past performance is not necessarily a reliable indicator of future results. Data that captures what people are buying, where and when they are buying it and how much they are paying for it, is not a perfect predictor of consumer preferences and trends. What it can do is take a lot of the guesswork out of working out what brands or offers are going to appeal to a particular customer. It can also dramatically shorten the odds of success for new brands and help brand owners to invest their marketing spend in a much more granular and targeted way and consequently to extract considerably more value from that investment.

All of this change coincides with a time when our industry is under greater scrutiny from regulators and the community and our social licence to operate is under threat. Digital transformation and the consumer expectations driving it have moved quickly, but the regulatory and legislative framework surrounding this channel of our industry has stood still. Governments are seeking to understand whether these new business models are meeting their obligations to serve the community in a responsible manner, and whether they have the will to regulate themselves or will need to have regulation imposed upon them.

The wine category – some retail trends

The last year has been a very challenging one for the wine category, volumes declined, with approximately 4.2 million litres lost in the retail market. Wine's share of the total packaged drinks category declined as customers increased their engagement with the bottled spirits and premix categories, which have done a great job of innovation and facing into customer trends. Red wine and rosé attracted more customer spend but white wine and champagne declined significantly.

Participation

The wine category has the highest customer penetration of all packaged drinks categories and is unique in a number of respects: firstly, demographic penetration by age increases in buyers over 50 years old, premium customers are the most highly engaged with the broadest repertoire, and bottled wine has both greatest frequency and highest spend of any category. However, it is the engagement of consumers between 25 and 50 years old that is cause for concern. As engagement with the wine category falls amongst these customers, the category becomes increasingly reliant on its core, ageing customer base which is unsustainable into the future. We have the opportunity to drive engagement with younger customers through pack types and wine styles that better fit their lifestyle. We can learn from the other drinks categories that are attracting these customers and bring those insights to the wine category. We can face into the moderation trend with low- and no-alcohol options.

Changing needs

Customers are no longer reliant on big brands for confidence, their needs and preferences are fragmenting, and they are more willing than ever to experiment. They want interesting and engaging varietals and lighter wine styles—not just more of the same. We have seen the importance of packaging design and innovation as it helps products stand out in a sea of sameness.

White wine

The dominance and commoditisation of Sauvignon Blanc has had a significant impact on the dynamics within the white wine category. What was once the driving force behind the growth of the category is now its Achilles heel. The scale of decline in Sauvignon Blanc is not being offset by growth in second tier varietals and white wine penetration is declining at twice the rate of 'total wine'. There is also limited price architecture to encourage customers to trade up.

Environment

Increasing temperatures are ripening grapes earlier, requiring more water and forcing wineries to adapt their plantings and vineyard management practices. Hotter temperatures have the potential to lead to higher alcohol levels, which flies in the face of customer trends towards lighter styles. There is an opportunity to build customer awareness and engagement with heat-tolerant varietals to better align with evolving consumer preferences. We can harness the environmental concerns and values of our customers and engage them in a conversation about sustainable winemaking practices and encourage them to discover emerging heat-tolerant varietals.

Macro-trends

In order to understand our customers' mindset and some of the factors that are driving the choices they make about where to shop and what to buy when they get there, we need to look first at some macro drivers.

We are very much a part of customers' discretionary spend. They pay the rent or the mortgage, the power bill, the insurance, basic food, fuel and all the other necessities and then they need to start making some choices and doing some prioritising. This means that the macro-economic environment can have a significant influence on what they choose to buy and how much they spend on it.

Wage growth whilst still barely positive is slowing, some key household expenses such as power and fuel costs are increasing at a rate well above overall inflation, and property prices are in a slump, particularly in the key New South Wales and Victorian markets. The general level of pessimism around slowing growth is impacting both consumer and business confidence[1].

Australians are feeling squeezed and their discretionary income is coming under increasing pressure. If they are going to spend it on a bottle of wine, they want to make sure that they're getting something they will love. They are also seeking our help to navigate through what is becoming a more diverse and confusing suite of brands within an increasingly complex product category.

Factors driving drinks' trends

So, when it comes time to make these choices what are the factors that come into play?

Health and well-being are becoming more important determinants in choosing anything that we consume. Wine is no exception, and while most people still have a balanced view of the role of alcohol in their lives, increasingly the 'no safe limit' narrative being prosecuted by the extreme elements of the neo-temperance movement is gaining traction. Wine drinkers are looking for alternatives that are lower in alcohol, sugar, preservatives and other additives, and our range of low- and no-alcohol brands across all categories has grown from a token offering a few years ago to a substantial and rapidly growing part of our range today. Mid-strength products now dominate sales of commercial beers, and zero-alcohol beer sales are growing rapidly. Lower-alcohol and no-alcohol wines are becoming more readily available as well as zero-alcohol spirits and cocktails.

New products and experiences drive engagement with the drinks category. To quote one non-wine example, the range of gins in our business has increased more than fivefold in the last four years. All of the net revenue growth in our business has come from brands that are new to our range, more than offsetting a net decline in sales of products within our existing range. Innovation is the life blood of the industry so a joint commitment to new product development will be one of the keys to future growth. As both brand owners and retailers, we need to be prepared to accept the occasional failure as the price of striving to keep our customers engaged with the products that we sell.

In order to satisfy their individual needs we can no longer take a one-size-fits-all approach. Customers' choices are making the market more fragmented and they are looking for products that not only fit with their lifestyle (organic, vegan, gluten-free, additive-free), but also ones that align with their values (local, authentic, biodynamic, ethically sourced, sustainable). Not only are they searching these products out but consumers, particularly millennials, have expressed a clear willingness to pay more for them[2].

It's this understanding of the fragmentation of consumer needs that will be a critical determinant of the future growth of the industry. Our customers are seeking quality, discovery, authenticity and provenance, and as much as there are pressures on the household income, they are prepared to pay for it if we can deliver.

The world of digital retail

If we are going to maintain the relationships that we have with our existing customers in the face of the threat from digital disruptors such as Amazon, and potentially capture emerging markets and customers looking to transact in new ways, we need to move quickly to enact the changes that doing business as a digital retailer will bring.

Our customers' expectations are being framed not only by our own industry but by the way they are transacting with other businesses. As retailers we need to be thinking about offering our wine range to our customers in the same way that Spotify offers music to theirs. Consider a balance between a back catalogue of your all-time-favourite wines, ones that you know and love, other stuff that's similar in style to the wines that you normally drink, and occasionally something that challenges you to take your taste in a different direction.

The definition of convenience is evolving—no longer is it just about physical presence, but about providing a consistent, familiar service through whatever channel suits the customers' changing needs. Convenience in the bricks and mortar world means that you're on my way home, you're still open when I get there, that I can park right out in front, that I can easily navigate the store, find what I want and get on my way again.

Convenience in the digital world means a seamless user experience, a website that's easy to navigate and search, content that helps me make the right choice but doesn't make me feel like a rejected member of the cognoscenti, not having to re-enter my details every time I transact, and being able to determine the time and place of delivery and to track its progress. At the same time I need to know that all of my personal details will be absolutely secure and only available to those who I have consented to share them with.

Convenience is only part of the story, the other drivers that sit behind what people buy and where they buy it are complex. It's data that is the key to unlocking this level of customer intimacy, and it's important to understand that the sharing of data is a transaction like any other. Our customers know that their data has value, and they share it with us on the expectation that they will receive something in return. They expect that the payback for them will be to help them navigate through the buying process as seamlessly as possible, and that we will use it to curate and offer a range that is relevant to their lifestyle and to their ever-changing tastes and preferences. In the new world of retail, the easiest way to turn your customers toward your competitors is to waste their time.

To paraphrase the financial advisers, past performance is not necessarily a reliable indicator of future results. Data that captures what people are buying, where and when they are buying it and how much they are paying for it, is not a perfect predictor of consumer prefer-

[1]Westpac consumer sentiment index 10 July 2019

[2]The Nielsen Global Survey of Corporate Social Responsibility and Sustainability: Feb-March 2015

ences and trends. What it can do is take a lot of the guesswork out of working out what brands or offers are going to appeal to a particular customer. A retailer who can tailor their range and promotional activity based on loyalty data can personalise not only product, but also eliminate the scatter-gun approach to pricing that erodes profitability all the way through the value chain. It can also dramatically shorten the odds of success for new brands and help brand owners to invest their marketing spend in a much more granular and targeted way, and consequently to extract considerably more value from that investment.

While it may seem that we are staring into a brave new world, there is nothing new about this type of personalisation. Looking back at retail in the early part of last century, your shopkeeper knew you, your family, what you liked to buy, knew what he had on his shelves, would ensure that what you needed was in stock, and would give you a good deal in the hope that you would return. The way that retailers served their customers barely changed in the century between 1900 and 2000.

Retail on a smaller scale with a curated range, a well-defined target market, and limited geography to cover is easier—note that I did not say it's easy, all retail is hard. But while there's an air of invincibility that comes with size and scale, there are plenty of examples of very good specialist operators who have carved out a profitable piece of the retail wine market due solely to the fact that they know their customers intimately and consequently are aware that the path to success is no longer determined by physical presence, but by providing a reliable and consistent experience through whatever channel suits the customer's needs.

Responding to customers' changing needs across 1,500 stores, a range of 10,000 SKUs, and millions of transactions every week is a different challenge entirely. To address this problem we need to accelerate our digital customer experience. In our business we've had a rude awakening over the past couple of years; we've rediscovered the value that our customers place on convenience and the importance of price has been put back into perspective. Price is only important on the product that you are buying. If you're buying a bottle of Oliver's Taranga Shiraz you may not necessarily be aware of or care about the price of a slab of Corona.

The challenge that we have as big retailers is to make the transition from our 20th century model—the model that has made us historically successful—to one that is both uncomfortable and unfamiliar. Volume and scale deliver economies and we need to capture that value but at the same time maintain and build on the connections that we have with our customers, and that is difficult.

If you are anything like me you are probably contemplating the amount of personal information you need to share to facilitate this intimate level of engagement with your favourite retailer, and contemplating taking the scissors to that loyalty card that you have in your wallet. It's a perfectly understandable position. The fact is that all of these relationships are built on trust. We have an obligation to ensure that we do not betray the trust that our customers have placed in us. We need to ensure that their data is secure and that it is not used for any purpose other than those we have disclosed.

Conclusion

Unfortunately, any realistic view of what the future of our industry looks like must take into account the significant threats to our social licence to operate. Despite decades of evidence, some elements of the public health lobby are vigorously prosecuting a 'no safe level of alcohol' consumption message. Calls for controls on price, availability and advertising mirror those which have been imposed on tobacco.

Research over the past 40 years has confirmed again and again that moderate consumption of the wines that we make and sell has a protective effect and actually lowers all-cause mortality compared with that cohort of the population who abstain from alcohol entirely.

I find it a difficult scenario to imagine that governments might some day enact a social marketing campaign that encourages teetotallers to enjoy a couple of glasses of wine a day for the good of their health. But we live in hope.

> There is no excuse to ignore regulatory interventions for access, advertisements, and unit cost that are shown to reduce alcohol consumption. Like tobacco, the longer the delay in effective control, the more severe future interventions for alcohol will need to be. It is not unimaginable that bottles of Château Mouton Rothschild, which once bore the artwork of Salvador Dali and Pablo Picasso, might one day be required to have plain packaging and images of oesophageal cancer or a cirrhotic liver. (The Lancet, Vol. 390: 18 November 2017)

What's happening internationally and why you need to care

S. Taylor

Sustainable Business International, Washington DC, USA
sandra@sustainablebizintl.com

Abstract

Men and women approach wine very differently. The collector profile is still predominantly male, while high-end wine buyers—those who regularly spend more than $15 on a bottle of wine—are mostly women. Women are purchasing wine more than ever before. The introduction of wine sales through supermarket stores in many nations has been one of the key factors that has dramatically changed wine purchasing gender roles. Research shows that 70 per cent of wines sold in the UK are purchased by women in supermarkets as part of their regular grocery shopping. In the US market, some have reported that women make over 60 per cent of wine purchases. Women are also more likely to purchase sustainably produced wines. This helps to illustrate why the female wine purchaser has been of such significant interest to wine producers and marketers in recent years. Women are also assuming leadership roles in every aspect of the industry, which will impact production, sustainability and marketing in the future. The observed increase in wine purchasing by females has resulted in wine marketers paying special attention to this consumer segment and featuring women more in their advertising. According to the Wine Market Council, 60 per cent of wine drinkers in the United States are women. Also 'highly involved' female wine drinkers are mostly older millennials who tend to be 'urban educated professionals' and are generally more ethnically diverse than the typical female wine drinker. Thus, the wine industry believes that women are the future of wine.

In countries around the world businesses and governments grapple with policies in response to the #MeToo movement, the Black Lives Matter movement, asylum seekers, immigration and sexual orientation.

So much is motivated by unconscious bias. I'd like to quote the Wine Enthusiast blog by Julia Coney, a wine writer in Washington, DC who is studying to be a sommelier:

> "Excuse me, you look like you work here." "Are you sure you're in the right room?" "I'm sorry, I thought you were the help." "How do you afford to travel like you do?"
>
> I grew up in a house where words meant things. And not just the words themselves; "it's not what you say, but how you say it," echoed daily. I used to mock my parents for saying it until I became an adult and realized the adage's simple truth. The questions and statements below are just a few things that have been said to me while I attended wine tastings.
>
> Did I mention I'm an African-American woman? Maybe I should have led with that. Now, read those statements again. Do you see a problem? Now multiply these statements with looks, comments and racial bias—real, not perceived. This is my wine life.
>
> My response to most of these statements was to ignore the person. Anything else would have given validation to their statements. I chose to act like they didn't exist. There were no words needed.
>
> The wine world is interesting. It's wide and vast, but the thinking about who wine represents still sits in a time lapse. My beloved industry is made of dynamic, smart people, some of the kindest people I know. There's an energy that makes me come alive when drinking, reading, writing and discussing wine. But, like most fields, there are issues around diversity that need to be addressed, and the lack of representation for people of color is a major problem.
>
> "Diversity" is a buzzword. It's right up there with "lean in," "woke" and "inclusive." Words mean things, but without action, they turn into old-school lingo. I'm often the only person of color at tastings. We represent less than 10% of attendees. How is this in 2018? I know many wine professionals of color. We're out here, it's not hard to find us. We just need to be welcomed in.
>
> So, if you see me at a tasting, say hello. That's a great place to start both change and a conversation. Don't judge, and don't make assumptions. Words mean things. Even the small ones.
>
> Whatever you do, don't mistake me for the help. (Coney 2018)

This resonated so much with me because this story of unconscious bias has been part of my journey in so many instances throughout my career where I was often the only person of colour in executive meetings.

I am starting to see some small progress in the wine industry in the US—there is a growing list of black winemakers in California and Oregon. And there are a few investors in wine brands—mostly wealthy athletes and entertainers including LeBron James, Dwayne Wade and John Legend, some who have wine brands developed for them in Napa, and this is drawing African-American consumers to fine wines. I created a wine education program in Washington for women from diverse backgrounds called Fine Wine Divas to help them experience wines from around the world. Small steps but so much more needs to be done by the industry to recognise and capture this huge consumer market of African-American consumers.

Lack of diversity creates barriers to entry, and we are missing talent.

I recently met a university professor who described his research on a diversity issue that is totally drowned out by gender, race and LGBTQ inclusion. It's the fact that 99 per cent of employees hired and employed by Fortune 500 corporations in the US come from some 25 top-ranked and top-dollar universities, and less than one per cent from other institutions and community colleges which are often the only paths for people trapped in poor neighbourhoods to escape the poverty trap.

Of course, naturally you feel sorry for the smart, ambitious kid from a poor neighbourhood who didn't have much opportunity and never made it up the ladder! But feel bad for industry too—we are missing out on some potentially great talent!

Understand the business case, not simply social justice

Fortunately, awareness of the business case for inclusion and diversity is on the rise. While social justice typically is the initial impetus behind these efforts, companies have increasingly begun to regard

inclusion and diversity as a source of competitive advantage, and specifically as a key enabler of growth.

Recent McKinsey research (Hunt et al. 2018) found that companies in the top quarter for female representation on executive teams were 21 per cent more likely to experience above-average profits than companies in the lowest quarter, and 27 per cent more likely to experience better long-term value creation. Closing the gender gap at the executive level is more than 'doing good'—it's a needed step to increase the bottom line.

Diverse teams tend to better reflect your customer base. They make better and more innovative decisions. Diversity is essential for recruiting and retaining quality talent today. And finally, the clincher: companies with diverse boards and senior executives make more money, period.

Adweek recently reported that only 25 of 77 Chief Marketing Officers in the food and beverage category were female. Of chief marketers surveyed, 87 per cent were white; only three per cent were black/African American. About five per cent of CMOs identified as Asian and Hispanic/Latino respectively (Oster 2018).

How can these teams successfully execute diversity and multicultural marketing campaigns?

As the wine industry forecasts future markets, women, millennials and diversity categories must be part of that strategy—and that requires hiring or retaining smart marketing managers who understand and represent those categories of potential customers.

How can you move forward?

Human nature tends to gravitate towards the familiar, to 'people like us'. In the business world, this is compounded by a long-standing system that has traditionally been male-oriented, male-dominated and influenced by gender bias. Working toward inclusivity starts with awareness—awareness of these trained inclinations, unconscious bias and established societal barriers.

Using diverse interview slates, implementing behaviour-based tools and training on unconscious bias will certainly make a difference. Measure inclusivity at all levels. Include diversity and inclusion measurements in compensation goals and performance metrics.

For the wine industry we also need to look at our supply chains and examine whether there are human rights abuses in the chain and also how we treat migrant/casual labour.

The immigration situation in the US has meant that many vineyard workers must now be legal residents and there are many in California who are. But we're seeing shortages of vineyard labourers, and the impact can be devastating; some vineyards simply aren't able to harvest their fruit because they don't have the people power.

The industry should take this opportunity to help labourers who are legal residents learn about the functions of the wine business, including viticulture, wine production, cellar operations, sales and hospitality management. A winery owner or manager should invite workers to educational tastings and host courses on these topics. Of course language training is also a prerequisite.

Second, it's important that vineyard labourers are treated with dignity and respect—they are the foundation of the operation. If they don't work, nothing happens.

We should recognise the value of these workers and give them opportunities for growth and education. Share information and resources to equip them for success. It's the right thing to do, and it makes business sense—when labourers learn more about what's being made, you develop a more passionate, engaged workforce. There are some good examples of where this is being done in Napa and in Willamette Valley. At Trefethen they employ their labourers year-round, having them train and work in the tasting room when the vineyard work is done in the winter. Trefethen is also proud to say they provide the same health care to their labourers as the owners receive.

We can do better!

References

Coney, J. (2018) Diversity in the World of Wine. Wine Enthusiast: 4 May: https://www.winemag.com/2018/05/04/diversity-in-wine/

Hunt, V.; Prince, S.; Dixon-Fyle, S; Yee, L. (2018) Delivering through Diversity. McKinsey & Company: January: https://www.mckinsey.com/business-functions/organization/our-insights/delivering-through-diversity

Oster, E. (2018) Brands Fail to Meet the ANA's Diversity Goals, Too. Adweek: 27 March: https://www.adweek.com/brand-marketing/brands-fail-to-meet-the-anas-diversity-goals-too/

Matching viticulture, weather and climate datasets to inform decision-making

M.J. Collopy, N. Benger

Australian Government Bureau of Meteorology, Adelaide, SA
matthew.collopy@bom.gov.au

Abstract

Modern viticultural practices involve a complex array of testing, analysis and response to critical points in grapevine phenology. The detail and volume of the information gathered through this process is increasing. Viticulturists typically have access to past datasets, which include timing of key stages in the plant cycle such as budburst, flowering, fruit set, veraison, sugar level changes and harvest.

In addition to phenology data, many vineyards hold climatology records of key weather elements such as rainfall, temperature and often more detailed soil moisture and irrigation records.

The Bureau of Meteorology maintains a high-quality national climatology dataset, derived from point sources with observations dating back over 100 years, and modern automated weather stations with very high temporal resolution measurements. The Bureau combines weather stations with satellite-derived elements and numerical methods to inform high-resolution gridded climatology for a range of weather elements. Recent advances include using Numerical Weather Prediction (NWP) models in back-cast mode to produce highly detailed datasets with hourly outputs on a 12 km grid for all weather parameters.

Matching vineyard phenology and viticultural information with climate and weather datasets can yield informative grapevine phenology predictors. Forecast predictions and related uncertainty measures can inform a range of important decisions including those involved in disease risk and control, canopy management and broader logistics planning.

Introduction

The goal of the Business Solutions group within the Bureau of Meteorology is to deliver impact and value through industry collaboration to develop specialist skills for focus sectors and deliver real solutions. The Agriculture team works closely with producers in all areas of agriculture, including the wine industry, to deliver real solutions which support profitable and sustainable production now and into the future.

The Australian wine industry faces increasing competition in national and international markets, combined with increasing input costs and pressures on per tonne prices. Staying competitive in this economic climate requires the adoption of the technologies and business processes which drive better crop outcomes for quality and yield. Variations in weather and climate create a constant challenge for producers, impacting all areas of the grapegrowing industry from fruit development and quality, to disease management, through to processing, storing and logistics. Developing a systematic approach to tracking and understanding how weather variables affect key phenology milestones can inform decision-making and minimise risk.

A systematic approach to using weather data combined with the knowledge of related key phenology milestones would contribute to efficient industry operations. Through well-informed decision-making processes based around weather data, producers can mitigate weather impacts (such as frost or heatwaves), improve disease/pest control and optimise harvesting and logistics planning.

Viticulturists are developing increasingly comprehensive and sophisticated datasets of both the vineyard inputs and grapevine phenology. Converting these datasets into predictive tools has typically been applied through analogue year approaches—comparing past years' outcomes to current. Changing climates and increasing variability in weather elements such as temperature and rainfall have limited the usefulness of this technique, as does the limited number of years of data used to develop such analogue predictors.

The Bureau of Meteorology maintains Australia's most comprehensive climate dataset based on over 100 years of digitised observational data, employing peer-reviewed and published data analysis techniques. Recent investment has linked the power of Numerical Weather Prediction (NWP) models with past data records to produce a 30-year (1990–present) reanalysis dataset, assimilating observation data to output very high-resolution spatial and temporal fields for over 100 weather parameters across the Australian region.

Bureau forecast services span very short-term detailed forecasts and warnings, through to 7-day forecast datasets for over 50 weather parameters for all of Australia at 6 km resolution. This graphical and point forecast service is based on a range of national and international NWP sources with expert meteorologist input for critical local weather phenomena.

The Bureau offers seasonal forecast services based on the Bureau's seasonal model (ACCESS-S) (Hudson et al. 2017) supplying information at weekly, monthly and seasonal time-scales about climate drivers and weather parameters. Information is probabilistic in order to capture the inherent uncertainty of longer-range forecasts; for example, the probability of warmer/cooler or wetter/drier conditions for time periods out to the seasonal time-scale.

Merging climate records, vineyard inputs and grape phenology datasets can yield predictors for key milestones such as flowering, veraison and harvest. These can be further enhanced by incorporating real-time weather forecasts at daily and seasonal time-scales: moving towards a more sophisticated analogue year approach to inform key resourcing and logistical decisions.

Observational data and climate analyses

The Bureau's Australian Data Archive for Meteorology (ADAM) has climate observations dating back to the mid-1800s, with over 200 million rainfall records from over 16,000 locations. In addition to rainfall observations, this record includes weather parameters such as air temperature, wind, sunshine and soil temperatures.

A modern, digital observational dataset dating from 1910 to the present provides the basis for current climate reference analyses, in line with peer-reviewed science and developed in collaboration with the CSIRO and the World Meteorological Organisation. This data is the basis for Bureau-gridded climatology—providing analyses at daily and monthly time-scales for parameters including rainfall (Figure

1) (Hope et al. 2015), max./min. temperatures and soil moisture at 5 km spatial resolution.

Recent advancements and investment have seen the development of the Bureau of Meteorology Atmospheric high-resolution Regional Reanalysis for Australia (BARRA, Figure 2) (Su et al. 2018). The regional reanalysis suite is based on the Australian Community Climate Earth-System Simulator (ACCESS) which ingests the full range of surface and satellite observations to produce an atmospheric reanalysis for the Australian domain at a 12 km resolution—downscaled to a 1.5 km resolution for selected sub-domains.

About 100 parameters are archived for a 29-year dataset (1990–present) at hourly time intervals for surface conditions such as temperature, precipitation, wind speed and direction, humidity, evaporation and soil moisture, as well as information on solar radiation and cloud cover. Providing an additional layer of detailed spatial and temporal weather climatology which can be applied to sub-daily time-scales and can be utilised to derive selected exceedance and probability elements.

Forecast datasets

Bureau service outputs and modelling provide gridded weather and climate forecasts for time-scales ranging from days to months. At 1- to 7-day time ranges, detailed weather data at 6 km resolution is provided through web-based graphical displays (MetEye®) (Figure 3) and the Australian Digital Forecast Database (ADFD) – weather parameters include temperatures, humidity, wind speed and direction, and probabilistic rainfall amounts.

Direct weather model and prediction systems outputs are available for many weather parameters—some through general, publicly available displays and others through registered user access to detailed NWP model data files. These systems span weather and marine forecast maps, through to the full model output at high-resolution in horizontal, vertical and temporal outputs.

Beyond the detailed seven-day outputs, the Bureau uses the recently operational ACCESS Seasonal (ACCESS-S) model which has land (60 km resolution) and ocean (25 km resolution) components providing predictions of key climate drivers such as El Niño and the Indian Ocean Dipole (Figure 4).

ACCESS-S produces map- and point-based rainfall and temperature outlooks for weekly, monthly and seasonal time-scales based on the output of a 99-member ensemble for the future climate (Figure 5). An ensemble prediction system runs the model a number of times with small perturbations in the initial starting point and physical processes. These perturbations represent the uncertainty in the state of the system; multiple runs then provide a measure in the spread of uncertainty in the outcome at varying time-scales.

The Bureau in collaboration with the CSIRO produces a range of climate prediction analyses and reports. These projections are based on an understanding of the climate system, historical trends and model simulations of the climate response to global scenarios of greenhouse gas and aerosol emissions. Simulations come from the archive of global climate models (GCMs) developed by modelling groups from around the world through the Coupled Model Intercomparison Project phase 5 (CMIP5) which

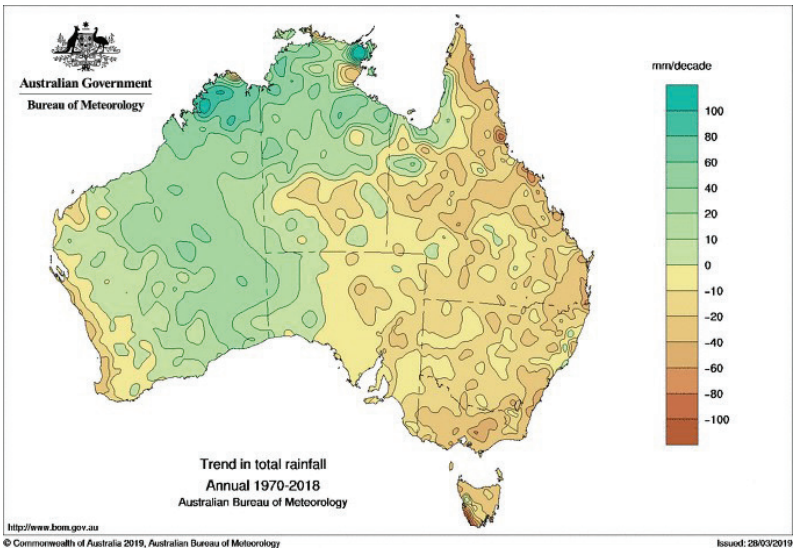


Figure 1. Bureau-gridded rainfall climatology 1970–2018 rainfall trend (Hope et al. 2015)

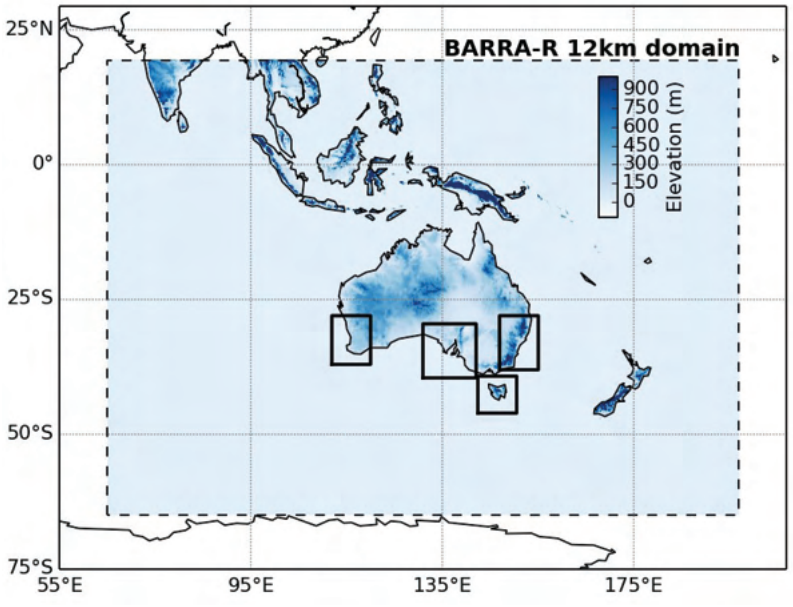


Figure 2. BARRA reanalysis 12 km resolution domain (with 1.5 km domain insets) (Su et al. 2019)

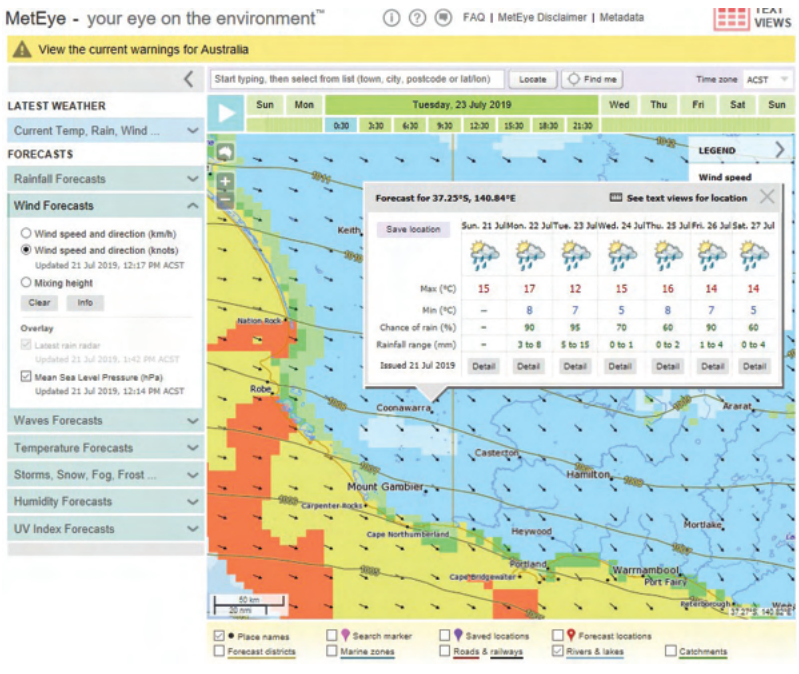


Figure 3. Bureau graphical MetEye® wind forecast, with detailed point (Coonawarra) forecast overlaid

also underpins the science of the Intergovernmental Panel on Climate Change.

Projected changes to Australia's climate based on the results of the CMIP5 models depict changes to mean temperature, rainfall, wind speed, solar radiation, relative humidity and potential evapotranspiration. Typical outputs provide ranges of predicted change based on 10-90th percentiles of the empirical distribution of the CMIP5 for decadal time-scales.

Tailored weather and climate services

Through close interaction with wine industry customers, a series of key weather sensitivities and risks were identified, these included:

- Temperature (both short- and long-term): a predictor of the rate of ripening and insight into canopy management at seasonal scales
- Extreme temperatures: maximum temperature – heat stress, minimum temperature – frost damage
- Rainfall and humidity: disease potential (critical close to harvest) – related to water uptake and fruit quality, leaf wetness preventing harvest.

These risks and sensitivities feed into vineyard management (machine and labour hire), business process and risk mitigation decisions.

To service these sensitivities and issues, a range of briefing and weather parameter displays were developed as maps, time-series plots, and tables with threshold alerts. These were accompanied by advice from climate and meteorological specialists to aid in the interpretation of the information. Table 1 shows detailed maximum and minimum temperature, plus median rainfall forecasts for a range of vineyard locations, with thresholds highlighted, in this case minimum temperatures below 4°C for potential frost.

Figure 6 depicts forecast time series for temperature (hourly and max./min.) with probalisitic spread; plus relative humidity, vapour pressure deficit, wind speed and rainfall with probability of exceeding thresholds.

Understanding uncertainty

In addition to the detailed location-specific tables and plotted time series, briefing materials provide insight into the predictability of weather outcomes. All weather forecasts contain a degree of uncertainty; however, some weather systems are inherently less predictable. This is borne in verification data and the recognition and understanding of this uncertainty is part of the expertise built up by Bureau meteorologists.

Figure 7 shows the modelled synoptic pressure pattern and 24-hour rainfall from two numerical weather prediction sources at a lead time of 7–8 days. The Bureau ACCESS model is shown on the left and the European centre ECMWF model on the right. Both the Bureau and European models represent the latest science in weather prediction but have slight differences in how physical processes are represented and modelled. This results in the differing forecast outcomes for the position of the low-pressure system, depicted to the south-west of the South Australian coast by the Bureau ACCESS system, whereas the European model has the low to the south of Tasmania. The uncertainty in the position of the low is typical for such as system at lead time beyond 5–6 days, and results in a significant difference in the rainfall pattern—where the ACCESS forecast is dry for South Australia and the ECMWF shows 10–20 mm of rainfall in the 24-hour forecast period. This complexity in the forecast outcome and possible rainfall scenarios is a layer of additional information which can inform decision-making, beyond that available through single-source values.

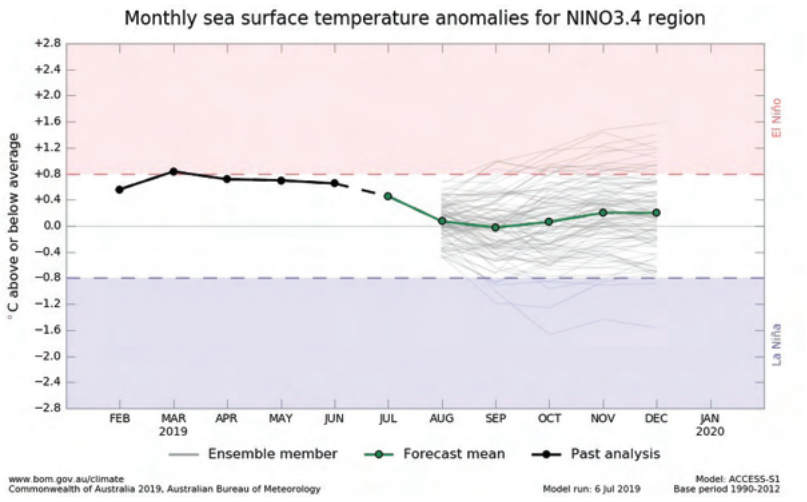


Figure 4. El Niño forecast – Pacific Ocean (NINO3.4 region) sea surface temperature anomalies ensemble output and mean depicted

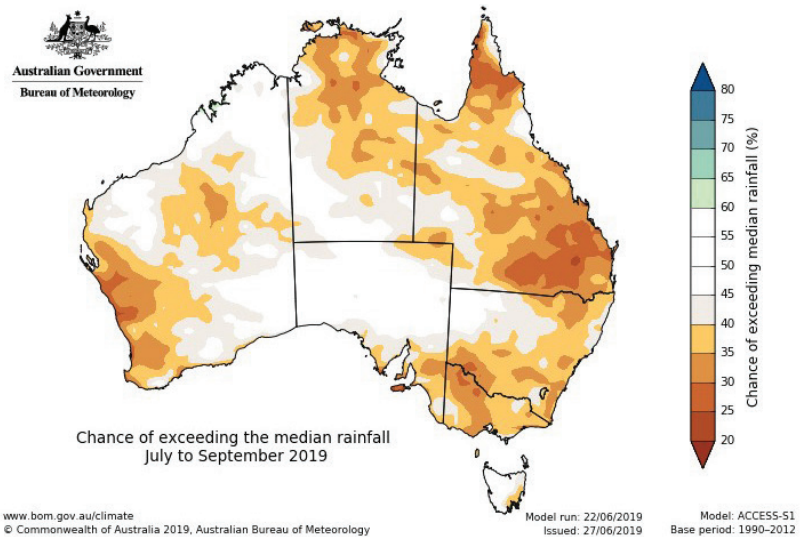


Figure 5. ACCESS-S seasonal outlook – chance of exceeding median rainfall July-September 2019

Table 1. Detailed temperature and rainfall forecast for defined location

Location	Mon 10/09/2018			Tue 11/09/2018			Wed 12/09/2018			Thu 13/09/2018			Fri 14/09/2018		
	Temp		Rain	Temp		Rain	Temp		Rain	Temp		Rain	Temp		Rain
	Max (°C)	Min (°C)	Rain (mm)	Max (°C)	Min (°C)	Rain (mm)	Max (°C)	Min (°C)	Rain (mm)	Max (°C)	Min (°C)	Rain (mm)	Max (°C)	Min (°C)	Rain (mm)
Barossa V	26	5	0	27	10	0.5	18	4	0	21	3	0	25	5	0
Eden V	23	5	0	20	11	1.7	13	4	0.2	15	3	0	19	5	0
Coonawarra	21	8	0	20	10	4.3	14	5	0.5	16	5	4.9	18	7	1.3
Robe	22	7	0	23	10	3.1	15	4	0.5	17	3	0.8	20	6	0.3
Murray D	27	5	0	28	10	0.6	19	4	0	21	3	0	26	5	0

Matching weather and phenology data

Viticultural records are an increasingly rich source of data which can be matched with key weather climatological data. With a sufficiently long record the data can be applied to known phenology predictors such as Growing Degree Days (GDD) for differing grape varieties—providing predictors based on temperatures across the grapevine seasonal pattern. Prediction with a measure of uncertainty can be provided for milestones such as budburst, flowering and veraison. Applying current season temperature records with short and seasonal forecasts of temperature for the remainder of the growing period can then inform vineyard planning and viticultural practices—in-line with the expected timing of the grapevine milestones. Application of this methodology depends on the quality and length of record of the past phenology data, aggregating this data across common varieties in a given region may yield positive outcomes for multiple growers. Figure 8 shows GDD accumula-

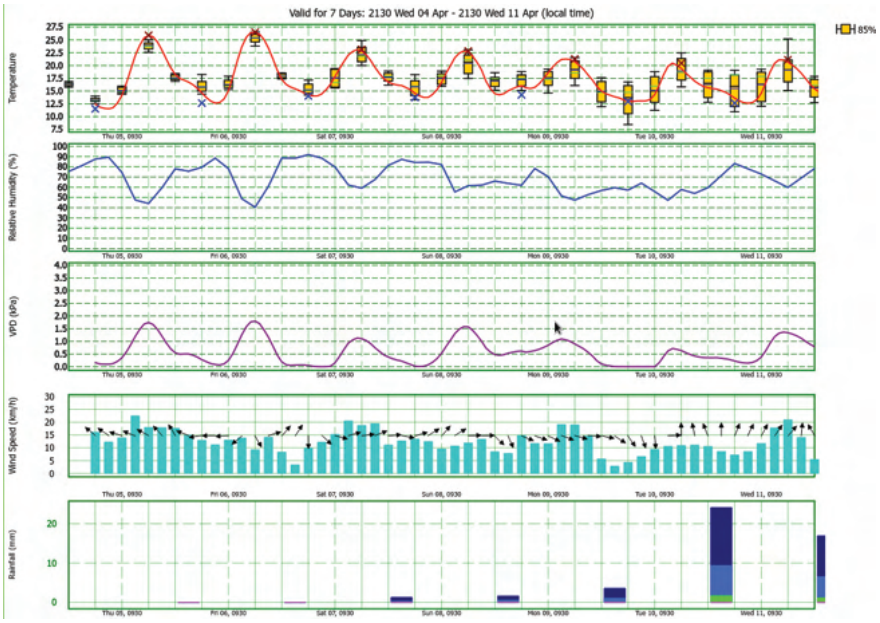


Figure 6. Detailed forecast time series for temperature, humidity, vapour pressure deficit, wind and rainfall

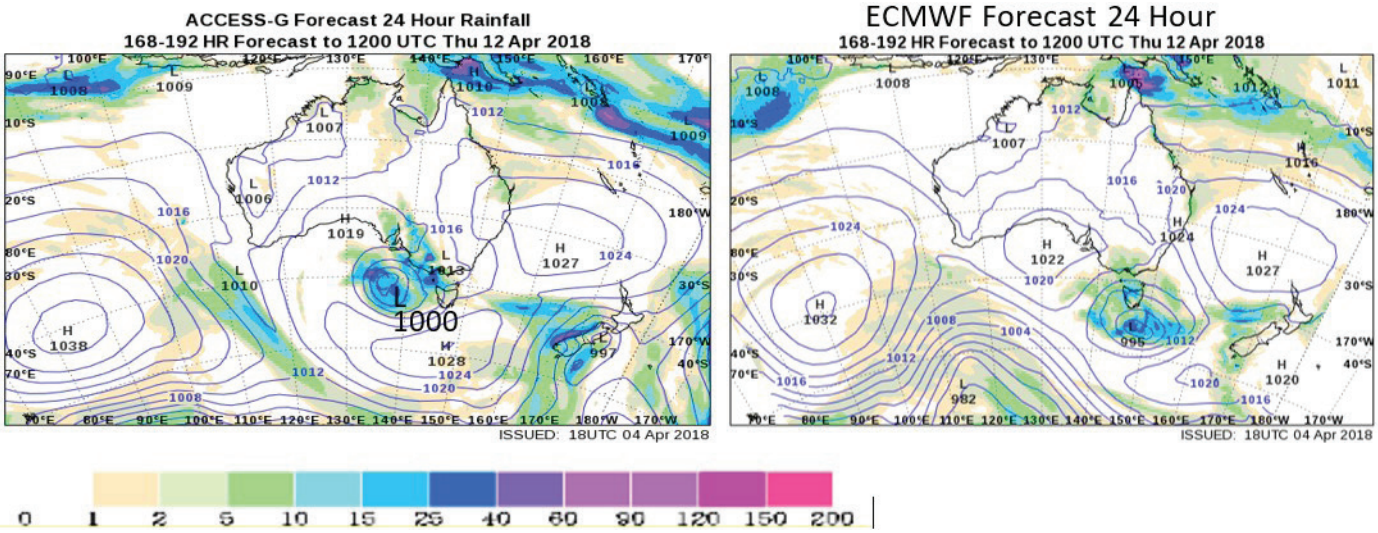


Figure 7. Model 24-hour rainfall forecast for lead time of 7–8 days (ACCESS on left, ECMWF right)

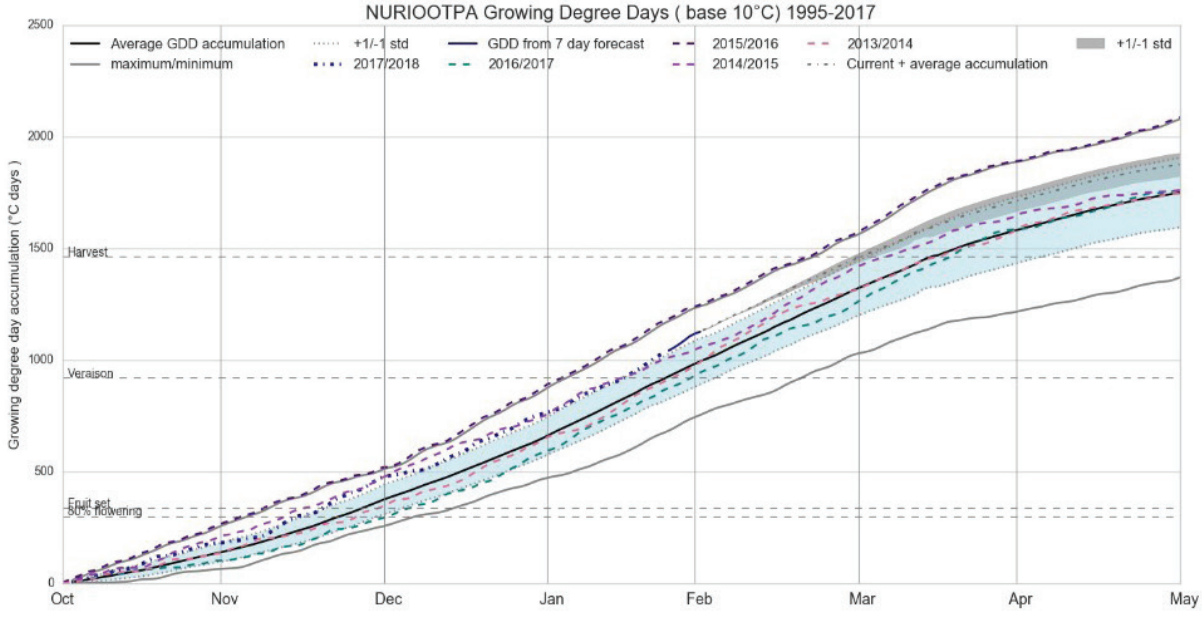


Figure 8. Growing Degree Day (base 10°C) for Nuriootpa (observation base record 1995–2017)

tion for Nuriootpa in the Barossa Valley with markers for flowering (80%), fruit set, veraison and a predictor for approximate harvest. This plot was provided in late-January 2018 and has forecast temperature values for the remainder of February to May, with increasing spread based on +/- standard deviation from expected seasonal temperatures. Also shown are the climate records for recent past analogue years, hottest and coldest on record, plus the median value.

Results: vineyard operational impacts

Working in partnership with vineyard operators and managers, Bureau services were adapted to provide weather and climate information most relevant to current challenges and business decisions. This close feedback and tailoring process resulted in benefits including:

- Improved short-term (weekly) vineyard planning – labour and machinery
- More efficient and effective forward planning through a common understanding of weather expectations across the business
- Stimulated innovative thinking around adaptive management strategies
- Advanced notice of extremes, allowing for mitigation decisions and actions.

Open dialogue between the Bureau as a weather service provider, and the viticulture customer with specific weather sensitivities aligned the knowledge of the two organisations. This resulted in greater impact from weather and climate information for vineyard management and business decisions.

Summary

In this paper we have presented some of the existing services provided by the Bureau of Meteorology to support winegrape growers. The goal of the Bureau's Agriculture Group is to deliver value to the agriculture industry. We do this through industry collaboration to identify where we can generate value using the Bureau's extensive resources to support profitable and sustainable production now and into the future.

Winegrape growers have shown interest in adapting operations to the future environmental conditions and competitive markets to mitigate the effects of variations in weather and climate, climate change and rapidly developing technology. To remain competitive both locally and internationally it is imperative to adapt and minimise the risk associated with decisions made on a daily, weekly and seasonal basis. A common practice amongst vineyard managers is the use of analogue years to predict the timing of crucial stages in the grapegrowing cycle. The service offered by the Bureau is a more sophisticated approach to using analogue years. It is also an evolving service, working with our industry and research partners to continually develop ways to exploit new weather and climate information, both historical and forecast, in conjunction with phenology data.

Future goals for this service are to broaden the scope to other crops, regional assessments for appropriate production conditions, calculation of chill hours/units, automated data ingestion for 'smart farms', heat stress management, strategic harvesting and integrated pest management. To continue to support the wine industry, and the Australian agriculture industry more broadly, we will proactively engage with producers and stakeholders to focus our future services on the areas where we can deliver the most impact.

Acknowledgements

The paper was produced with the support of the Bureau of Meteorology Business Solutions, Agricultural Program. Contacts and partnerships in the Australian wine industry included Treasury Wines Estates and Taylors Wines.

References

Hope, P.; Grose, M.R.; Timbal, B.; Dowdy, A.J.; Bhend, J.; Katzfey, J.J.; Bedin, T.; Wilson, L.; Whetton, P.H. (2015) Seasonal and regional signature of the projected southern Australian rainfall reduction. Aust. Meteorol. Oceanogr. J. 65: 54–71.

Hudson, D.; Alves, O.; Hendon, H.H.; Lim, E.-P.; Liu, G.; Luo, J.-J.; MacLachlan, C.; Marshall, A.G.; Shi, L.; Wang, G.; Wedd, R.; Young, G.; Zhao, M.; Zhou, X. (2017) ACCESS-S1: The new Bureau of Meteorology multi-week to seasonal prediction system. J. South. Hemisph. Earth Syst. Sci. 67(3): 132–159.

Su, C.-H.; Eizenberg, N.; Steinle, P.; Jakob, D.; Fox-Hughes, P.; White, C.J.; Rennie, S.; Franklin, C.; Dharssi, I.; Zhu, H. (2019) BARRA v1.0: The Bureau of Meteorology Atmospheric high-resolution Regional Reanalysis for Australia. Geosci. Model Dev. Discuss. 12: 2049–2068.

CSIRO (2015) Climate Change in Australia Technical Report. Ekström, M.; Gerbing, C.; Grose, M.; Bhend, J.; Webb, L.; Risbey, J. (eds). Available from: https://www.climatechangeinaustralia.gov.au/en/publications-library/technical-report/

Adapting to climate change in Europe

C. van Leeuwen[1], A. Destrac[1], M. Dubernet[2], E. Duchêne[3], M. Gowdy[1], E. Marguerit[1], P. Pieri[1], A. Parker[4], L. de Rességuier[1], N. Ollat[1]

[1]EGFV, Bordeaux Sciences Agro, INRA, Université de Bordeaux, ISVV, Villenave d'Ornon, France
[2]Laboratoires Dubernet, ZA du Castellas, Montredon des Corbières, France
[3]UMR 1131 Santé de la Vigne et Qualité du Vin, INRA, Université de Strasbourg, Colmar, France
[4]Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand
vanleeuwen@agro-bordeaux.fr

Abstract

Temperatures have increased by approximately 1°C over the course of the 20th century and will continue to rise over the next century at a rate depending on greenhouse gas emissions. Modifications of rainfall patterns show local variability, but most winegrowing regions worldwide are being affected by more frequent and intense periods of summer drought, because reference evapotranspiration increases with rising temperatures. Wine quality and yield are strongly influenced by climatic conditions and depend on complex interactions between plant material, temperatures and water availability. In established winegrowing regions growers have optimised output in terms of yield and quality by choosing plant material and viticultural techniques according to local climatic conditions. When the climate changes, plant material and cultural practices need to be adjusted. Winegrowers worldwide are facing this challenge. In Europe, awareness about the potential impact of climate change on viticulture rose at the end of the 20th century and created a strong research focus on potential adaptations. Adaptations to higher temperatures include all possible techniques (trunk height, leaf area to fruit weight ratio, timing of pruning, etc.) and modifications in plant material (rootstocks, cultivars and clones) which maintain harvest dates in the optimal period at the end of September or early October in the northern hemisphere. Vineyards can be made more resilient to drought by planting drought-resistant plant material (rootstocks and cultivars), planting goblet-trained bush vines or trellised vineyards at wider row spacing or selecting soils with greater soil water-holding capacity. Most vineyards in Europe are dry farmed. Implementation of irrigation is also an option to grow sustainable yields under dry conditions but should be avoided when possible because of environmental impacts.

Introduction

Like other agricultural crops, grapegrowing is impacted by environmental conditions such as soil and climate. The profitability of agricultural production is driven largely by yield; however, for winegrape growing, the quality potential of the grapes is also important, as it can significantly affect the quality of the resulting wine and the price consumers are willing to pay. In fact, wine prices can vary by a factor up to 1,000 (from 1 to 1,000 € per bottle), while yields generally vary by a factor of about 10 (from 3 to 30 tonnes/ha). Environmental conditions play an important role, not only in yield but also grape quality potential, and hence the overall profitability of wine production.

The output of grape production in terms of yield and quality can be optimised through the choice of plant material (variety, clone, rootstock) and viticultural techniques (training system, vineyard floor management, etc.). Profitability is also impacted by production costs which can be reduced through mechanisation. In established winegrowing regions, growers have historically adjusted their plant material and viticultural techniques through trial and error and research to achieve the best possible compromise between yield, quality and production costs. Because environmental conditions are different in each location, there is no general recipe that can be applied to all. This explains why plant material and viticultural techniques vary so much across the winegrowing regions of the world.

Depending on environmental conditions and access to market, high profitability can be more easily achieved in some regions by optimising yields and reducing production costs, while in other locations profitability can be driven by high quality and high wine prices. High yields can be obtained when soil and climate induce little or no limiting conditions for photosynthesis: moderately high temperatures, non-limiting light, nitrogen and water availability. When soil and climate induce a limitation of water and nitrogen, these can be supplied through irrigation and fertilisation.

Highest possible quality potential is generally achieved when environmental conditions are moderately limiting. Ideal balance in grape composition at ripeness—sugar/acid ratio, colour and aroma—is obtained when grape ripening occurs under moderate temperatures. Excessively cool climatic conditions during ripening can result in 'green' and acidic wines. On the other hand, temperatures between veraison and harvest that are too high can result in unbalanced fruit composition, with sugar levels too high, acidity too low and aromatic expression dominated by 'cooked fruit' aromas (van Leeuwen and Seguin 2006; Pons et al. 2017), resulting in wines that lack freshness and aromatic complexity. Mild temperatures during grape ripening, favourable to wine quality, are generally met late in the growing season, roughly between 10 September and 10 October in the northern hemisphere and in March or early April in the southern hemisphere. White wine production is optimised under cool ripening conditions which are of particular importance in obtaining intense and complex aroma expression. When varietal heat requirements match the critical temporal window to obtain ripeness, the best wine quality ensues. For red wine production, water deficits at specific stages of grape development are favourable for wine quality, because they reduce berry size and increase phenolic compounds in grape skins (Matthews and Anderson 1988; Ojeda et al. 2002; van Leeuwen et al. 2009; Triolo et al. 2019). Recently, it has also been shown that vine water deficits positively influence aromatic expression in mature wines (Picard et al. 2017; Le Menn et al. 2019). Moderate nitrogen uptake induces similar effects on grape composition, reducing berry size and increasing skin phenolics (van Leeuwen et al. 2018). For improved quality in white wines, a limitation in vine water status is also desirable, although this limitation should be milder than for red wine production (Peyrot des Gachons et al. 2005). For white wine from thiol-driven aromatic varieties such as Sauvignon Blanc, Colombard, Sémillon and Riesling, vine nitrogen status should not be limiting (Helwi et al. 2016).

Given the factors promoting yield versus quality, it makes sense to optimise profit by maximising yield in warm areas on rich soils, while under cool climate conditions and in poor soils maximum profitability is better achieved by producing premium wine quality, to be sold at the highest possible price.

Although soil and climate are both major environmental components in wine production, the latter is of greater importance for the development of yield components, vine phenology and grape composition (van Leeuwen et al. 2004; van Leeuwen et al. 2018). Until the end of the 20th century, soil and climate were considered stable in a given site, with the exception of year-to-year climatic variability. In the 1990s some European researchers became aware that the shifting climatic conditions due to climate change might possibly have a great impact on viticulture worldwide (Schultz 2000). Progressively, over the first two decades of the 21st century, climate change has become a topic of increasing importance in the viticulture and oenology research community. In 2011, 23 French research laboratories collaborated in the LACCAVE (long-term adaptation to climate change in viticulture and enology) project to study the effect of climate change in viticulture and potential growers' adaptations (Ollat et al. 2017). Several peer-reviewed scientific journals, including the *Journal of Wine Economics* (Storchmann 2016) and *OENO One* (Ollat et al. 2017) released special issues on this subject. Today, a substantial body of literature is available to assess the effects of climate change in viticulture and wine production, including effects on vine physiology, phenology, grape composition and wine quality. Also, potential adaptations have been studied to help continue production of high-quality wines with economically sustainable yields under changing climatic conditions.

Temperature and drought effects of climate change

Temperature changes associated with climate change are not homogeneous around the globe. Temperatures are currently 1°C higher on average than pre-industrial revolution (IPCC 2014), but the increase is even higher in some regions. In Bordeaux for example, Average Growing Season Temperature (AvGST; Jones et al. 2005) has increased by approximately 2°C over the past 70 years, with a remarkable jump between 1985 and 2006 (Figure 1A). Temperatures have become increasingly warm during the period of grape ripening, as is shown by temperature summations >30°C during 45 days before harvest (Figure 1B for Bordeaux). This can significantly affect the rate and timing of vine phenology and the eventual quality of the grapes. Also, as increased temperatures increase the evaporative demand driving both vine transpiration and soil evaporation, the soil water balance over the season will become increasingly negative (Figure 1D; van Leeuwen and Darriet 2016). And while annual rainfall has not seen much change in long-term trends, there has been an increase in extreme wet and dry years (Figure 1C for Bordeaux). Taken together, increased temperatures resulting in higher reference evapotranspiration values (Figure 1D) and more frequent years with low rainfall have induced, and will continue to induce, more intense and frequent drought conditions for vineyards in Bordeaux and around the world.

Temperature effects

Temperature is the major driver of vine phenology (Parker et al. 2011, 2013). Increased temperature as a consequence of climate change leads to advanced phenology (van Leeuwen and Darriet 2016; Duchêne and Schneider 2005; Figure 2). Similar trends are observed in many winegrowing regions around the world (van Leeuwen and Darriet 2016). Advanced budbreak may expose vines more frequently to spring frost, although this risk depends on the climatic situation of each specific winegrowing region (Sgubin et al. 2018). Varieties which have historically been selected for performing best in a given winegrowing region may move out of their ideal ripening window. Harvest dates in Alsace (France) for Riesling used to occur in the first two weeks of October. Today in this region, harvests more frequently occur in the first week of September and sometimes even at the end of August. This evolution can be detrimental for the quality potential

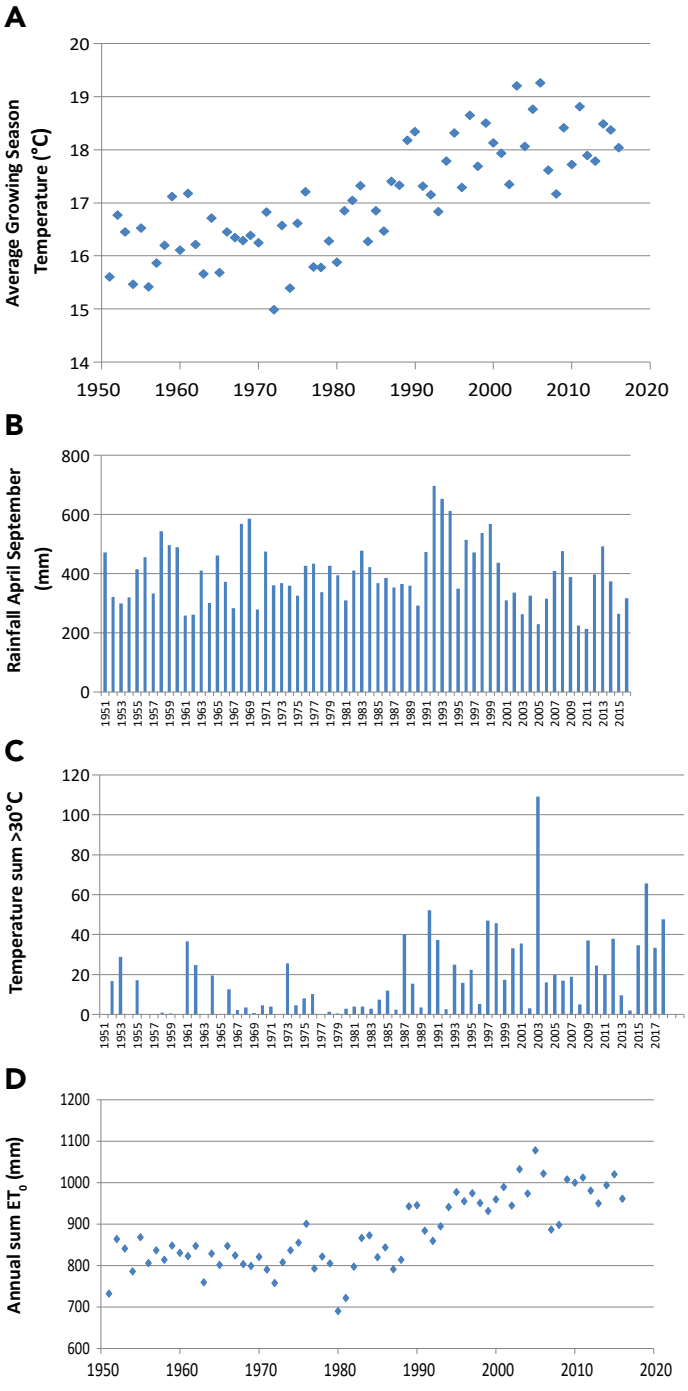


Figure 1. Climate data for Bordeaux (Bordeaux Mérignac weather station) from 1951–2018. A. Average growing season temperature; B. Temperature sum >30°C during 45 days prior to harvest; C. Rainfall April – September; D. Annual sum of reference evopotranspiration (ET_0)

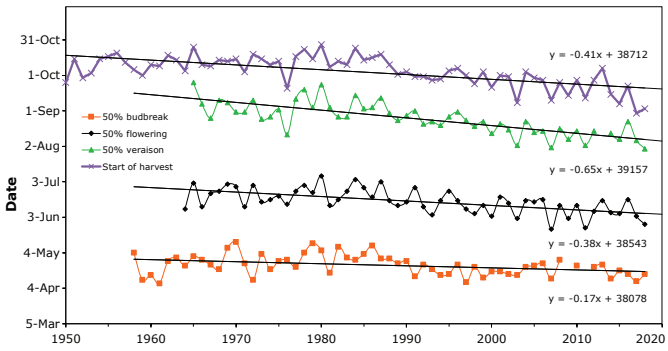


Figure 2. Long-term evolution of vine phenology for Riesling in Alsace. Data source: budbreak, flowering and veraison adapted from Duchêne and Schneider (2005); harvest dates from Conseil Interprofessionnel des Vins d'Alsace (CIVA)

of the grapes, which are increasingly high in sugar content (Duchêne and Schneider 2005) and may eventually become less aromatic.

In Bordeaux, major grapevine varieties include Sauvignon Blanc, Merlot, Cabernet Franc and Cabernet Sauvignon. Harvest dates can be modelled by using the Grapevine Sugar Ripeness model (GSR) to predict sugar ripeness (Parker et al. 2019). According to this model, 200 g/L of grape sugar is attained when a daily mean temperature summation reaches a value F^* (base temperature of 0°C, start date day of the year 91, which is 1 April in the northern hemisphere). F^* is variety specific, where a higher value indicates a later ripening variety (Figure 3).

In the following example, the GSR model was used to predict the day when four major grapevine varieties grown in Bordeaux (Merlot, Cabernet Sauvignon, Cabernet Franc and Sauvignon Blanc) would reach 200 g/L of sugar, with input temperature data from the Bordeaux Mérignac weather station and F^* values retrieved from Parker et al. 2019 (Figure 3). To predict harvest dates, five days were added for Sauvignon Blanc, which is picked at around 210 g/L of grape sugar (12.5% potential alcohol). For harvest dates of the three red varieties, 15 days were added, because they are generally picked at 230 g/L of grape sugar (13.5% potential alcohol). When the model was run with average historical temperature data from 1951–1980, modelled ripeness was 22 September for Sauvignon Blanc, 4 October for Cabernet Franc, 7 October for Merlot and 14 October for Cabernet Sauvignon (Figure 4). These projections are perfectly in line with observed harvest dates from Bordeaux (van Leeuwen and Darriet 2016). If the ideal window for grape ripeness is defined from 10 September to 10 October, when temperatures are not excessive but still high enough to achieve full ripeness, all varieties fall within this window except Cabernet Sauvignon. This is consistent with the observation that during this period high-quality Cabernet Sauvignon wines could only be produced in early ripening locations on warm gravel soil. In the cooler parts of Bordeaux, wines from Cabernet Sauvignon used to be 'green' (high in methoxypyrazine content) and acidic. When the same projection is made with average climate data from 1981–2010, the following harvest dates were obtained: 7 September for Sauvignon Blanc, 18 September for Merlot, 21 September for Cabernet Franc and 28 September for Cabernet Sauvignon (Figure 4). At the turn of the millennium, Bordeaux became suitable for growing high-quality Cabernet Sauvignon over most of the region but marginally too warm for Sauvignon Blanc. It is predicted that it will still be possible to grow high-quality Sauvignon Blanc in cooler locations of the region on north-facing slopes or on cool soils. When 1°C is added to the average 1981–2010 temperatures (which is close to temperature projections for around 2050), the Bordeaux climate is still perfectly suitable for producing high-quality wines from Cabernet Franc and Cabernet Sauvignon (projected harvest 11 and 18 September respectively), but Merlot is moving out of the ideal ripening window (8 September) and the Bordeaux climate will be too warm to produce crisp and aromatic wines from Sauvignon Blanc (29 August; Figure 4). Hence, among the traditional Bordeaux varieties, Sauvignon Blanc and Merlot will be the first victims of climate change. During the past decade, Bordeaux wines containing a majority of Merlot, which is still the most widely planted variety in this region, are increasingly dominated by 'cooked fruit' aromas and excessively high alcohol content (Pons et al. 2017).

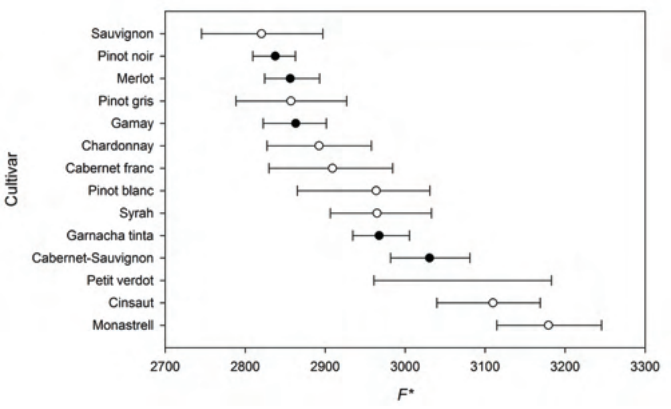


Figure 3. Temperature summation (F^*) to reach 200 g/L of grape sugar according to grapevine sugar ripeness (GSR) model for 15 major grapevine varieties

In general, grape and wine compositions have dramatically changed over the past three decades worldwide. Mean data from Languedoc, France shows that over a 35-year time span, grape sugar content expressed in potential alcohol increased from 11% to 14%, pH from 3.50 to 3.75 and total acidity decreased from 6.0 to 4.5 g/L (Figure 5). Similar observations have been made in many regions around the world (Schultz 2000; Duchêne and Schneider 2005; Petrie and Sadras 2008; Mira de Orduña 2010).

Drought effects

Climate change will also expose vines to increased drought, either because of reduced rainfall, or because of higher reference evapotranspiration due to elevated temperatures. This may lead to lower yields, because several yield parameters are impacted by water deficits, in particular berry size (Ojeda et al. 2002; Triolo et al. 2019) and bud fertility (Guilpart et al. 2014). On the other hand, water deficit has a positive effect on red wine quality because grape skin phenolics increase (Ojeda et al. 2002; van Leeuwen et al. 2009; Ollé et al. 2011) and wines develop more complex aromas during bottle ageing (Picard et al. 2017; Le Menn et al. 2019). So far, the best vintages in Bordeaux (where vines are not irrigated) are dry vintages (van Leeuwen and Darriet 2016). The frequency of dry vintages has increased over the past three decades and this has resulted in better vintage ratings in recent years. In white wine production only very mild water deficits are positive for wine quality, while more severe water deficits are detrimental (Peyrot des Gachons et al. 2005). For red wines, the

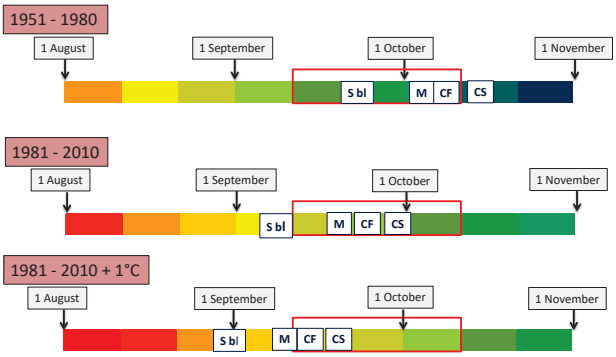


Figure 4. Modelled harvest dates for Sauvignon Blanc (S bl), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) in Bordeaux for the following periods: 1951–1980, 1981–2010 and 1981–2010 + 1°C. Sugar ripeness is modelled with the Grapevine Sugar Ripeness model (GSR; Parker et al. 2019). Temperature data is from the Bordeaux Mérignac weather station. Warm colours indicate higher temperatures and cool colours indicate lower temperatures.

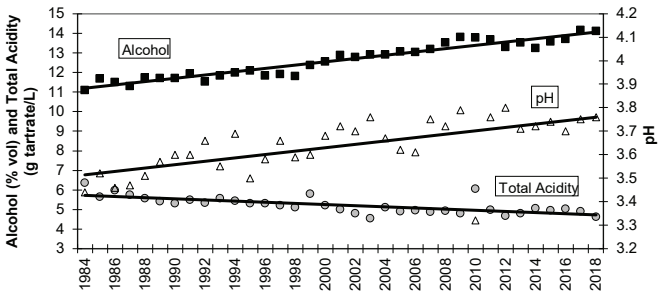


Figure 5. Evolution of red wine composition in the Languedoc region (France) from 1984 to 2018. Each data point is the average of several thousands of analyses of red wines just after alcoholic fermentation (data: Dubernet laboratory, F-11100 Montredon des Corbières)

general tendency under increased drought is lower yields and better quality (except situations of severe water stress); for white wine, not only yields can be negatively affected but quality can also be jeopardised.

In established winegrowing regions, growers have optimised output in terms of quality and yield by choosing plant material, viticultural techniques and winemaking that are most adapted to their local environment. Now that the climate has become warmer and drier in most winegrowing regions, this balance is threatened. Specific adaptations are needed to continue to produce optimum quality and yield in a changing environment.

Adaptations to higher temperatures

Higher temperatures advance grapevine phenology. Hence, grapes ripen earlier in the season. When grapes achieve full ripeness in the warmest part of the season (July–August in the northern hemisphere; January–February in the southern hemisphere) grape composition can be unbalanced (e.g. high sugar levels and low acidity), with red grapes containing less anthocyanins. Wines from these grapes will lack freshness and aromatic complexity. Adaptations to higher temperatures encompass all changes in plant material or modifications in viticultural techniques with the purpose of delaying ripeness.

Later ripening varieties

In all traditional winegrowing regions in Europe, growers have planted varieties that ripen between 10 September and 10 October under local climatic conditions. This is the case for Riesling in the Rheingau; Chardonnay and Pinot Noir in Burgundy; Merlot, Cabernet Franc and Cabernet Sauvignon in Bordeaux; Grenache and Carignan in Languedoc; Tempranillo in La Rioja; Sangiovese in Tuscany; Nebbiolo in Barolo; Touriga Nacional in Douro and Monastrell (Mourvèdre) in Alicante. Now that temperatures have increased, traditional varieties may move out of the ideal ripening window with detrimental effects on wine quality. In this context, one potential adaptation to a changing climate is to plant later ripening varieties. The Ecophysiology et Génomique Fonctionelle de la Vigne research unit (EGFV) from the Institut des Sciences de la Vigne et du Vin (ISVV) near Bordeaux planted the VitAdapt vineyard experiment in 2009, where 52 varieties are planted with five replicates to study physiology, phenology, ripening dynamics and wine quality (by small-scale vinifications) to assess how these varieties behave differently in a warming climate (Destrac-Irvine and van Leeuwen 2016). The experimental set-up includes later ripening varieties from warm locations like Touriga Nacional, Tinto Cão (Portugal, red varieties) and Assyrtiko (Greece, white variety; Figure 6). Data from this vineyard shows average veraison dates (2012 to 2018) spanning over 34 days, demonstrating the extent to which later ripening can be achieved by simply changing the variety (Figure 7).

In European wine appellations, the choice of varieties is regulated to allow only varieties that perform best in terms of quality and typicity under local climatic conditions. Under a changing climate, however, these regulations will need to be modified. Recently seven new varieties, including Touriga Nacional, were accepted for planting in up to 5% of area in Bordeaux winegrowing estates to allow testing with full-scale vinifications. This percentage may be increased if the experiments are conclusive. The choice of the varieties allowed for testing was based directly on results from the VitAdapt experiment.

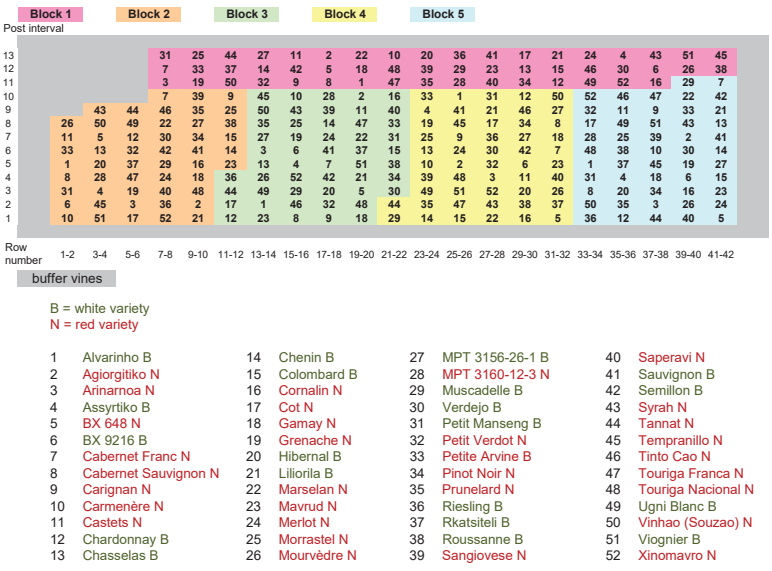


Figure 6. Layout of the 52 varieties planted in the VitAdapt experiment, with five replicates per variety and 10 vines per replicate

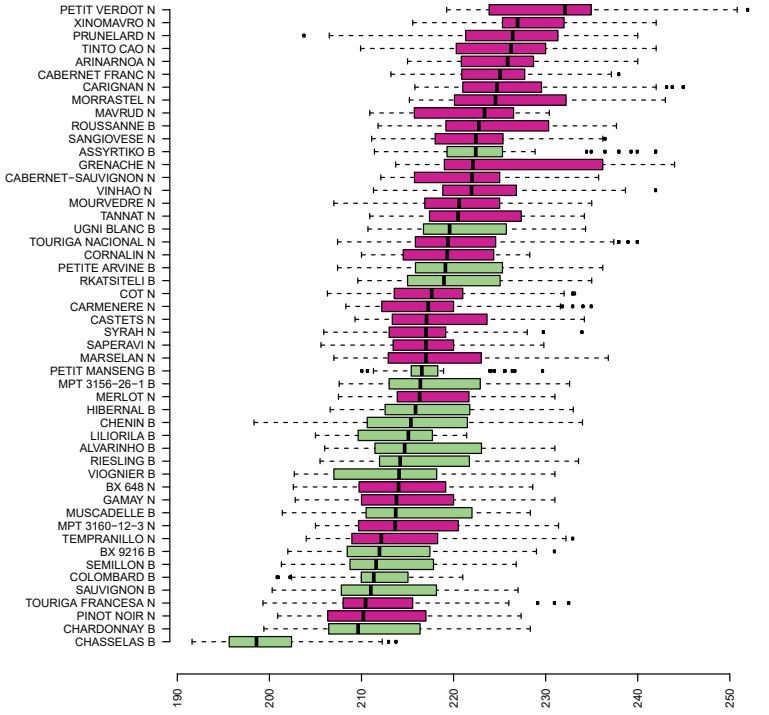


Figure 7. Box-plot of observed mid-veraison dates of varieties planted in the VitAdapt experiment (average day of the year from four replicates per variety over the period 2012–2018)

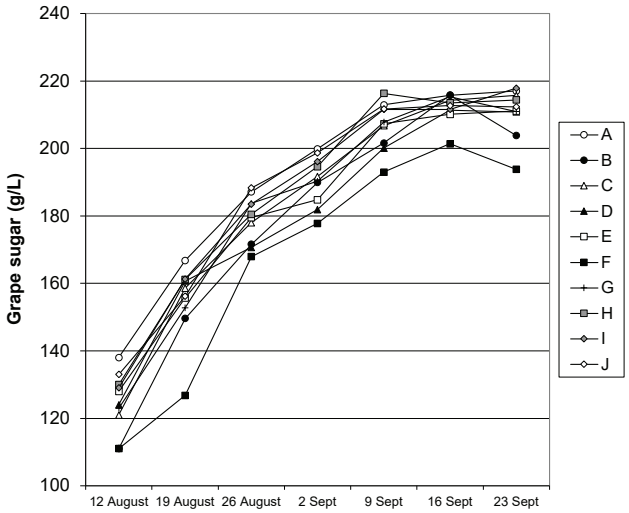


Figure 8. Sugar accumulation dynamics in 2013 from a private clonal selection program on Cabernet Franc. A – J represent 10 different clones (van Leeuwen et al. 2013)

Later ripening clones

Within a given variety a certain level of genetic variability exists, referred to as clonal variability. Historically, clones have been selected for traits such as high productivity, early ripening and high sugar content in grapes. In the context of a changing climate it may be preferable to select new clones with the opposite characteristics. Sugar accumulation dynamics vary among clones, as shown from an example of a clonal selection trial on Cabernet Franc (van Leeuwen et al. 2013; Figure 8). At ripeness, differences in grape sugar concentration among clones can be more than 17 g/L (1% potential alcohol). In the same clonal collection, differences in mid-veraison dates ranged from six to nine days depending on the vintage (data not shown).

Later ripening rootstocks

Rootstocks can influence the phenology of the grafted scion. Some rootstocks induce earlier phenology and ripening, while others induce a longer cycle (Bordenave et al. 2014; van Leeuwen and Destrac 2017). Precise data on this effect is scarce in scientific literature. In 2015 the GreffAdapt experiment was planted by the EGFV research unit from the ISVV. In this project, 55 rootstocks are phenotyped with five different scions in field conditions. Each combination is planted with three replicates (Marguerit et al. 2019). Over the coming years this experimental vineyard will yield precise information regarding whether and how rootstocks may induce differences in grapevine phenology and the timing of ripeness.

Increasing trunk height

Trunk heights determine the distance from the soil to the grapes and can vary according to training systems from 30 cm to over one metre. Maximum temperatures are higher close to the soil and the resulting vertical temperature gradient can be used to fine-tune the microclimate in the bunch zone through variations in trunk height. In Bordeaux, where the climate historically has been marginal for ripening Cabernet Sauvignon, growers planted this variety on warm gravel soils and trained the vines with short trunks to have the bunches as close as possible to the soil. In warmer climatic conditions as caused by climate change, the temperatures may be too high close to the soil surface, in particular for early ripening varieties in the Bordeaux context like Sauvignon Blanc and Merlot. An experiment was set up in the Saint-Emilion winegrowing region where temperature sensors were installed at 30, 60, 90 and 120 cm on vine posts with three replicates in four different vineyard blocks (Figure 9A). The Winkler Index as measured in these canopies was 60 degree days lower at 120 cm compared to 30 cm (Figure 9B). Based on a 19°C average temperature (which corresponds to 9°C above a base of 10°C) this difference may induce a delay of seven days in grape ripening.

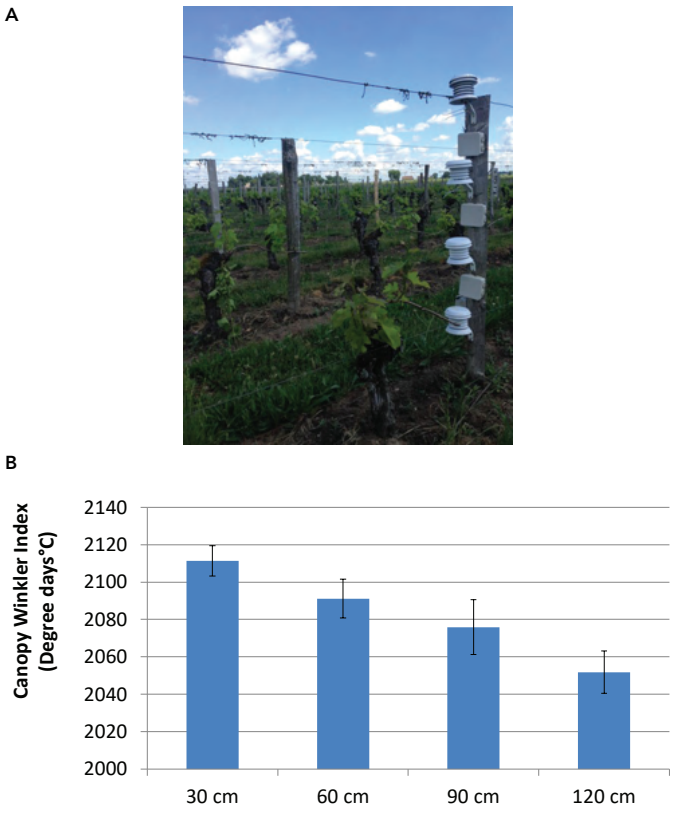


Figure 9. A. Temperature sensors installed at 30, 60, 90 and 120 cm heights in a vineyard in Saint-Emilion (France). B. Variations in Canopy Winkler Index between 30 and 120 cm in height

Reducing leaf area to fruit weight ratio

A leaf area to fruit weight ratio (LA:FW) of at least 1 m^2/kg of fruit is generally considered as necessary to ensure optimum ripening conditions and in particular sugar accumulation (Kliewer and Dokoozlian 2005). Reducing LA:FW ratios can considerably delay veraison and sugar accumulation in grapes, with limited effect on total acidity (Parker et al. 2014, 2015). Lower LA:FW ratios, however, adversely affect anthocyanin accumulation in grapes, which makes this technique more applicable in white wine production than red wine production.

Late pruning

When winter pruning is carried out late, budbreak is delayed by a few days (Friend and Trout 2007). However, differences tend to become smaller for subsequent phenological stages. Maturity is more substantially delayed when vines are pruned a second time, well after budburst (Fiend and Trout 2007; Martínez-Moreno et al. 2019). This technique, however, is still experimental and long-term carry-over effects on vigour need to be studied.

Moving to higher altitudes

In mountainous areas, temperatures decrease by 0.65°C per 100 m of elevation. If other vineyard adaptations are not adequate, and if topography permits (Douro, Portugal; Mendoza, Argentina), moving vineyards to higher altitudes can be an effective adaptation to a warming climate. In Mendoza, varieties are grown according to the altitude—in very warm conditions at 800 m above sea level (a.s.l.) entry-level wines are produced from high-yielding vines. Finer wines are produced from Malbec and Cabernet Sauvignon planted at 1,100 m a.s.l. and early ripening Chardonnay and Pinot Noir planted at 1,500 m a.s.l. Moving vineyards to higher elevations, however, may have detrimental environmental effects associated with disruption to wildlife habitat and ecosystem services, which need to be considered (Hannah et al. 2013).

Combination of adaptations

The previously mentioned changes in plant material and viticultural techniques can be progressively implemented. Some of them do not require major changes in viticultural management (e.g. late pruning), while others may involve replanting vineyards with a potential change in wine typicity (e.g. change of varieties). To a certain extent, these techniques can be combined, but further research is needed to assess if the delaying effect of combining several techniques is additive. Overall, depending on the rate of climate warming, such adaptations should be effective for decades to come, except perhaps for already very hot winegrowing areas.

Adaptations to increased drought

Water deficits reduce yield but, except in situations of severe stress, can have a positive effect by promoting red wine quality. The production of high-quality white wines requires mild water deficits. With

increasing water deficits as a consequence of climate change, yields are negatively impacted, decreasing profitability of wine production. Hence, adaptations to drier growing conditions is becoming increasingly pertinent in viticulture worldwide. The vine is a highly drought-resistant species. In the Mediterranean basin there are thousands of years of experience growing vines in warm and dry conditions. In a context where water is an increasingly scarce resource it is important to take advantage of this expertise. Potential adaptations to increased drought include the use of drought-resistant plant material, the implementation of specific training systems, locating vineyards where soils have greater soil water-holding capacity, and possible use of irrigation.

Drought-resistant rootstocks

Since phylloxera reached Europe in the second half of the 19th century, most vines in the world are grafted on rootstocks. Rootstocks vary considerably in their ability to resist drought. Several authors have addressed this issue (Carbonneau 1985) and recently a compilation was made by Ollat et al. 2015 (Table 1). Physiological mechanisms behind drought tolerance in rootstocks (as measured on the scion) were studied by Marguerit et al. (2012). This issue will be further investigated in field conditions in the GreffAdapt experiment in the EGFV research unit in Bordeaux (Marguerit et al. 2019). The use of drought-resistant rootstocks to sustain yields and avoid quality losses from excessive water stress is a powerful and environmentally friendly adaptation to increased drought, and once planted such rootstocks do not increase production costs.

Table 1. Drought tolerance among rootstocks (adapted from Ollat et al. 2015)

Rootstocks	Usual name	Phylloxera resistance	Water stress adaptation
Riparia Gloire de Montpellier	Riparia Gloire	High to Very High	Low
Grézot 1	G1	Low to Medium	Low
Foëx 34 École de Montpellier	34 EM	High	Low to Medium
Millardet et de Grasset 420 A	420 A	High	Very Low to Medium
Kober-Téléki 5 BB	5 BB	High	Low to Medium
Téléki 5 C	5 C	High	Low to Medium
Couderc 1616	1616 C	High	Low to Medium
Rupestris du Lot (St. George)	Rupestris	Medium to High	Low to Medium
Millardet et de Grasset 101-14	101-14 MGt	High	Very Low to Medium
Couderc 3309	3309 C	High	Very Low to High; mostly Low to Medium
Téléki-Fuhr Selection Oppenheim n°4	SO4	High	Very Low to High; mostly Low to Medium
Téléki 8 B	8 B	High	Low to Medium
Dog Ridge	Dog Ridge	High	Very Low to High
Schwarzmann	Schwarzmann	High to Very High	Very Low to Medium
Couderc 1613	1613 C	Low to Medium	Low to Medium
Couderc 161-49	161-49 C	High	Low to Medium
Kober-Téléki 125 AA	125 AA	High	Medium
Millardet et de Grasset 41B	41B	Medium to High	Very Low to High, mainly Medium
Castel 216-3	216-3 Cl	High	Medium
Fercal INRA Bordeaux	Fercal	Medium to High	Medium
Gravesac INRA Bordeaux	Gravesac	High to Very High	Medium
Freedom	Freedom	Medium to High	Medium
Harmony	Harmony	Low to Medium	Medium to High
Foëx 333 École de Montpellier	333 EM	Medium to High	Low to High, mainly Medium to High
Richter 99	99 R	High	Medium to Very High
Börner	Börner	Very High	High
Castel 196-17	196-17 Cl	Low to Medium	Medium to High
Georgikon 28	Georgikon 28	High	High
Malègue 44-53	44-53 M	High	Medium to Very High
Ramsey	Ramsey	High	Medium to Very High
Paulsen 1103	1103 P	High	High to Very High
Paulsen 1447	1447 P	High	High to Very High
Richter 110	110 R	High	High to Very High
Ruggeri 140	140 Ru	High	High to Very High

Drought-resistant varieties

Grapevine varieties are highly variable in their tolerance to drought (Chaves et al. 2007). This may be linked to the way different varieties regulate their water potential in response to increasing atmospheric demand and decreasing soil water content. Some varieties appear to control their water potential more closely (isohydric behaviour) under drought conditions (Schultz 2003), although the characterisation of this response has recently been challenged (Charrier et al. 2018).

The way varieties modify their water use efficiency in response to drought is another useful indication of varietal drought tolerance. At the leaf level, water use efficiency is the amount of carbon assimilation (i.e. carbohydrates produced by photosynthesis) for a given amount of transpiration through the stomata (i.e. water loss). At the plant level, it is the yield of grapes and change in vine biomass compared to the amount of water consumed by the vine over the season (Tomás et al. 2012). Clonal differences in water use efficiency have been observed (Tortosa et al. 2016) and may be a useful tool for assessing the drought tolerance of different varieties. Analysing the carbon isotope discrimination in grape berry juice sugars provides an integrative measure of the water use efficiency of a grapevine over the course of the berry ripening period (Bchir et al. 2016). A comparison of changes in carbon isotope discrimination (i.e. water use efficiency) between wet versus dry years can help characterise the drought resistance of different varieties.

Most grapevine varieties originating from the Mediterranean basin (Grenache, Cinsault, Carignan) are considered drought tolerant, while varieties like Merlot, Tempranillo or Sauvignon Blanc are not. Some local varieties from Mediterranean islands, like Xinisteri from Cyprus are reported to have a very high drought resistance and deserve experimentation outside this original region of production (Manganaris, pers. comm.). A study of the underlying physiological mechanisms of drought resistance is currently being undertaken in the VitAdapt projects (EGFV research unit, ISVV Bordeaux; Gowdy et al. 2019). Planting drought-resistant varieties in dry environments is a logical step in adapting to climate change, and therefore these varieties deserve increased attention.

Training systems

Over centuries, winegrowers in the Mediterranean basin have developed a training system which is particularly resistant to drought and high temperatures: the so-called Mediterranean goblet or bush vine. With this training system it is possible to dry farm vines in extremely dry environments, down to a mere 350 mm of rainfall/year. Although goblet-trained vines generally produce low yields, they are easy to cultivate at reduced production costs on a per hectare basis. Despite low yields, production costs expressed on a per kilogram basis are not necessarily high. They present the drawback, however, of being difficult to harvest by machine. If harvesting goblet-trained vines could be mechanised, this would further reduce production costs for this otherwise drought-resistant training system.

An alternative solution to increasing the drought resistance of a vineyard is to increase row spacing. Row spacing is traditionally high in regions where water deficit is not a major issue, like Bordeaux, Champagne and Burgundy (France). Close row spacing optimises sunlight interception, which allows the production of high-quality wines at moderately high yields. When water is, or becomes, a limiting factor, close row spacing increases water use because sunlight interception is providing the driving energy for transpiration. The effect of row spacing on water balance was recently modelled by van Leeuwen et al. (2019) for three row spacings (2 m = 5,000 vines/ha; 3 m = 3,333 vines/ha and 4 m = 2,500 vines/ha) and three levels of total transpirable soil water (TTSW), a concept similar to soil water-holding capacity (Lebon et al. 2003). The output of the water balance model is the fraction of transpirable soil water (FTSW), where the lower the FTSW, the greater the water deficit experienced by the vines. The output of the water balance modelling demonstrated that vine spacing had an important effect on water balance and water availability during grape ripening, except when TTSW was already low (Figure 10). It should be noted that increased vine spacing reduces both yield (and related revenue) and production costs, with profitability depending on the trade-off between these two effects. Modelling found production cost savings outweighing yield-related revenue loss when producing lower-value grapes, while the opposite was true for production of higher-value grapes (van Leeuwen et al. 2019).

Soil water-holding capacity

TTSW or soil water-holding capacity has a major impact on vine water status. In the analysis described above and presented in Figure 10, average FTSW for the 30 days prior to modelled harvest is 0.43, 0.26 and 0.19 for TTSW of 300 mm, 200 mm and 100 mm respectively. Note that vines do not face any water deficit when FTSW is between 1.00 and 0.40 and that water deficits are increasingly intense for FTSW between 0.40 and 0.00 (Lebon et al. 2003). TTSW depends on soil type (texture and content in coarse elements) as well as rooting depth. In dry climates it makes sense to plant vineyards in soils with at least medium TTSW. Rooting depth can be promoted by thorough soil preparation, such as deep ripping.

Irrigation

To avoid yield losses due to drought, irrigation is also an option when adequate water resources are available. Vineyard irrigation is not a historical technique in the Mediterranean basin, where the vast majority of vines are still dry farmed. Although the acreage of irrigated vineyards is increasing, it is likely that there will never be enough water to irrigate the total area which is currently under vines. Hence, dry farming should be considered as a precious skill, of which the underlying mechanisms need to be better understood. Another drawback of irrigation is that in some situations (in particular when winters are dry), it can lead to increased soil salinity, which results in reduced long-term suitability of vineyard soils for cultivation.

When irrigation is chosen as a technique for vineyard management in dry climates, consideration must also be given to the potential negative impacts on regional surface and groundwater resources, including the effect on other potential users of water and the surrounding environment. If irrigation is implemented, techniques such as deficit irrigation should be used with precise vine water status monitoring (e.g. by measuring stem water potential) in order to limit, as much as possible, the amount of irrigation water applied. However, even with finely tuned irrigation management, the blue water footprint of an irrigated vineyard is generally at least 100 times higher than a dry farmed vineyard.

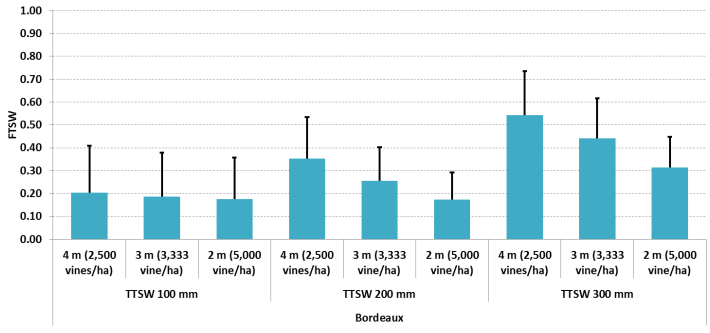


Figure 10. Modelled average fraction of transpirable soil water (FTSW) during 30 days prior to modelled harvest dates for three vine spacings (2 m, 3 m and 4 m) and three levels of total transpirable soil water (100 mm, 200 mm and 300 mm). Input weather data from 1981–2010, Bordeaux Mérignac weather station

Conclusion

Due to climate change, vines are facing increasingly warm and dry growing conditions. The vine is, however, a plant of Mediterranean origin which is well adapted to these conditions. But higher temperatures shift phenology and the ripening period to a time in the season which is less favourable for the production of quality wine and increasingly dry conditions lead to yield reduction. In some situations this improves wine quality, in particular in the production of red table wines, while excessive water stress may jeopardise wine quality. Adaptations to climate change include modifications in plant material and viticultural techniques which delay phenology and grape ripening and increase drought tolerance. The use of late-ripening and drought-resistant plant material (varieties, clones and rootstocks) is an environmentally friendly and cost-effective tool for adaptation. The vast genetic diversity in vines for these traits constitutes a precious resource to continue to produce high-quality wines with sustainable yields in a changing climate.

References

Bchir, A.; Escalona, J.M.; Gallé, A.; Hernández-Montes, E.; Tortosa, I.; Braham, M.; Medrano, H. (2016) Carbon isotope discrimination ($\delta^{13}C$) as an indicator of vine water status and water use efficiency (WUE): Looking for the most representative sample and sampling time. Agric. Water Manag. 167: 11–20.

Bordenave, L.; Tandonnet, J.P.; Decroocq, S.; Marguerit, E.; Cookson, S.J.; Esmenjaud, D.; Ollat, N. (2014) Wild *Vitis* as a germplasm resource for rootstocks. In: Exploitation of autochtonous and more used vines varieties – Oenoviti International Network meeting. 3 November; Geisenheim, Germany.

Carbonneau, A. (1985) The early selection of grapevine rootstocks for resistance to drought conditions. Am. J. Enol. Vitic. 36: 195–198.

Charrier, G.; Delzon, S.; Domec, J.-C.; Zhang, L.; Delmas, C., Merlin I.; Corso, D.; Ojeda, H.; Ollat, N.; Prieto, J.; Scholash, T.; Skinner, P.; van Leeuwen, C.; Gambetta, G. (2018) Drought will not leave your glass empty: Low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. Sci. Adv. 4(1): eaao6969.

Chaves, M.; Santos, T.; Souza, C.; Ortuño, M.; Rodrigues, M.; Lopes, C.; Maroco, J.; Pereira, J. (2007) Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. Ann. Appl. Biol. 150: 237–252.

Destrac-Irvine, A; van Leeuwen, C. (2016) VitAdapt: an experimental program to study the behavior of a wide range of *Vitis vinifera* varieties in a context of climate change in the Bordeaux vineyards. Ollat, N. (ed.) Proceedings of the conference Climwine, sustainable grape and wine production in the context of climate change. 11-13 April, Bordeaux: 165–171.

Duchêne, E.; Schneider C. (2005) Grapevine and climatic change: a glance at the situation in Alsace. Agron. Sustain Dev. 25: 93–99.

Friend, A.; Trought M. (2007) Delayed winter spur-pruning in New Zealand can alter yield components of Merlot grapevines. Aust. J. Grape Wine Res. 13: 157–164.

Gowdy, M.; Destrac, A.; Marguerit, E.; Gambetta, G.; van Leeuwen C. (2019) Carbon isotope discrimination berry juice sugars: changes in response to soil water deficits across a range of *Vitis vinifera* cultivars. Koundouras, S. (ed.) Proceedings of the 21st International GiESCO meeting. 24-28 June, Thessaloniki, Greece: 813–814.

Guilpart, N.; Metay, A.; Gary C. (2014) Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. Eur. J. Agron. 54: 9–20.

Hannah, L.; Roehrdanz, P.R.; Ikegami, M.; Shepard, A.V.; Shaw, M.R.; Tabor, G.; Zhi, L.; Marquet, P.A.; Hijmans, R.J. (2013) Climate change, wine, and conservation. Proceedings of the National Academy of Sciences 110(17): 6907–6912.

Helwi, P.; Guillaumie, S.; Thibon, S.; Keime, C.; Habran, A.; Hilbert, G.; Gomes, E.; Darriet, P.; Delrot, S.; van Leeuwen C. (2016) Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. BMC Plant Biol. 16: 173.

IPCC (2014) Climate Change 2014. Synthesis Report. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Core writing team; Pachauri, R.K.; Meyer, L.A.; (eds) IPCC; Geneva, Switzerland: 151 p.

Jones, G.; White, M.; Cooper, O.; Storchmann, K. (2005) Climate change and global wine quality. Clim. Change 73: 319–343.

Kliewer, W.; Dokoozlian, N. (2005) Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. Am. J. Enol. Vitic. 56: 170–181.

Lebon, E.; Dumas, V.; Pieri, P.; Schultz, H. (2003) Modelling the seasonal dynamics of the soil water balance of vineyards. Funct. Plant Biol. 30: 699–710.

Le Menn, N.; van Leeuwen, C.; Picard, M.; Riquier, L.; de Revel G.; Marchand, S. (2019) Effect of vine water and nitrogen status, as well as temperature, on some aroma compounds of aged red Bordeaux wines. J. Agric. Food Chem. 67: 7098–7109.

Marguerit, E.; Brendel, O.; Lebon, E.; Decroocq, S.; van Leeuwen, C.; Ollat N (2012) Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. New Phytologist 194(2): 416–429.

Marguerit, E.; Lagalle, L.; Lafargue, M.; Tandonnet, J.-P.; Goutouly, J.-P.; Beccavin, I.; Roques, M.; Audeguin, L.; Ollat, N. (2019) GreffAdapt: a relevant experimental vineyard to speed up the selection of grapevine rootstocks. Koundouras, S. (ed.) Proceedings of the 21st International GiESCO meeting. 24-28 June, Thessaloniki, Greece: 204–208.

Martínez-Moreno, A.; Sanz, F.; Yeves, A.; Gil-Muñoz, R.; Martínez, V.; Intrigliolo, D; Buesa, I. (2019) Forcing bud growth by double-pruning as a technique to improve grape composition of *Vitis vinifera* L. cv. Tempranillo in a semi-arid Mediterranean climate. Sci. Hortic. 256: 108614.

Matthews, M.; Anderson, M. (1988) Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. Am. J. Enol. Vitic. 39(4): 313–320.

Mira de Orduña R. (2010) Climate change associated effects on wine quality and production. Food Res. Int. 43: 1844–1855.

Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A; Deloire, A. (2002) Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Syrah. Am. J. Enol. Vitic. 53: 261–267.

Ollat, N.; Peccoux, A.; Papura, D.; Esmenjaud, D.; Marguerit, E.; Tandonnet, J.-P.; Bordenave, L.; Cookson, S.; Barrieu, F.; Rossdeutsch, L.; Lecourt, J.; Lauvergeat, V.; Vivin, P.; Bert, P.-F.; Delrot, S. (2015) Rootstock as a component of adaptation to environment. In: Grapevine in a changing environment: a molecular and ecophysiological perspective. Geros, H.; Chaves, M.; Medrano, H.; Delrot, S. (eds) John Wiley & Sons, Ltd: 68–108.

Ollat, N.; van Leeuwen, C.; Garcia de Cortázar-Atauri, I.;Touzard, J.-M. (2017) The challenging issue of climate change for sustainable grape and wine production. OENO One 51(2): 59–60.

Ollé, D.; Guiraud, J.L.; Souquet, J.M.; Terrier, N.; Ageorges, A.; Cheynier, V.; Verries, C. (2011) Effect of pre-and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. Aust. J. Grape Wine Res. 17: 90–100.

Parker, A.; Garcia de Cortázar-Atauri, I.; van Leeuwen; Chuine I. (2011) General phenological model to characterise the timing of flowering and veraison of *Vitis vinifera* L. Aust. J. Grape Wine Res. 17(2): 206–216.

Parker, A.; Garcia de Cortázar-Atauri, I.; Chuine, I.; Barbeau, G.; Bois, B.; Boursiquot, J.-M.; Cahurel, J.-Y.; Claverie, M.; Dufourcq, T.; Gény, L.; Guimberteau, G.; Hofmann, R.; Jacquet, O.; Lacombe, T.; Monamy, C.; Ojeda, H.; Panigai, L.; Payan, J.-C.; Rodriquez-Lovelle, B.; Rouchaud, E.; Schneider, C.; Spring, J.-L.; Storchi, P.; Tomasi, D.; Trambouze, W.; Trought, M.; van Leeuwen C. (2013) Classification of varieties for their timing of flowering and veraison using a modelling approach. A case study for the grapevine species *Vitis vinifera* L. Agric. For. Meteorol. 180: 249–264.

Parker, A.; Hofmann, R.; van Leeuwen, C.; McLachlan, A; Trought M. (2014) Leaf area to fruit mass ratio determines the time of veraison in Sauvignon blanc and Pinot noir grapevines. Aust. J. Grape Wine Res. 20: 422–431.

Parker, A.; Hofmann, R.; van Leeuwen, C.; McLachlan, A.; Trought, M. (2015) Manipulating the leaf area to fruit mass ratio alters the synchrony of soluble solids accumulation and titratable acidity of grapevines: implications for modelling fruit development. Aust. J. Grape Wine Res. 21: 266–276.

Parker, A.; Garcia de Cortázar-Atauri, I.; Gény, L.; Spring, J.-L.; Destrac, A.; Schultz, H.; Stoll, M.; Molitor, D.; Lacombe, T.; Graça, A.; Monamy, C.; Storchi, P.; Trought, M.; Hofmann, R.; van Leeuwen, C. (2019) The temperature based Grapevine Sugar Ripeness (GSR) model for adapting a wide range of *Vitis vinifera* L. cultivars in a changing climate. Koundouras, S. (ed.) Proceedings of the 21st International GiESCO meeting. 24-28 June; Thessaloniki, Greece: 303–308.

Petrie, P.; Sadras, V. (2008) Advancement of grapevine maturity in Australia between 1993 and 2006: putative causes, magnitude of trends and viticultural consequences. Aust. J. Grape Wine Res. 14: 33–45.

Peyrot des Gachons, C.; van Leeuwen, C.; Tominaga, T.; Soyer, J.-P.; Gaudillere, J.-P.; Dubourdieu, D. (2005) Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L. cv Sauvignon blanc in field conditions. J. Sci. Food Agric. 85(1): 73–85.

Picard, M.; van Leeuwen, C.; Guyon, F.; Gaillard, L.; de Revel, G.; Marchand, S. (2017) Vine water deficit impacts aging bouquet in fine red Bordeaux wine. Front. Chem. 5: 56.

Pons, A.; Allamy, L.; Schüttler, A.; Rauhut, D.; Thibon, C.; Darriet P. (2017) What is the expected impact of climate change on wine aroma compounds and their precursors in grape? OENO One 51(2): 141–146.

Schultz, H.R. (2000) Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine Res. 6: 2–12.

Schultz, H.R. (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field grown *Vitis vinifera* L. cultivars during drought. Plant Cell Environ. 26: 1393–1405.

Sgubin, G.; Swingedouw, D.; Dayon, G.; Garcia de Cortázar-Atauri, I.; Ollat, N.; Pagé, C.; van Leeuwen, C. (2018) The risk of tardive frost damage in French vineyards in a changing climate. Agr. Forest Meteorol. 250–251: 226–242.

Storchmann, K. (2016) Introduction to the special issue devoted to wine and climate change. J. Wine Econ. 11: 1–4.

Tomás, M.; Medrano, H.; Pou, A.; Escalona, J. M.; Martorell, S.; Ribas-Carbó, M.; Flexas, J. (2012) Water-use efficiency in grapevine cultivars grown under controlled conditions: Effects of water stress at the leaf and whole-plant level. Aust. J. Grape Wine Res. 18(2): 164–172.

Tortosa, I.; Escalona, J.; Bota, J.; Tomas, M.; Hernandez, E.; Escudero, E.; Medrano, H. (2016) Exploring the genetic variability in water use efficiency: Evaluation of inter and intra cultivar genetic diversity in grapevines. Plant Sci. 251: 35–43.

Triolo, R.; Roby, J.-P.; Pisciotta, A.; Di Lorenzo, R.; van Leeuwen, C. (2019) Impact of vine water status on berry mass and berry tissue development of Cabernet franc (*Vitis vinifera* L.) assessed at berry level. J. Sci Food Agric. 99(13): 5711–5719.

van Leeuwen, C.; Darriet P. (2016) The impact of climate change on viticulture and wine quality. J. Wine Econ. 11: 150–167.

van Leeuwen, C.; Destrac A. (2017) Modified grape composition under climate change conditions requires adaptations in the vineyard. OENO One 51(2): 147–154.

van Leeuwen, C.; Friant, Ph.; Chone, X.; Tregoat, O.; Koundouras, S.; Dubourdieu D. (2004) Influence of climate, soil and cultivar on terroir. Am. J. Enol. Vitic. 55(3): 207–217.

van Leeuwen, C.; Pieri, P.; Gowdy, M.; Ollat, N.; Roby C. (2019) Reduced density is an environmental friendly and cost effective solution to increase resilience to drought in vineyards in a context of climate change. OENO One 53(2): 129–146.

van Leeuwen, C.; Roby, J.-P.; Alonso-Villaverde, V.; Gindro, K. (2013) Impact of clonal variability in *Vitis vinifera* Cabernet franc on grape composition, wine quality, leaf blade stilbene content and downy mildew resistance. J. Agric. Food Chem. 61(1): 19–24.

van Leeuwen, C.; Roby, J.-P.; de Rességuier L. (2018) Soil related terroir factors, a review. OENO One 52: 173–188.

van Leeuwen, C.; Seguin, G. (2006) The concept of terroir in viticulture. J. Wine Res. 17(1): 1–10.

van Leeuwen C.; Trégoat, O.; Choné, X.; Bois, B.; Pernet, D.; Gaudillère, J.-P. (2009) Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? J. Int. Sci. Vigne Vin 43(3): 121–134.

Managing climate risk in a wine business

L.E. Clements, W. Birchmore

Accolade Wines, Reynella, SA
lucy.clements@accoladewines.com

Abstract

The world's changing climate offers significant challenges to the running of any agricultural business, right through the production and supply chains. The unpredictability of weather events coupled with more predictable summer warming guarantees qualitative and quantitative concerns and a need for a strong climate change strategy. In order to best manage the risks of climate change and create such a strategy a detailed understanding of foundational technical and operational challenges is required. This understanding is underpinned by a real-life view from the vineyard and cellar. This paper offers insight into the realities of climate change in a large wine business and honest, often simple, strategies from an operational and technical perspective.

Introduction

This short paper should not be viewed as a technical research document. Instead it is a collaboration with Warren Birchmore on the climate change impacts on grapegrowing and winemaking across Australia within the Accolade Wines network of eight wineries (as at December 2019). The footprint covers diverse territory from Western Australia to Tasmania, with almost all major growing regions covered (Figure 1).

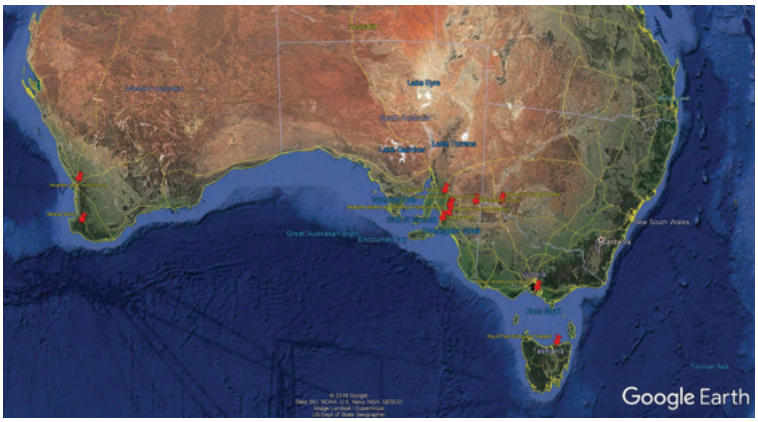


Figure 1. Map of Accolade Wines' wineries as at December 2019

Major climate change factors for a wine business

The overwhelming volume of research on climate change is too broad to mention in detail. This paper is focusing on discussion surrounding heatwaves, increased average temperatures in the growing season, and the increased prevalence of bushfires.

Bushfires

Regrettably, bushfires have historically been part of our nation and have increased in prevalence in recent years (Figure 2). As an industry we have had myriad opportunities to build our knowledge about compounds that cause smoke taint, the distance from the fire, smoke duration and intensity effects, grapevine varietal susceptibility, fuel type, and burn temperature effects. However, what we lack is a clear and practical solution once our vineyard resources have been exposed to smoke that does not compromise the quality of our wines. We can reduce the effects, but not eliminate them. To that end, Accolade Wines—like many other companies—is forced to either not pick, or pick and ferment separately and assess post-fermentation and apply remedial action via reverse osmosis. A solution in the vineyard would be ideal but this is yet to be developed.

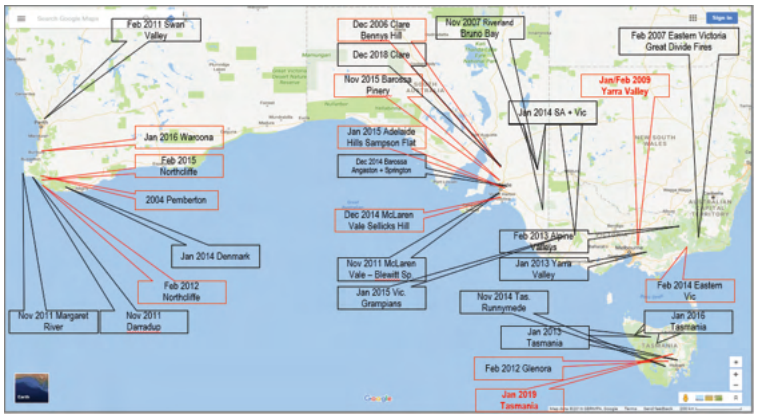


Figure 2. Map of fires recorded by Accolade Wines since 2006

Increased average temperatures

An increase in average temperatures has the overwhelming effect of compressing the vintage picking window.

Vintage compression typically refers to the situation where vintage has a similar start date but, due to warming weather, the end date is earlier than 'normal'. This climate change phenomenon has the net effect of increasing the peak intake. Figure 3 illustrates that end dates in 2019 were approximately 14 days earlier than in 2002. The major limiting factor of vintage compression is intake and tank capacity. A compressed vintage that results in the ripening of many grape varieties at the same time cannot be mitigated easily unless throughput and tank capacity are increased.

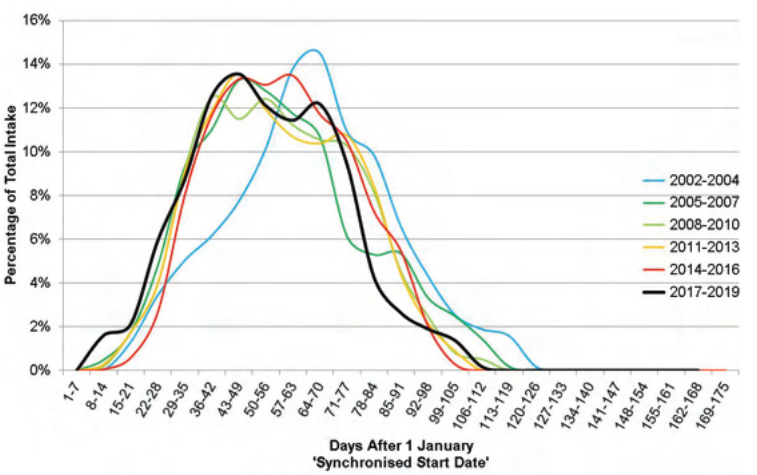


Figure 3. Vintage picking days at Berri Estates, Glossop from 2002 to 2019. Source: Accolade Wines

The inability to pick, crush and ferment any faster is a difficulty in all of our wineries.

Practical remedial actions for when vintage compression is compounded by an inability to process vintage faster:

- Harvest scheduling for cooler parts of night to early morning to take advantage of refrigeration load scheduling efficiency
- Multiregional sourcing program to take advantage of subregional ripening differences
- Start vintage early by picking earlier (below optimal Baumé) to blend away and lower alcohol
- Strategic development of new styles and new product development to take advantage of early picked grapes (not just use them for sparkling wine)
- Potential early intake for yeast cultures, acid adjustment and/or Baumé adjustment
- Later intake can be used for fortified/'jammy' styles for international markets or de-alcoholised via reverse osmosis or dilution with condensate ('cooked fruit' character considerations)
- Optimise intake between wineries – a major advantage of Accolade's network of five SA wineries
- Consider chain of responsibility compliance and biosecurity requirements of fruit fly/phylloxera
- Thermovinification to divert throughput to off skins and blow off 'green' characters of underripe fruit if early picked (considerable expense)
- Ferment reds off skins through white system if style/quality allows, once whites are crushed and pressed
- Manipulation of skin contact and press fractions for excessive phenolic extraction from sunburnt berries
- Water addition for overripe grapes and condensate after fermentation
- Saignée (drain-off) to concentrate remaining flavours.

Practical considerations for viticulture to mitigate higher average temperatures to delay/manipulate ripening:

- Crop load variation
- Heavier crops ripen later and can spread varietal intake
- Irrigation management
- Irrigation scheduling based on vine and soil requirements
- Crop load manipulation
- Canopy structure
- Cooling effects, either directly via evaporation or mid-row sward growth
- Night irrigation versus midday irrigation
- Irrigation before heatwaves
- Earlier/later pruning
- Sunscreens for sunlight intensity management
- Anti-transpirants
- Bunch exposure and varietal susceptibility to sunburn/overheating
- Review of fungicides for temperature sensitivity
- Salinity impacts
- New varieties – for example, heat-loving varieties such as Touriga Nacional and Zinfandel.

Conclusion

The realities of climate change are simply everyday vintage challenges for winemakers and grapegrowers around Australia. The reality of dealing with a compressed vintage owing to higher average temperatures cannot be easily mitigated unless significant winery investment is built to increase capacity at vintage. Many Australian wineries cannot justify extra crushers and fermenters that are unused for much of the year when price points are squeezed in many markets. Therefore, a very practical approach is commonplace. It is our aspiration that this short paper has illuminated the difficult reality faced by our industry as a result of climate change and revealed some simple, practical considerations for managing climate risk in a wine business.

When do grapes stop accumulating sugar?

P.R. Petrie[1,2,3,4], W. Jiang[2], K.A. Bindon[2], V.O. Sadras[1,3]

[1]Crop Sciences, South Australian Research and Development Institute, Urrbrae, SA
[2]The Australian Wine Research Institute, Urrbrae, SA
[3]School of Agriculture, Food and Wine, The University of Adelaide, Urrbrae, SA
[4]School of Mechanical and Manufacturing Engineering, University of New South Wales, Sydney, NSW
paul.petrie@sa.gov.au

Abstract

Over the last two decades grapegrowers and winemakers have observed grapes ripening earlier and over a shorter period. This can lead to a harvest with higher than ideal sugar concentrations and less desirable flavour characteristics. To help manage high-sugar grapes, Food Standards Australia and New Zealand regulations now allow the pre-fermentation dilution of must to 13.5°Baumé. By establishing a lower limit for sugar concentration this has shifted the focus towards the mass of sugar produced, in addition to the mass of grapes harvested. Under the revised regulations, the point when sugar allocation into the fruit ceases becomes an additional trait to inform harvest decisions, in addition to fruit maturity and flavour profile. Berry sugar content can be tracked by measuring berry weight and sugar concentration. We compiled data on the dynamics of sugar per berry from 36 Australian research articles to investigate management and environmental factors that can putatively stop sugar accumulation. Quantile regression was used to empirically determine the maximum sugar concentration where the sugar content per berry plateaued: this returned thresholds of at ≈13°Baumé for Shiraz and ≈14.5°Baumé for Cabernet Sauvignon. As grapegrowers are paid based on the yield of grapes, conflict might occur between them and wineries when high sugar concentrations occur due to fruit dehydration. Knowing the sugar concentration above which dehydration is likely to occur will complement flavour assessment and maturity analysis when prioritising harvest decisions.

Introduction

Over the last two decades grapegrowers and winemakers have observed that their fruit is ripening earlier (Petrie and Sadras 2008) and over a shorter period (Petrie and Sadras 2016). This can lead to difficulties harvesting fruit in the optimal time frame and fruit being picked at higher than ideal sugar concentrations. In turn, this can cause problems with fermentations 'sticking' before all the sugars have fermented (Chaney et al. 2006) or lead to wines with undesirably high alcohol (Varela et al. 2015). In the USA, the dilution of must to facilitate fermentation is legal and widely accepted (Chaney et al. 2006). More recently, to help manage fruit with a high sugar concentration, Food Standards Australia and New Zealand changed the regulations to allow the limited addition of water to high-sugar musts and juice in Australia (Anon. 2016). The revised regulations allow musts to be diluted with water until they reach 13.5°Baumé. In a similar position to the USA, the rationale behind this change was to reduce the chances of problems arising during fermentation; but an additional benefit may be to help industry manage the logistical problems caused by compressed vintage periods. By establishing a lower limit for sugar concentration, this has shifted the focus towards the mass of sugar produced, in addition to the mass of grapes harvested. Under the revised regulations, the point at which sugar allocation in the fruit ceases becomes an additional trait to inform harvest decisions, in addition to fruit maturity and flavour profile. Conflict between grapegrowers—who are paid on the basis of yield (mass) of grapes—and wineries can arise when high sugar concentrations are potentially achieved due to fruit dehydration as opposed to the importation of sugar into the grape berries (Gogoll 2017; Smart 2005). As the vineyard yield is determined in part by the sugar content of the fruit, the stage of development at which sugar accumulation ceases becomes critical. While other key quality parameters such as acids, anthocyanins and phenolics may change and the quality of the final wine improve due to metabolism, concentration or changes in extractability of critical compounds (Bindon et al. 2013; Coombe and McCarthy 2000), the further increase in sugar concentration in the fruit is due to dehydration of the berries.

The cultivar, environmental conditions and management can all potentially impact the plateau of sugar accumulation in berries, and this can occur before the theoretical maximum is reached. The manipulation of yield and canopy size are classic viticultural practices to advance or delay fruit maturity and their interaction has been assessed at least since the early 1900s (Ravaz 1903). For example, Etchebarne et al. (2010) observed that berries on Grenache Noir vines with five leaves per shoot stopped accumulating sugar earlier than berries on shoots with 10 or 18 leaves. They also observed that irrigation allowed the berries to continue to accumulate sugar later into the season. The assessment of the environmental effects on sugar accumulation of field vines is more difficult as a control treatment is hard to establish. However, Greer and Weston (2010) were able to show that heat stress at veraison or mid-ripening could stop the accumulation of sugar in potted Semillon vines. Sadras et al. (2008) compared a range of wine and table grape cultivars and found that the date at which they reached 95% of their maximum sugar concentration varied significantly.

Changes in sugar content are normally tracked by the assessment of berry size, and in conjunction with sugar concentration, the sugar content per berry can be calculated. In a commercial context the assessment of berry size in addition to the traditional berry maturity measures of sugar concentration, titratable acidity and pH is prohibitive due to the additional labour requirements. In a research context, the average berry weight is more often recorded; however, it is normally assessed at a point in time or at a specified maturity (Dai et al. 2011) and the impact of the experimental treatments or environmental conditions on the point when sugar accumulation stops is rarely determined. For some cultivars, especially Shiraz, the dynamics of sugar accumulation and its partitioning into flows of solutes and water is well defined, as it is prone to shrivel during late berry development (McCarthy 1999; McCarthy and Coombe 1999). While the magnitude of shrivel has been well characterised, even for a single cultivar there is divergence within the literature as to the sugar concentration where sugar content reaches a plateau. For example, McCarthy and Coombe (1999) reported '…without shrinkage, the juice °Brix of Shiraz berries under these conditions would not rise above 20-21°!'; while Keller (2015) states that '…Syrah berries reach a maximum amount of sugar when their sugar concentration approaches 25°Brix'.

We analysed trajectories of grape ripening in published studies to determine the maximum sugar concentration at which sugar accumu-

lation by grape berries ceases in Shiraz and Cabernet Sauvignon under a range of Australian conditions. These figures could be used as a guide to determine when fruit dehydration is likely to occur and therefore support harvest decisions.

Methods

A systematic search of literature was performed to find Australian-based studies that assessed the ripening of grape berries using the Scopus (https://www.scopus.com/) database that is maintained by Elsevier (Amsterdam, The Netherlands). The search was completed on 16 March 2018, and there was no restriction imposed on the year of publication, language or scientific subject area. A Boolean search was completed using the following combination of search terms: ((viti* or grape*) and (matu* or ripe*)) and affiliation Austral*); where the asterisk denoted that the balance of the search term could be comprised of any characters. A list of 411 potential articles was generated and the articles were initially reviewed using the Scopus interface on the basis of the title and abstract; this resulted in the selection of 144 articles. Each article was inspected and included in the study based on the following criteria: i) the research was completed in Australia; ii) the study was completed in the field as opposed to a glasshouse or other protected cropping system; and iii) the study contained three or more records of berry weight and sugar concentration for at least one treatment, within one season. This resulted in 36 articles that provided data for the analysis. When data was presented in a tabular form it was transcribed into a spreadsheet; when it was presented in graphical form the image was copied from the portable document format file using the 'take a snapshot' function from Adobe Acrobat Reader (Adobe Systems Incorporated, San Jose, CA, USA). The data was extracted from the image of the graph using GraphGrabber V2.0 (Quintessa Limited, Henley-on-Thames, Oxfordshire, UK). Data was only extracted from the control treatment. The cultivar, date and location of the trial were also recorded giving a total of 47 datasets containing unique cultivar, by season, by location combinations. Data was collected across a wide range of cultivars; however, there were only enough results to analyse Shiraz (n = 220) and Cabernet Sauvignon (n = 97).

Sugar per berry was calculated by multiplying the treatment means of the percent total soluble solids (°Brix) by the berry mass. When the berry sugar concentration was presented in °Baumé this was converted to °Brix by multiplying by 1.8. To allow the full range of berry sizes to be compared and avoid the dataset being skewed by the trials with the larger berries (e.g. due to more severe pruning), the sugar per berry data was standardised by dividing the results for a treatment by year combination by the last sample collected. This meant that values close to one represented minimal change in sugar per berry, relative to the final sample date. Boundary-line analysis (Webb 1972) was used to estimate the point beyond which sugar per berry no longer increased. The boundary line was calculated using a method similar to Sadras and Petrie (2011), where the entire dataset was divided into 0.56°Baumé (1°Brix) sections and the 10th percentile for each section calculated. The regressions were then completed on the first decile values and the sugar concentration (°Brix) where the sugar content reached 100% of the final value denoted the point where berries stopped accumulating sugar.

Results and discussion

Despite the sugar concentration (Baumé) continuing to increase, there were very few observations where the berry sugar content increased beyond approximately 13°Baumé for Shiraz (Figure 1) and beyond approximately 14.5°Baumé for Cabernet Sauvignon. This is close to the Keller (2015)-reported threshold of 13.8°Baumé. As the dataset used for this analysis includes the results from McCarthy and Coombe (1999), where sugar accumulation stopped at 20-21°Brix (11.1-11.7°Baumé), it is likely that other environmental (e.g. water stress), management or vine factors (e.g. low source:sink ratio) halted the sugar accumulation for the berries at a lower concentration.

At maturity, grape berries contain very high soluble sugar concentrations compared to other fruit crops (Coombe 1976). As grapes ripen, the sugar unloading pathway moves from symplasmic to apoplasmic from veraison (Zhang et al. 2006), which indicates that transmembrane transport of sugars occurs during this period (Dai et al. 2010). The process of transport of sugar into berries is yet to be fully defined; and potentially involves passive diffusion, turgor-driven mass transport or active flow transport regulated by sugar transporters (Davies and Robinson 1996; Hayes et al. 2007). However, all of the potential models of sugar movement into berries are in part regulated by the differing concentration of sugars (osmotic potential) between the phloem and fruit cells (Dai et al. 2010). This implies that as the concentrations in the fruit cells increases the importation of sugar will slow or eventually stop and that berries can reach a theoretical maximum sugar concentration beyond which further increases are only due to dehydration (Bondada et al. 2017; Coombe and McCarthy 2000) and is supported by our empirical observations. The loss of cellular functionality related to mesocarp cell death, particularly in Shiraz, might have also contributed to these differences (Xiao et al. 2018a, b).

As the Shiraz stopped accumulating sugar at a lower concentration, there were many more samples collected that were at or close to the maximum sugar content per berry compared to the Cabernet Sauvignon. This means that the upper limit for sugar accumulation was easier to define for the Shiraz. In addition, Cabernet Sauvignon ripens later than Shiraz (Petrie and Sadras 2008), so there is less opportunity to collect samples of riper fruit (above 14.5°Baumé). The magnitude of the difference in the sugar concentration beyond which the Cabernet Sauvignon and Shiraz cease accumulating sugar was also surprisingly large given that the processes that regulate sugar accumulation are likely to be common across many fruit crops. However, the propensity for Shiraz berries to shrivel at relatively low sugar concentrations compared to other cultivars is well defined (McCarthy 1999; McCarthy and Coombe 1999) and has been related to differences in other physiological processes such as a loss of membrane integrity and cell death (Tilbrook and Tyerman 2008; Xiao et al. 2018a, b). The collection of berry weight and sugar concentration metrics from other cultivars, especially for ripe and overripe fruit, would be beneficial to understand the range that can occur.

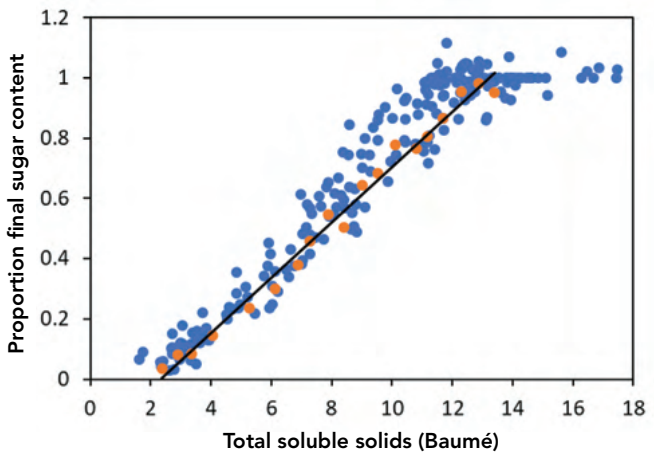


Figure 1. The relationship between Shiraz berry sugar concentration and the proportion of the final berry sugar content for each trial. The orange symbols represent the 10th percentile for arbitrary 0.56°Baumé sections of the data. Data was collected from 30 experiments and includes 220 sample points. Proportion final sugar content = 0.094*°Baumé - 0.228; R^2 = 0.98

When detached fruit was dried in a controlled environment (drying tunnel) a 1°Baumé increase in sugar concentration was associated with a weight loss of approximately 9% due to dehydration (Muganu et al. 2011). We would expect a similar trend once sugar importation into the berries has ceased; but this was not directly analysed. The implication is that if a Shiraz vineyard was harvested at 15°Baumé, its yield would be over 20% lower than if it was harvested at 13°Baumé. Desirable flavour and aroma characters will develop between 13 and 15°Baumé (Bindon et al. 2013; Coombe and McCarthy 2000), so while the yield is decreasing, the value of the fruit is likely to increase even if the mass of sugar (and potential final quantity of wine) does not change.

There were many trials where the fruit stopped accumulating sugar earlier or did not reach this value at all (often due to harvest or inclement conditions). Tracking sugar per berry in addition to berry sugar concentration will potentially aid in making better harvest decisions, especially in these situations. If the fruit has reached its desired flavour profile and sugar accumulation has ceased, then there is little to be gained by delaying harvest. While it is currently prohibitively expensive to manually count and weigh berries, as technology to automate the measurement of berry size improves (Liu et al. 2015), growers or wineries may be able to easily measure sugar per berry for their own blocks as an aid to harvest decisions.

References

Anon. (2016) Addition of Water to Facilitate Wine Fermentation. Food Standards Australia and New Zealand: Kingston, ACT, Aust. and Wellington, N.Z.

Bindon, K.; Varela, C.; Kennedy, J.; Holt, H.; Herderich, M. (2013) Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. Food Chem. 138: 1696–1705.

Bondada, B.; Harbertson, E.; Shrestha, P.M.; Keller, M. (2017) Temporal extension of ripening beyond its physiological limits imposes physical and osmotic challenges perturbing metabolism in grape (*Vitis vinifera* L.) berries. Sci. Hortic. 219: 135–143.

Chaney, D.E.; Rodriguez, S.B.; Fugelsang, K.C.; Thornton, R.J. (2006) Managing high-density commercial scale wine fermentations. J. Appl. Microbiol. 100: 689–698.

Coombe, B.G. (1976) The development of fleshy fruits. Annu. Rev. Plant Physiol. 27: 507–528.

Coombe, B.G.; McCarthy, M.G. (2000) Dynamics of grape berry growth and physiology of ripening. Aust. J. Grape Wine Res. 6: 131–135.

Dai, Z.W.; Ollat, N.; Gomès, E.; Decroocq, S.; Tandonnet, J.P.; Bordenave, L.; Pieri, P.; Hilbert, G.; Kappel, C.; Van Leeuwen, C.; Vivin, P.; Delrot, S. (2011) Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: A review. Am. J. Enol. Vitic. 62: 413–425.

Dai, Z.W.; Vivin, P.; Barrieu, F.; Ollat, N.; Delrot, S. (2010) Physiological and modelling approaches to understand water and carbon fluxes during grape berry growth and quality development: A review. Aust. J. Grape Wine Res. 16: 70–85.

Davies, C.; Robinson, S.P. (1996) Sugar accumulation in grape berries. Cloning of two putative vacuolar invertase cDNAs and their expression in grapevine tissues. Plant Physiol. 111: 275–283.

Etchebarne, F.; Ojeda, H.; Hunter, J.J.K. (2010) Leaf:Fruit ratio and vine water status effects on Grenache Noir (*Vitis vinifera* L.) berry composition: water, sugar, organic acids and cations. S. Afr. J. Enol. Vitic. 31: 106–115.

Gogoll, N. (ed.) (2017) Water into wine. Aust. N.Z. Grapegrower Winemaker 636: 50–53.

Greer, D.H.; Weston, C. (2010) Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. Funct. Plant Biol. 37: 206–214.

Hayes, M.A.;Davies, C.; Dry, I.B. (2007) Isolation, functional characterization, and expression analysis of grapevine (*Vitis vinifera* L.) hexose transporters: Differential roles in sink and source tissues. J. Exp. Bot. 58: 1985–1997.

Keller, M. (2015) Ch. 6. Developmental Physiology. In: The Science of Grapevines (2nd ed.) Keller, M. (ed.) San Diego: Academic Press: 193–265.

Liu, S.; Whitty, M.; Cossell, S. (2015) A lightweight method for grape berry counting based on automated 3D bunch reconstruction from a single image. Proceedings of ICRA, International Conference on Robotics and Automation (IEEE), 30 May: Seattle, USA.

McCarthy, M.G. (1999) Weight loss from ripening grape berries of Shiraz grapevines (*Vitis vinifera* L. cv. Shiraz). Aust. J. Grape Wine Res. 5: 10–16.

McCarthy, M.G.; Coombe, B.G. (1999) Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? Aust. J. Grape Wine Res. 5: 17–21.

Muganu, M.; Bellincontro, A.; Barnaba, F.E.; Paolocci, M.; Bignami, C.; Gambellini, G.; Mencarelli, F. (2011) Influence of Bunch Position in the Canopy on Berry Epicuticular Wax during Ripening and on Weight Loss during Postharvest Dehydration. Am. J. Enol. Vitic. 62: 91–98.

Petrie, P.R.; Sadras, V.O. (2008) Advancement of grapevine maturity in Australia between 1993 and 2006: Putative causes, magnitude of trends and viticultural consequences. Aust. J. Grape Wine Res. 14: 33–45.

Petrie, P.R.; Sadras, V.O. (2016) Practical options to manage vintage compression. Beames, K.S.; Robinson, E.M.C.; Dry, P.R.; Johnson, D.L. (eds) Proceedings of the 16th Australian wine industry technical conference, 24-28 July 2016, Adelaide, SA. Adelaide: Australian Wine Industry Technical Conference Inc.: 63–67.

Ravaz, L. (1903) Sur la brunissure de la vigne. Les Comtes Rendus de l'Académie des Sciences 136: 1276–1278.

Sadras, V.O.; Collins, M; Soar, C.J. (2008) Modelling variety-dependent dynamics of soluble solids and water in berries of *Vitis vinifera*. Aust. J. Grape Wine Res. 14: 250–259.

Sadras, V.O.; Petrie, P.R. (2011) Quantifying the onset, rate and duration of sugar accumulation in berries from commercial vineyards in contrasting climates of Australia. Aust. J. Grape Wine Res. 17: 190–198.

Smart, R. (2005) Scrutinizing hang-time trend. Pract. Winery Vineyard J. 27: 10–12.

Tilbrook, J.; Tyerman, S.D. (2008) Cell death in grape berries: Varietal differences linked to xylem pressure and berry weight loss. Funct. Plant Biol. 35: 173–184.

Varela, C.; Dry, P.R.; Kutyna, D.R.; Francis, I.L.; Henschke, P.A.; Curtin, C.D.; Chambers, P.J. (2015) Strategies for reducing alcohol concentration in wine. Aust. J. Grape Wine Res. 21: 670–679.

Webb, R.A. (1972) Use of the boundary line in the analysis of biological data. J. Hortic. Sci. 47: 309–319.

Xiao, Z.; Liao, S.; Rogiers, S.Y.; Sadras, V.O.; Tyerman, S.D. (2018a) Effect of water stress and elevated temperature on hypoxia and cell death in the mesocarp of Shiraz berries. Aust. J. Grape Wine Res. 24: 487–497.

Xiao, Z.; Rogiers, S.Y.; Sadras, V.O.; Tyerman, S.D. (2018b) Hypoxia in grape berries: the role of seed respiration and lenticels on the berry pedicel and the possible link to cell death. J. Exp. Bot. 69: 2071–2083.

Zhang, X.Y.; Wang, X.L.; Wang, X.-F.; Xia, G.H.; Pan, Q.H.; Fan, R.C.; Wu, F.Q.; Yu, X.C.; Zhang, D.P. (2006) A shift of phloem unloading from symplasmic to apoplasmic pathway is involved in developmental onset of ripening in grape berry. Plant Physiol.142: 220–232.

Climate change and its influence on scale insects and sooty mould occurrence

P.D. Cooper[1], J. Venus[2]

[1]Research School of Biology, The Australian National University, Canberra, ACT
[2]Brad Case Contracting, Langhorne Creek, SA
paul.cooper@anu.edu.au

Abstract

Discussions of potential changes in climate rarely consider how changes may affect the impact of pest insects on the yield of agricultural crops. Grain crops in temperate regions have been suggested to experience greater reductions in yield compared with more tropical-grown crops, such as rice. Grapevines are typical of temperate plants and therefore may be more susceptible to the effects of various pests as temperature and humidity change in the future. Soft scales of the genus Parthenolecanium *have been reported to infest grapevines in Australia since early last century and have become more noticeable in the last 10 years as climate patterns have changed. By producing honeydew and initiating the growth of sooty mould on grapes and leaves, vineyards have suffered yield reduction and economic losses. This paper includes a series of graphical models that indicate how temperature and humidity changes associated with climate change can result in increased likelihood of sooty mould production on leaves and fruit. Increases in temperature can lead to two complete life cycles being present during the growing season. Increases in both scale population and honeydew production rate coupled with increases in humidity and temperature can result in honeydew residues that persist longer on leaves and fruit. As abiotic conditions change, differences in the cultivars with respect to scale infestation may provide ways of controlling the extent of sooty mould in the future.*

Introduction

Grapevines are susceptible to several insect pests (Bostanian et al. 2012). In Australia, among the current pest insects are several species of scale insects that have become more apparent in vineyards (Rakimov et al. 2013; Simbiken 2014). The most common species present on grapevines are *Parthenolecanium persicae* (grapevine scale) and *P.* nr *pruinosum* (frosted scale). Although the scales can cause damage to vines directly, more commonly the damage is associated with the growth of sooty mould on leaves and grapes (Essling and Petrie 2018; Venus 2017). The damage is not to the plant, but to the ability for vineyards to market their grapes that have sooty mould, as most wineries will reject grapes if the presence of sooty mould exceeds 2% (Venus 2017). In the last five years the level of damage, both directly by scale feeding and by subsequent presence of sooty mould, has become critical to the industry as both plant and fruit damage is resulting in potentially large financial losses by grapegrowers (Venus 2017).

Scale insects on grapevines in Australia are currently recognised as being univoltine; that is, only one generation occurs a year (Simbiken et al. 2017). Females are present in late-September to mid-October with first instar larvae appearing in November. The first instars feed on phloem or parenchyma cells (Simbiken 2014) through January-February and then moult into second instars. The second instars of grapevine scales moult to third instars by March and overwinter as third instars. In contrast, frosted scales overwinter as second instars, with the third instar not developing until August when it is present for only about two weeks before becoming an adult. Adults of both species are therefore present at the beginning of spring (Simbiken et al. 2017). Although the beginning of a second generation may occur in March-April (Venus 2017), it is unknown whether any of the offspring are able to survive to the following spring, as leaves are senescing.

The distribution and abundance of scales is strongly influenced by the environment, with both abiotic (temperature, wind, general habitat) and biotic (host plant, life history pattern and response to other arthropods) factors (Andrewartha and Birch 1954, 1984). Temperature is the most important abiotic influence on an insect as it directly determines scale insect body temperature (Angilletta 2009; Schowalter 2016). The lowest temperature for survival is the critical minimum temperature, which we have determined in previous work to be -13˚C when conditions are dry and 0˚C when scale insects are wet (Hayes et al. 2017). As temperature increases above this minimal temperature, the scale insects can demonstrate other biological processes, with a putative minimal temperature for development for *Parthenolecanium* sp. around 10˚C (Camacho et al. 2017). The upper temperature for development and the maximum critical temperature are still unknown, and further work is necessary to determine to what extent the instars (first and second) present in the summer can survive high temperatures and still moult to the next instar.

The temperature will also determine the rate of growth of sooty mould, as well as the minimum temperature for the initiation of growth. In addition, increased humidity leads to the growth of sooty mould in tropical, subtropical and adjacent regions (Chomnunti et al. 2014; Shukla et al. 2017), and increased humidity has previously been suggested to be partly responsible for the increased presence of sooty mould in South Australian vineyards (Venus 2017). However, without high humidity and a carbohydrate source, sooty moulds do not usually grow. Honeydew produced by scale insects can at least provide the source of carbohydrates that permits sooty mould growth (Chomnunti et al. 2014). Therefore, the interaction of the abiotic external humidity and temperature are only one aspect of what is necessary for sooty mould growth, and is supplemented by the biotic interactions of the scale insect and the host plants.

To better understand how the abiotic and biotic effects can interact to contribute to sooty mould formation on grapevines, a graphical model is presented that highlights how the effects of temperature and humidity interact to contribute to sooty mould formation. The model incorporates the role that different cultivars may play as the cultivars differ in the ability of scale insects to colonise and survive, leading to differences in honeydew accumulation, and therefore differences in cultivar susceptibility to sooty mould within a vineyard.

Insect pest species and climate change

Several models are available for predicting how insect populations will change in the future with most being conceptual (Castex et al. 2018; Reineke and Thiéry 2016), although mechanistic models also have been recently used (Maino et al. 2016). Deutsch and co-authors (2018) have used a mathematical model to indicate that climate change will benefit temperate more than tropical insect species, as the increase in temperature will permit temperate species to develop faster with more generations over a host plant growth period. However, the conceptual models have suggested that more parameters are important for the pest species effects than just temperature increase (Castex et al. 2018; Reineke and Thiéry 2016). The model that is represented here uses conceptual, mechanistic and mathematical approaches to develop the graphical representation of the interaction between the abiotic environment and the biotic components of insect pest, grapevine cultivar and the fungal development of sooty mould. The conceptual aspect is necessary as the empirical information needed for understanding the development of sooty mould is limited, although the presence of sooty mould in relation to sucking insects on mango orchards in tropical regions has been reported (Shukla et al. 2017).

Conceptual model

Sooty mould growth and development is dependent upon both abiotic and biotic environments (Figure 1).

The cultivar provides a surface, either leaves or fruit, for the growth of the sooty mould (Figure 2). Honeydew from the scale insects drips onto these locations and provides nutrients (mostly sugars) for sooty mould that stimulates growth. However, without a sufficiently high humidity, water loss by evaporation results in honeydew becoming crystallised and unsuitable for sooty mould growth. Cultivars may vary in their susceptibility to either scale insects or sooty mould growth (differences in leaf chemistry or evapotranspiration) (Simbiken et al. 2015; Venus 2017) that can either reduce or enhance these responses.

Scale insects (either grapevine scale or frosted scale) are present on the leaves of grapevines for both first and second instars. The instars are dorsoventrally flattened and are typically located on the underside of leaves, although at high densities they will occupy the leaf topside. Their body shape is such that they will be enclosed within the boundary layer of the leaf and will be the same temperature of the leaf on which they live, although the humidity may be different from the external humidity depending upon the water loss from the plant (Figure 3). The insects will feed (presumably from parenchyma cells; Simbiken 2014) and produce honeydew as a result of feeding.

Currently, scale insects in Australian vineyards have a single generation during grapevine development (Figure 4). The timing of scale egg production is late-spring/early-summer, and then it takes three to four months for the hatching and growth to reach third (*P. persicae*) or second (*P.* nr *pruinsum*) instars prior to overwintering.

Temperature effects on scale insects

Temperature influences all aspects of insect and plant development (Castex et al. 2018). However, this model will only consider how scale insect populations are affected. As temperature increases, the metabolic rate of insects increases (Harrison et al. 2012), as does ingestion and reproduction (Figure 5). With an increase in ingestion, the quantity of excreta increases. The increases are relatively exponential until insects approach their maximum critical temperature, when

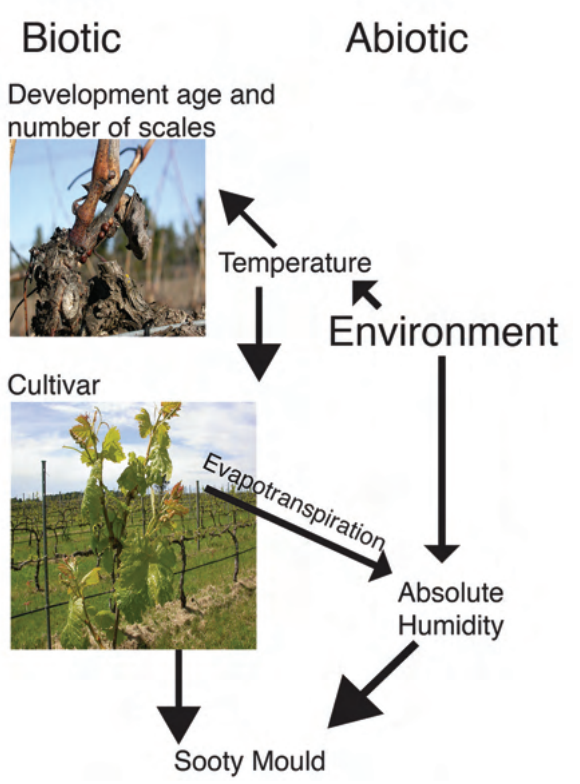


Figure 1. Biotic and abiotic determinants of sooty mould growth on grapevines. Age and development of scales determine how much honeydew is available and the cultivar is partially important for the amount of water vapour that is present to maintain the honeydew as a fluid. The environment provides the temperature and absolute humidity that allows for increase in insect population, honeydew production and the ability for fluid honeydew to act as a substrate for sooty mould growth.

Figure 2. Grapes with sooty mould indicating the extent of damage that can occur when conditions are appropriate

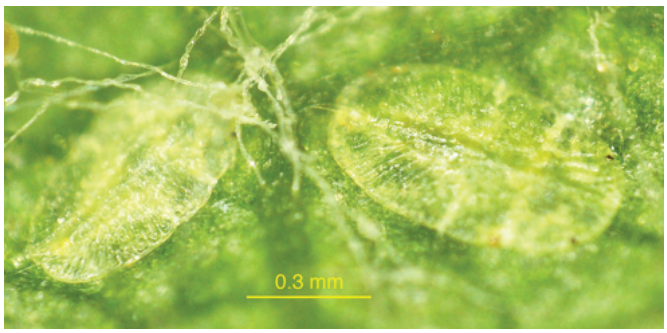


Figure 3. Two second instar grapevine scales on leaf. They are under the leaf hairs that are shown. Typically the scales will also be near a leaf vein, but will be found anywhere as their density increases.

the performance starts declining and then decreases towards zero as the insects start dying (Figure 5).

A component of the increase in scale population is an increase in the number of generations that can be produced by the scale insects. However, as temperature increases, the time for emerging from the overwintering period may be advanced and the timing for each moult to the next stage of development is also shortened and advanced (Figure 6). The reduction in time for the entire process from egg to adult will be shorter and this could lead to at least a second generation during the period when leaves are still growing.

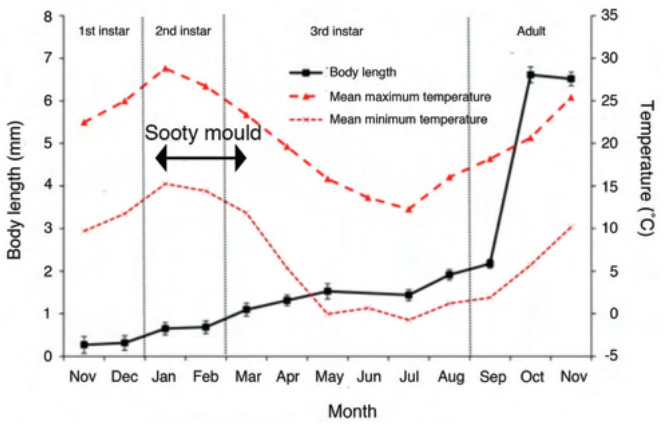


Figure 4. Current pattern of development for scale insects observed, with first instars appearing in late-spring and adults appearing in early-spring. Sooty mould is observed starting in summer. The figure represents scale development in vineyards in the NSW/ACT region.

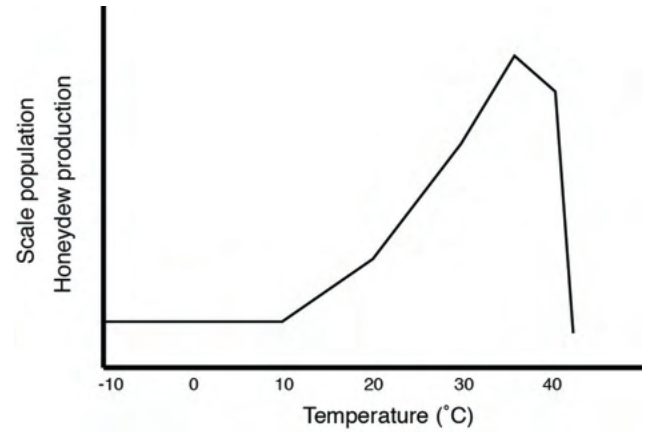


Figure 5. Thermal performance curve that indicates changes in both population and honeydew production as temperature increases. The minimum critical temperature for scale insects is 0˚C in wet conditions, but -13˚C in dry conditions. The flat line between -10 to 10˚C indicates that no development or feeding occurs until 10˚C is reached. The maximum critical temperature is assumed to be between 40 and 43˚C.

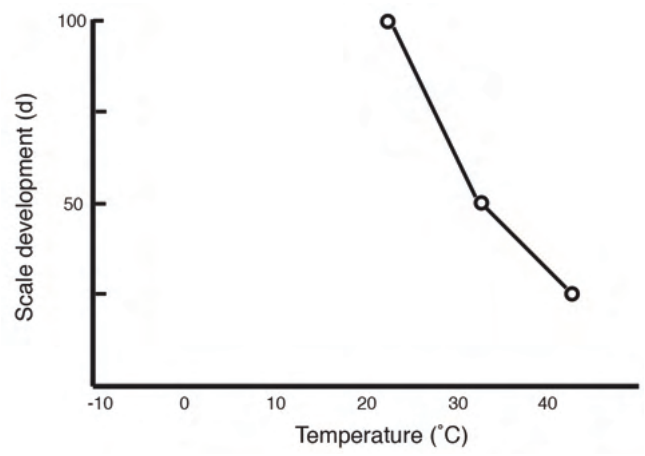


Figure 6. As temperature increases from 22˚C the development rate increases. For each 10˚C increase in temperature the time for development decreases by half. Data for figure at 22˚C are taken from Simbiken (2014) and extrapolated to higher temperatures assuming a doubling of development rate for each 10˚C increase.

Although Figure 6 was determined using constant temperature, the values can be changed to more realistic terms of a 2.5˚C increase in temperature with degree days (Cooper 2017). If temperature increases from an average of 27.5˚C to 30˚C, development time for first and second instars decreases by 10% from 20 to 18 days. That means complete development to the third instar would occur in just over one month. Depending upon when scales emerged in spring, the potential for a second generation would be increased.

Humidity, honeydew and sooty mould

Honeydew has a high concentration of oligosaccharides (compound sugars) as a result of the removal of excess sugar that is ingested with food by the scale insects (Wilkinson et al. 1997). Although the concentration of the honeydew is the same as the hæmolymph of insects, once excreted onto a leaf the fluid will evaporate, thereby concentrating the sugars. Most sugars are in equilibrium with a relative humidity of 75–85% (Maudru and Paxson 1950; Money and Born 1951), and as humidity increases water can be absorbed from the surrounding air (Figure 7). As humidity increases, more dissolved sugars are present to act as a substrate for sooty mould growth.

The sugar solution that is present with honeydew can thus be present longer on leaves and fruits as humidity increases, such as occurs in tropical and subtropical environments (Chomnunti et al. 2014). It is the persistence of the solution that allows for the establishment and growth of the sooty mould. If temperatures are just increased without increased humidity, the rapid loss of water from the honeydew limits the growth of sooty mould, indicating that sooty mould is not indicative of scale insect abundance in South Australian vineyards (Venus 2017). Potentially, differences in humidity within the plant boundary layer may also contribute to the differences in sooty mould formation among cultivars (Simbiken et al. 2015; Venus 2017), but further work is needed to determine if that is enough to result in the observed differences in sooty mould.

Sooty mould is assumed to be limited in growth at low temperatures and will increase in occurrence as temperatures increase as long as humidity is high enough. However, if temperatures are too high and humidity is low, then sooty mould will not grow (Figure 8). The assumption for this conclusion is that the insect scale population is high enough to provide the appropriate honeydew levels to cause sooty mould occurrence. As sooty mould is not always present even when scale insects are abundant, further work is needed to determine the minimum humidity that initiates sooty mould occurrence.

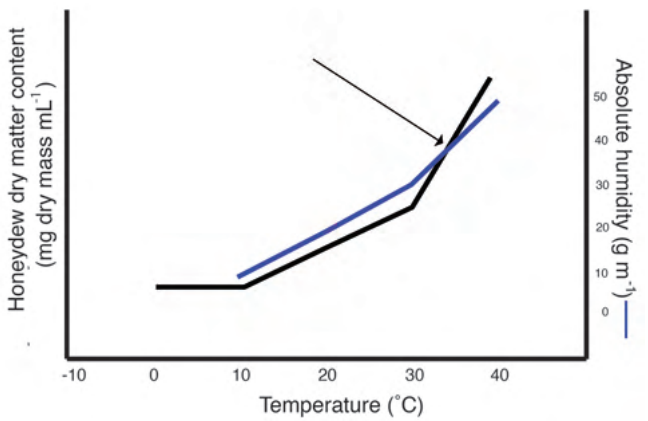


Figure 7. As temperature increases evaporation from honeydew increases, but so does absolute humidity. The figure suggests that the two lines intersect near 30˚C but depending upon the actual environmental humidity surrounding the honeydew that includes evapotranspiration from the cultivar leaves, the exact point of intersection is unknown. To the right of the intersection honeydew will absorb water from the environment.

Current work

Reduction of sooty mould and its economic impact on vineyards is the goal of current research. In a changing climate there are limited ways of attacking the problem as abiotic conditions are difficult to predict, other than understanding that temperatures are likely to increase. The best option is to use the information about differences in the response of cultivars to the invasion of scale insects or the presence of sooty mould. Cultivars that have apparent resistance to scale insects are Pinot Noir, Sauvignon Blanc, Merlot and Cabernet Sauvignon (Simbiken et al. 2015; Venus 2017), although the reason for such resistance is unknown.

As leaf chemistry is a likely source of variation among cultivars, an investigation of the potential variation was initiated. This started with measurement of all compounds in digested freeze-dried leaves collected in the field using gas chromatography–mass spectroscopy (GC-MS) for chemical identification. The instrument used was a single quadrupole GC-MSD (Agilent Technologies, Palo Alto, CA, USA) consisting of a 7890A series gas chromatograph with a split/splitless injector and a 5975C inert XL MSD mass selective detector (Triple-Axis Detector). This analysis permitted comparison of peak retention time and peak amplitude. Using data from the retention times and amplitudes, a principal component analysis was performed to determine whether there was any pattern to the distribution of cultivars based on the unknown compounds that were present. The data suggests that grape cultivars were separated along the first principal component, with the scale-resistant cultivar Pinot Noir separating from the scale-susceptible cultivars Shiraz, Chardonnay and Riesling (Figure 9).

The GC-MS system was also retrofitted with an MPS 2 Gerstel Multipurpose sampler with liquid injection, static headspace (HS) and solid phase microextraction (SPME) capability (GERSTEL GmbH & Co. KG, Germany) that allows for volatiles and other compounds to be measured and identified from leaf pieces (Rivers et al. 2019). Using some of the leaves, Shiraz and Pinot Noir differed in relative quanti-

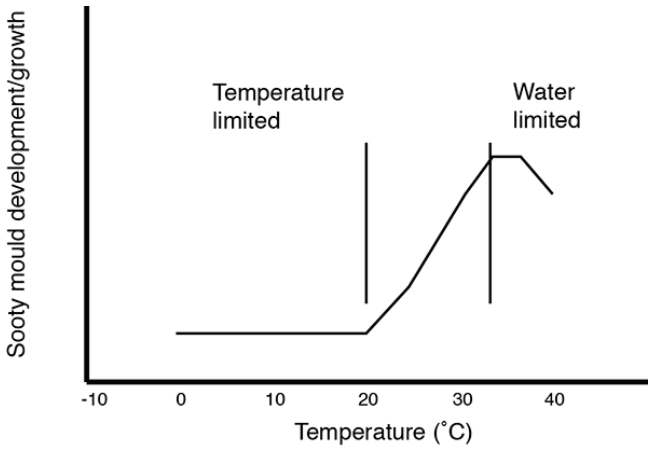


Figure 8. Sooty mould colony growth as temperature increases. Growth of sooty mould is limited at temperatures below 20˚C, increases up to 33˚C and then starts to decrease in this figure. However, if humidity increases with climate change, then water is not limiting the growth of sooty mould and as conditions become more tropical sooty mould will become more apparent in vineyards.

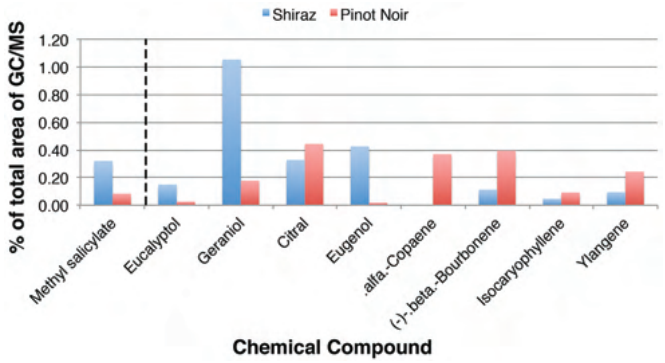


Figure 10. Comparison of relative quantities of methyl salicylate and several volatiles measured in field-collected leaves of Shiraz and Pinot Noir from vineyards in the ACT/NSW region. These measurements were made on five leaves of each cultivar (three vials per cultivar) by sampling the air within a vial using solid phase microextraction (SPME) sampling followed by GC-MS analysis.

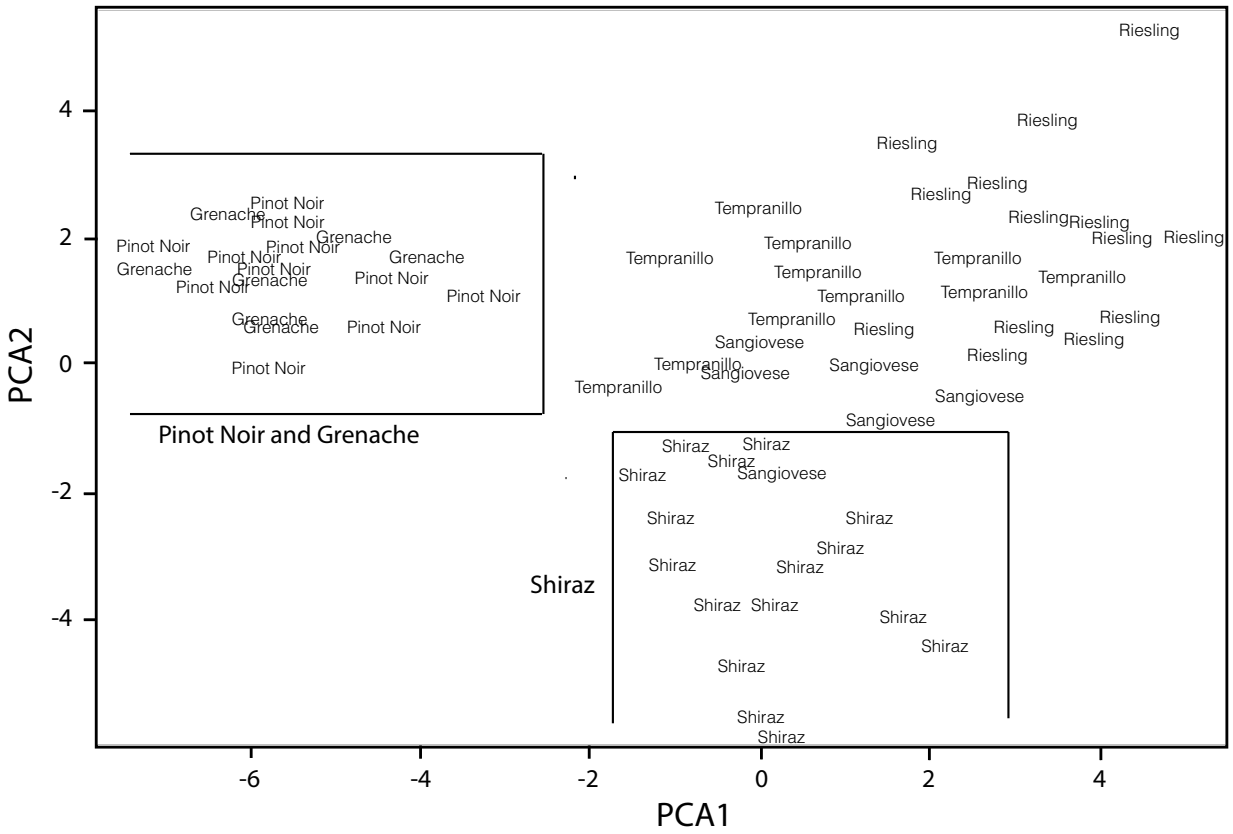


Figure 9. Plot of principal component 1 against principal component 2 that shows divisions between leaves from resistant and susceptible cultivars based upon chemical compound retention and amplitude. The leaves were digested in solvents and the solvent was used in GC-MS to measure the released chemical compounds. This figure is based on vineyards in the ACT/NSW region, but further work is needed to see if this is limited to vineyards in this region. Figure from Cooper (2017)

ties of various volatile compounds (Figure 10). Methyl salicylate, a known component of plant defense systems against piercing and sucking insects (Aljbory and Chen 2018; Erb 2018), was also found. These field-collected plants were not necessarily infested with scale insects, but the same measurement system is being used to determine the quantity of these compounds in grapevines that have been intentionally infested with scale insects compared with uninfested plants kept in a polycarbonate greenhouse. Further work is needed to ensure that any chemicals that may correlate with scale resistance are identified, and understand how this might be translated into a scale control program.

Preliminary data indicate that there are differences between resistant cultivars in their response to scale insects. Sauvignon Blanc had a local response that was not transmitted to other plants within the greenhouse, but Pinot Noir had a much wider effect with apparent resistance conferred to nearby plants, even though each plant was in its own pot. Currently work is underway to determine the different chemicals involved.

Conclusions

Climate change will potentially expose grapevines to varying abiotic conditions, but vineyards have been exposed to varying conditions historically and their pests may be sensitive to climate changes as well (Daane et al. 2018). Certain abiotic conditions (increased temperature and humidity) will favour increases in scale insect numbers, honeydew production and possibly sooty mould. By using the variation in susceptibility to scale infestation among cultivars, it may be possible to control scale insect increases and therefore limit the economic damage of sooty mould. Using IPM (integrated pest management) and harnessing the natural resistance systems present in different cultivars may allow both better control and reduced cost of vineyard management.

Acknowledgements

This work was done in collaboration with the Langhorne Creek wine region and Lian Jaensch, Executive Officer, Langhorne Creek Grape and Wine Incorporated. Leaf chemical measurements were done with the help of Thy Truong, Kenneth Webster and Andras Kesrei. Funding was provided by the Research School of Biology, ANU and Wine Australia through the R&D Regional Program for SA Central (incorporating the wine regions of Langhorne Creek, Adelaide Hills, McLaren Vale, Kangaroo Island, Currency Creek and Southern Fleurieu). Several local vineyards in both ACT/NSW and SA Central region cooperated by allowing access to their properties for undertaking field assessments of scale insect infestation.

References

Aljbory, Z.; Chen, M.-S. (2018) Indirect plant defense against insect herbivores: a review. Insect Sci 25: 2–23.

Andrewartha, H.G.; Birch, L.C. (1954) The distribution and abundance of animals. Chicago, Illinois: The University of Chicago Press.

Andrewartha, H.G.; Birch, L.C. (1984) The ecological web: more on the distribution and abundance of animals. Chicago, Illinois: The University of Chicago Press.

Angilletta, M.J. (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford: Oxford University Press.

Bostanian, N.J.; Vincent, C.; Isaacs, R. (2012) Arthropod management in vineyards: pests, approaches and future directions. Heidelberg: Springer.

Camacho, E.R.; Chong, J.H.; Braman, S.K.; Frank, S.D.; Schultz, P.B. (2017) Life history of *Parthenolecanium* spp. (Hemiptera: Coccidae) in urban landscapes of the southeastern United States. J. Econ. Entomol. 110: 1668–1675.

Castex, V.; Beniston, M.; Calanca, P.; Fleury, D.; Moreau, J. (2018) Pest management under climate change: the importance of understanding tritrophic relations. Sci. Total Environ. 616–617, 397–407.

Chomnunti, P.; Hongsanan, S.; Aguirre-Hudson, B.;Tian, Q.; Persoh, D.; Dhami, M.K.; Alias, A.S.; Xu, J.C.; Liu, X.Z.; Stadler, M.; Hyde, K.D. (2014) The sooty moulds. Fungal Divers. 66: 1–36.

Cooper, P.D. (2017) Scale insects and the *Vitis vinifera* cv. Shiraz-reason for high incidence of scales. Wine Australia: 6 December: http://www.wineaustralia.com/getmedia/0c13513e-caae-4816-b2e8-dd66b9fc73c1/Final-Report-AGW-1702

Daane, K.M.; Vincent, C.; Isaacs, R.; Ioriatti, C. (2018) Entomological Opportunities and Challenges for Sustainable Viticulture in a Global Market. Ann. Rev. Entomol. 63: 193–214.

Deutsch, C.A.; Tewksbury, J.J.; Tigchelaar, M.; Battisti, D.S.; Merrill, S.C.; Huey, R.B.; Naylor, R.L. (2018) Increase in crop losses to insect pests in a warming climate. Science 361: 916–919.

Erb, M. (2018) Plant defenses against herbivory: closing the fitness gap. Trends Plant Sci. 23: 187–194.

Essling, M.; Petrie, P. (2018) Understanding factors leading to sooty mould. Wine Australia: 10 January: http://www.wineaustralia.com/getmedia/6274ffc6-9ccb-4d4e-9e76-dcfecab12851/Sooty-Mould_20180321

Harrison, J.F.; Woods, H.A.; Roberts, S.P. (2012) Ecological and environmental physiology of insects. Oxford: Oxford University Press.

Hayes, A.; Neeman, T.; Cooper, P.D. (2017) Overwintering survival of grapevine scale *Parthenolecanium persicae* (Hemiptera:Coccidae) in the Canberra region of Australia. Austral Entomol. 55: 346–353.

Maino, J.L.; Kong, J.D.; Hoffmann, A.A.; Barton, M.G.; Kearney, M.R. (2016) Mechanistic models for predicting insect responses to climate change. Curr. Opin. Insect Sci. 17: 81–86.

Maudru J.E.; Paxson, T.E. (1950) The Relationship of Sugar Moisture to Relative Humidity. American Society of Sugar Beet Technologists, 6th Biennial Meeting; Detroit, Michigan: pp. 538–540: http://assbt-proceedings.org/1950 Proceedings.htm

Money, R.W.; Born, R. (1951) Equilibrium Humidity of Sugar Solutions. J. Sci. Food Agric. 2: 180–185.

Rakimov, A.; Ben-Dov, Y.; White, V.; Hoffmann, A.A. (2013) Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. Aust. J. Entomol. 52: 371–378.

Reineke, A.; Thiéry, D. (2016) Grapevine insect pests and their natural enemies in the age of global warming. J. Pest Sci. 89: 313–328.

Rivers, J.Y.; Truong, T.T.; Pogson, B.J.; McQuinn, R.P. (2019) Volatile apocarotenoid discovery and quantification in *Arabidopsis thaliana*: optimized sensitive analysis via HS-SPME-GC/MS. Metabolomics 15: 79.

Schowalter, T.D. (2016) Insect Ecology. Amsterdam: Academic Press, Elsevier.

Shukla, P.K.; Gundappa; Adak, T. (2017) Development of sooty moulds in mango orchards in relation to weather parameters and major sucking pests. J. Environ. Biol. 38: 1293–1300.

Simbiken, N.A. (2014) Feeding and ecology of grapevine scales, *Parthenolecanium persicae* and *P. pruinosum*, on different varieties of grapevine. PhD thesis. The Australian National University: 256 p.

Simbiken, N.A.; Cooper, P.D.; Powell, K.S. (2015) Development and feeding effect of frosted scale *Parthenolecanium pruinosum* Cocquillet (Hemiptera: Coccidae) on selected *Vitis vinifera* L. cultivars. Aust. J. Grape Wine Res. 21: 451–457.

Simbiken, N.A.; Powell, K.S.; Cooper, P.D. (2017) Scale back on scale in the vineyard. Aust. NZ Grapegrower Winemaker 645: 42–47.

Venus, J. (2017) Scale in vineyards – identification and control. Wine Australia: 31 July: http://www.wineaustralia.com/getmedia/ac2d8790-432e-4f3d-9824-d509b9e903a0/SA-Central-2016_17-AOP-Activity-2-Scale-in-Vineyards-Final-REPORT

Wilkinson, T.L.; Ashford, D.A.; Pritchard, J.; Douglas, A.E. (1997) Honeydew sugars and osmoregulation in the pea aphid *Acyrthosiphon pisum*. J. Exp. Biol. 200: 2137–2143.

Evaluating activated carbons for removal of phenols and their glycosides from smoke-affected juice and wine

J.A. Culbert, W. Jiang, M.P. Krstic, M.J. Herderich

The Australian Wine Research Institute, Urrbrae, SA
julie.culbert@awri.com.au

Abstract

Exposure of grapes to smoke can result in the uptake of volatile phenols, which, once absorbed into the berry, are enzymatically converted into a range of non-volatile phenolic glycosides. In wine, volatile phenols and their glycosides can cause an unpleasant 'ashy' and 'smoky' sensory sensation and lingering aftertaste, commonly known as smoke taint. In the last 15 years smoke taint has had a substantial financial impact on the wine industry around the world. Activated carbon products are highly porous carbon-rich materials capable of adsorbing organic compounds, including undesirable contaminants. Fourteen commercially available activated carbon products were evaluated for their abilities to remove smoke taint compounds from grape juice and wine. The efficiency of carbon products in removing smoke taint compounds was found to be highly dependent on (i) the type of carbon used; (ii) the matrix (i.e. juice versus wine; red versus white); and (iii) the dose rate of the carbon. Not all activated carbons were created equal, with some carbons found to be better at targeting phenolic glycosides, while others exhibited good selectivity for removing volatile phenols. Generally, activated carbon products were more effective at removing phenolic glycosides in juice than wine. Certain carbon products could reduce the concentration of phenolic glycosides in juice by 90% or more, but at best the reduction was 20% for a smoke-affected Pinot Noir wine and 50-60% for a smoke-affected Sauvignon Blanc wine. Volatile phenols could be effectively removed in both matrices but higher dose rates were required for wine (e.g. >0.5 g/L). These research findings emphasise the importance of selecting the right activated carbon product for the right application (i.e. depending on whether the removal of phenolic glycosides or volatile phenols is being targeted and in what matrix). Benchtop evaluation allowed quick screening of activated carbon products for their ability to remove smoke taint molecules in juice and wine and allowed two products to be shortlisted for small-scale (50 L) winemaking trials.

Introduction

Exposure of grapes to smoke can result in the uptake of volatile phenols such as guaiacol, cresol and syringol. Volatile phenols, once absorbed into the berry, are enzymatically converted into a range of non-volatile phenolic glycosides (Hayasaka et al. 2013). During winemaking, sugar unit(s) from the glycosides can be cleaved, releasing the volatile phenols back into the wine. In wine, volatile phenols and their corresponding glycosides can cause an unpleasant 'ashy' and 'smoky' sensory sensation and lingering aftertaste, commonly known as smoke taint (Hayasaka et al. 2013). Since 2003, major fire events in 2003, 2004, 2007, 2009, 2013 and 2015 have resulted in over $400M worth of grapes and wine being rendered worthless, or products being downgraded, as a result of smoke taint (Krstic et al. 2015). Thus, it is important that the Australian wine industry manages this major financial and reputational risk and develops cost-effective remediation tools for smoke-affected grapes and wine.

Activated carbon products are highly porous carbon-rich materials capable of adsorbing organic compounds including undesirable contaminants. In the wine industry, carbon treatment has been used for decolourising wine, removing Ochratoxin A from red wines (Castellari et al. 2001), reducing the levels of *Brettanomyces* metabolites 4-ethylphenol and 4-ethylguaiacol in wine (Lisanti et al. 2008; Milheiro et al. 2017; Filipe-Ribeiroa et al. 2017), reducing fungicidal residues in white wines via addition during fermentation (Nicolini et al. 2016) and reducing the perception of smoke taint in wine (Fudge et al. 2012). Studies on remediation of smoke taint using carbon have been limited, not only in the number of studies but in the number of activated carbon products evaluated. Such studies have also often focused on treating wine rather than grape must and/or juice. In addition, earlier studies focused solely on the fate of volatile phenols and did not include glycosides, as the discovery of glycosidic derivatives occurred later, first reported by Hayasaka and colleagues in 2010 (a-c).

Activated carbons can be derived from various raw materials (e.g. vegetables, coal, coconut and synthetic materials). Depending on the activation process, which can be either chemical or physical, the resulting carbon will have varying characteristics and therefore possess varying adsorption properties. The aim of this study was to evaluate a comprehensive range of activated carbon products for their ability to remove volatile phenols and their corresponding phenolic glycosides from smoke-affected juice and wine.

Methods

Fourteen commercially available activated carbon products (Table 1) were evaluated for their ability to remove smoke taint compounds from grape juice and wine. The grape varieties evaluated were: Merlot, Cabernet Sauvignon, Pinot Noir, Mataro, Sauvignon Blanc, Riesling and Chardonnay.

Table 1. List of 14 commercially available activated carbon products evaluated

Activated carbon product number	Activated carbon product	Manufacturer/supplier
1	Acticarb PC1000	Activated Carbon Technologies
2	Acticarb PS1000	Activated Carbon Technologies
3	Acticarb PS1300	Activated Carbon Technologies
4	CA-50	CarboChem
5	P-1000	CarboChem
6	PC-900	CarboChem
7	Carbocromos super	Vason/IMCD Australia Ltd
8	FPS	Vason/IMCD Australia Ltd
9	Smartvin	Vason/IMCD Australia Ltd
10	Toxical	Laffort
11	Norit D10	Cabot/IMCD Australia Ltd
12	Norit SX Plus	Cabot/IMCD Australia Ltd
13	Bentonorit DX	Cabot/IMCD Australia Ltd
14	Norit CASPF	Cabot/IMCD Australia Ltd

Juice or wine (50 mL) was treated with a carbon dose rate of 0.5-2.0 g/L (addition of 25 to 100 mg) with contact times of 2-24 hours. Experiments were performed with constant stirring using a magnetic bar and a multi-position magnetic stirrer (Figure 1). After the given contact time, samples were centrifuged (3,750 rpm for 5 minutes) and the supernatant subsampled for volatile phenol and/or phenolic glycoside analysis. Carbon-treated samples were compared against the control (i.e. no carbon treatment) to determine the amount of volatile phenols and/or phenolic glycosides removed by the treatment.

Phenolic glycoside analysis

Smoke phenolic glycosides were detected and quantified using a previously published method using stable isotope dilution analysis and liquid chromatography mass spectrometry (LC-MS) (Hayasaka et al. 2013). Sample preparation for juice and wine samples was as follows:

Sample preparation for juice

Samples were prepared according to the method described by Hayasaka et al. (2010b) with the exception of the use of d_3-syringol gentiobiocide (d_3-SyGG) as internal standard instead of d_3-guaiacol monoglucoside. In brief, juice (2 mL) was spiked with d_3-SyGG (50 µL of 20 µg/mL solution) to give a final concentration in the juice of 500 µg/L. The dosed juice was mixed by vortex for 30 seconds and extracted by solid phase extraction (SPE). The sample was loaded onto a conditioned (1 × 5 mL methanol, 1 × 5 mL MilliQ water) Extract Clean C18-HF SPE 500 mg/4 mL cartridge (Manufacturer: S*Pure Pte. Ltd, Singapore; Supplier: Adelab Scientific, Australia). The cartridge was washed with MilliQ water (10 mL) and eluted with methanol (2 mL). The methanol extract was evaporated to dryness under nitrogen, the resulting residue reconstituted in MilliQ water (300 µL) and filtered (0.45 µm) into an autosampler vial containing a small-volume (250 µL) insert ready for analysis by LC-MS.

Preparation for wine

For phenolic glycoside analysis, wine (1 mL) was spiked with d_3-SyGG (50 µL of 20 µg/mL solution) to give a concentration in the wine of 1,000 µg/L, mixed by vortex and filtered (0.45 µm) directly into a HPLC sample vial (1.5 mL) ready for analysis.

Volatile phenols

For quantification of volatile phenols in juice and wine, samples were submitted to AWRI Commercial Services for extraction and analysis by gas chromatography–mass spectrometry (GC-MS) using stable isotope dilution analysis (Pollnitz et al. 2004). The AWRI's volatile phenols screen includes the analysis of guaiacol, 4-methylguaiacol, *m*-, *o*- and *p*-cresols, syringol and methylsyringol.

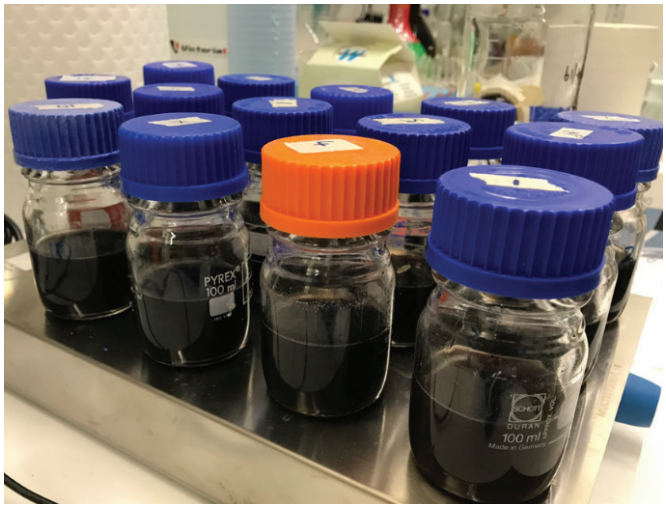


Figure 1. Multiple carbon experiments performed at a 50 mL-scale on a multi-position magnetic stirrer

Table 2. Levels of phenolic glycosides in each of the juice and wine matrices prior to activated carbon treatment

Sample	Concentration (µg/kg SyGG equivalents)						
	SyGG	CrRG	GuRG	MGuRG	MSyGG	PhRG	Total
2018 NSW Sauvignon Blanc juice	55	12	14	14	20	7	122
2018 NSW Sauvignon Blanc wine	26	9	11	9	8	7	70
2018 NSW Pinot Noir wine	28	5	8	7	2	5	55
2016 SA Merlot juice*	55	13	11	5	6	10	100
2016 Vic Pinot Noir wine	43	3	<1	2	12	<1	60
2019 Tas Chardonnay juice	142	15	23	56	13	3	252
2019 Tas Pinot Noir rosé juice	193	31	28	48	22	13	335

SyGG = syringol gentiobioside; CrRG = cresol rutinoside; GuRG = guaiacol rutinoside; MGuRG = methylguaiacol rutinoside; MSyGG = methylsyringol gentiobioside; PhRG = phenol rutinoside; *obtained from artificially smoked grapes

Table 3. Levels of volatile phenols in each of the juice and wine matrices prior to activated carbon treatment

Sample	Concentration (µg/L)							
	4-MG	Guaiacol	*o*-Cresol	*p*-Cresol	*m*-Cresol	Syringol	MSy	Total
2018 NSW Sauvignon Blanc juice	<1	<1	<1	<1	<1	<1	<1	<1
2018 NSW Sauvignon Blanc wine	<1	2	<1	6	<1	4	<1	12
2018 NSW Pinot Noir wine	3	17	5	2	6	12	3	48
2016 SA Merlot juice*	<1	<1	<1	<1	<1	<1	<1	<1
2016 SA Merlot juice* – dosed with volatile phenols	34	29	27	<1	10	3	3	106
2016 Vic Pinot Noir wine	9	26	6	2	2	28	13	86
2019 Tas Chardonnay juice	<1	2	<1	2	<1	<1	<1	4
2019 Tas Pinot Noir rosé juice	2	17	5	6	5	<1	<1	35

4-MG = 4-methylguaiacol; MSy = methylsyringol; *obtained from artificially smoked grapes

Results and discussion

The phenolic glycosides and volatile phenol content of the juices and wines evaluated in this study prior to activated carbon treatment are given in Tables 2 and 3, respectively.

Removal of phenolic glycosides by activated carbon treatment

The removal efficiency of phenolic glycosides from smoke-affected juice and wine was dependent on the activated carbon product type, dose rate and matrix (Figures 2, 3, 4). Even at a low dose rate of 0.5 g/L (contact time of 24 hours), differences in efficiency of the various carbon products were seen (Figure 2). For instance, activated carbon products 3, 4, 9 and 14 were amongst the best performers, reducing total phenolic glycosides in the smoke-affected 2018 NSW Sauvignon Blanc juice by up to 20%. Their superior performance was further highlighted when a dose rate of 2 g/L was used, with these carbon products all reducing the total phenolic glycosides by 80% or more. Activated carbon product 5 also performed well, reducing levels by almost 80% and carbons 2 and 12 had moderate performance (reduction of approximately 60%). Of the remaining seven carbon products evaluated, the majority did not reduce the total phenolic glycosides by more than 20%, even at the higher dose rate of 2 g/L.

Higher removal efficiency of phenolic glycosides was observed in white versus red wine (Figure 3). Even at a dose rate of 2 g/L, there was little to no removal of the phenolic glycosides in the smoke-affected 2018 NSW Pinot Noir wine. The activated carbons fared slightly better in the Sauvignon Blanc wine, with the best-performing carbons (numbers 3 and 5) removing 50-60% of phenolic glycosides from this white wine. Generally, activated carbon products are more effective at removing phenolic glycosides from juice than wine, perhaps as a consequence of pigmented polymers and tannins competing for sites in the activated carbons, an effect which is also more enhanced in red wines than white.

The greater affinity for phenolic glycosides of some activated carbon products than others was further highlighted when the smoke-affected 2019 Tasmanian Chardonnay and Pinot Noir rosé juices were treated with the various carbons at a dose rate of 2 g/L and a contact time of 24 hours (Figure 4). Activated carbon products 3, 4, 5, 9 and 14 were again the best performers, showing similar performance trends regardless of the juice matrix treated. This experiment also highlighted differences in percentage removals between white and red (rosé) juices, which followed that observed for wine (i.e. greater removal in white than red).

Removal of volatile phenols by activated carbon treatment

Differences in the ability of activated carbon products to remove smoke taint molecules were also observed with smaller volatile phenols (Figure 5). Since many of the juice matrices were low in volatile phenols (Table 2), the artificially smoked Merlot juice was spiked with volatile phenols (total 106 µg/L after spiking) prior to carbon treatment. The removal efficiencies of volatile phenols by the 14 activated carbon products at a dose rate of 0.5 g/L were compared in the spiked Merlot juice matrix and in the naturally smoke-affected 2016 Victorian Pinot Noir wine (Figure 5). Volatile phenols were effectively removed in both matrices but higher dose rates were required for the wine (i.e. >0.5 g/L). For the best performers (activated carbon products 7 and 8), up to 40% removal could be achieved in the Pinot Noir wine at a dose rate of 0.5 g/L and over 90% removal could be achieved in the spiked Merlot juice at the same dose rate. A comparable removal rate could be achieved in the Pinot Noir wine but only when it was treated at a dose rate of 2 g/L (data not shown). Greater removal of volatile phenols than phenolic glycosides was observed in wine, perhaps due to those larger molecules found in wine, such as pigmented polymers and tannins, being too large to compete for sites in the activated carbons containing smaller pore sizes, which are better suited for encapsulating the smaller volatile phenols. Interestingly, activated carbons 7 and 8 that performed best for removing volatile phenols were ineffective at removing phenolic glycosides.

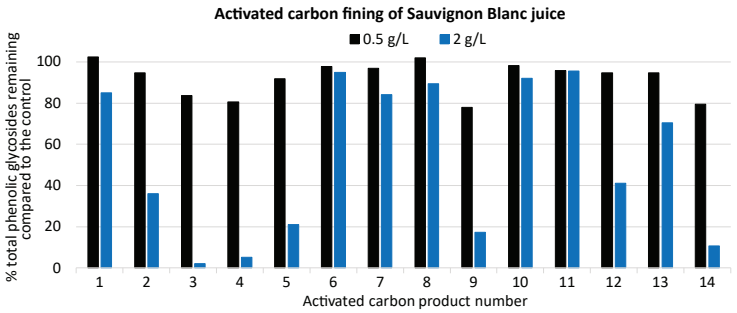


Figure 2. Percentage of total phenolic glycosides (n=6) remaining in smoke-affected 2018 NSW Sauvignon Blanc juice after treatment with various activated carbon products at two dose rates (0.5 and 2 g/L) for 24 hours

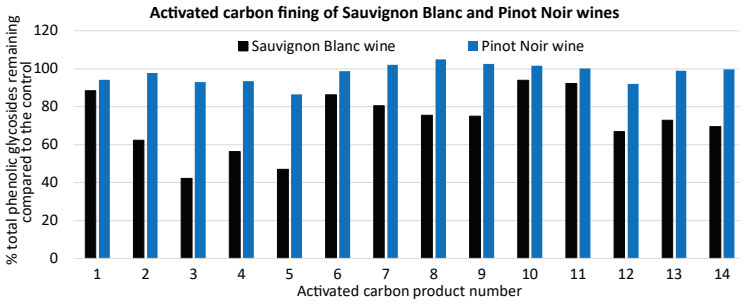


Figure 3. Percentage of total phenolic glycosides (n=6) remaining in smoke-affected 2018 NSW Sauvignon Blanc and Pinot Noir wines after treatment with various activated carbon products at 2 g/L for 24 hours

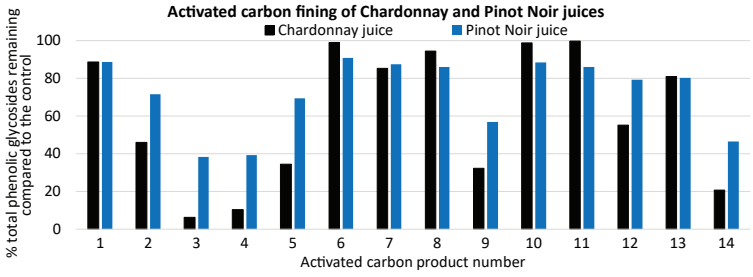


Figure 4. Percentage of total phenolic glycosides (n=6) remaining in smoke-affected 2019 Tasmanian Chardonnay and Pinot Noir juices after treatment with various activated carbon products at 2 g/L for 24 hours

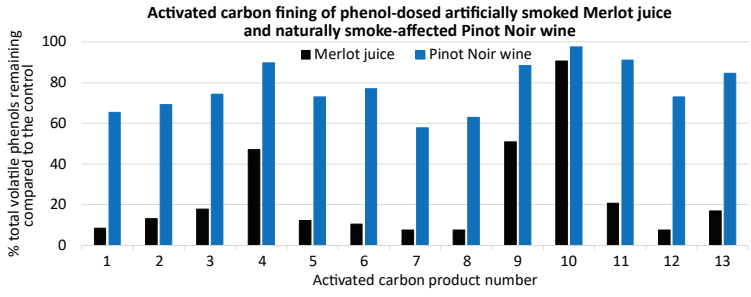


Figure 5. Percentage of total volatile phenols (n=7) remaining in volatile phenol-dosed artificially smoked 2016 Merlot juice and naturally smoke-affected Pinot Noir wine after treatment with various activated carbon products at 0.5 g/L for 24 hours.

Conclusions

Benchtop evaluation allowed quick screening of activated carbon products for their ability to remove smoke taint compounds from grape juice and wine. Not all activated carbon products were created equal, with some better at targeting phenolic glycosides, and others more selective for smaller volatile phenols. Generally, activated carbon products were more effective at removing phenolic glycosides from juice than wine. Volatile phenols could be effectively removed in both matrices but higher dose rates were required for wine (i.e. >0.5 g/L). These research findings emphasise the importance of selecting the right activated carbon product for the right application (i.e. depending on whether the removal of phenolic glycosides or volatile phenols is being targeted and in what matrix). It is best to target phenolic glycoside removal by carbons in juices rather than in finished wine, particularly for white and rosé styles. As a result of these studies, activated carbon products 3 and 14 were shortlisted for small-scale (50 litres) winemaking trials.

Acknowledgements

The authors gratefully acknowledge the members of the Australian wine industry who provided smoke-affected grapes and wine for this project. This project is supported by the AWRI, and through funding from the Australian Government Department of Agriculture, Water and Environment as part of its Rural R&D for Profit program and Wine Australia. The AWRI is a member of the Wine Innovation Cluster in Adelaide, South Australia.

References

Castellari, M.; Versari, A.; Fabiani, A.; Parpinello, G.P.; Galassi, S. (2001) Removal of Ochratoxin A in red wines by means of adsorption treatments with commercial fining agents. J. Agric. Food Chem. 49: 3917–3921.

Filipe-Ribeiro, L.; Milheiro, J.; Matos, C.C.; Cosme, F.; Nunes, F.M. (2017) Reduction of 4-ethylphenol and 4-ethylguaiacol in red wine by activated carbons with different physicochemical characteristics: Impact on wine quality. Food Chem. 229: 242–251.

Fudge, A.L.; Schiettecatte, M.; Ristic, R.; Hayasaka, Y.; Wilkinson, K.L. (2012) Amelioration of smoke taint in wine by treatment with commercial fining agents. Aust. J. Grape Wine Res. 18: 302–307.

Hayasaka, Y.; Baldock, G.A.; Pardon, K.H.; Jeffery, D.W.; Herderich, M.J. (2010a) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine *Vitis vinifera* L. cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS Analysis. J. Agric. Food Chem. 58: 2076–2081.

Hayasaka, Y.; Baldock, G.A.; Parker, M.; Pardon, K.H.; Black, C.A.; Herderich, M.J.; Jeffery, D.W. (2010b) Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke. J. Agric. Food Chem. 58: 10989–10998.

Hayasaka, Y.; Dungey, K.A.; Baldock, G.A.; Kennison, K.R.; Wilkinson, K.L. (2010c) Identification of a β-d-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. Anal. Chim. Acta 660: 143–148.

Hayasaka, Y.; Parker, M.; Baldock, G.A.; Pardon, K.H.; Black, C.A.; Jeffery, D.W.; Herderich, M.J. (2013) Assessing the impact of smoke exposure in grapes: development and validation of a HPLC-MS/MS method for the quantitative analysis of smoke-derived phenolic glycosides in grapes and wine. J. Agric. Food Chem. 61: 25–33.

Krstic, M.P.; Johnson, D.L.; Herderich, M.J. (2015) Review of smoke taint in wine: smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint. Aust. J. Grape Wine Res. 21: 537–553.

Lisanti, M.T.; Piombino, P.; Gambuti, A.; Genovese, A.; Siani, V.L.; Moio, L. (2008) Analytical evaluation of remedial treatments for red and white wines contaminated by volatile phenols. Bulletin de l'OIV 81: 45–55.

Milheiro, J.; Filipe-Ribeiro, L.; Cosme, F.; Nunes, F.M. (2017) A simple, cheap and reliable method for control of 4-ethylphenol and 4-ethylguaiacol in red wines. Screening of fining agents for reducing volatile phenols levels in red wines. J. Chromatogr. B 1041–1042: 183–190.

Nicolini, G.; Villegas, T.R.; Tonidandel, L.; Moser, S.; Larcher, R. (2016) Small amounts of charcoal during fermentation reduce fungicide residues without penalising white wine aroma compounds and colour. Aust. J. Grape Wine Res. 22: 376–383.

Pollnitz, A.P.; Pardon, K.H.; Sykes, M.; Sefton, M.A. (2004) The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. J. Agric. Food Chem. 52: 3244–3252.

Grape seed powder as a novel and sustainable bentonite alternative

E. Romanini[1,2], J.M. McRae[2,3], D. Colangelo[1], M. Lambri[1]

[1]Università Cattolica del Sacro Cuore, Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile - DiSTAS, Piacenza, Italy
[2]The Australian Wine Research Institute, Urrbrae, SA
[3]Current address: The University of Adelaide, Adelaide, SA
elia.romanini@unicatt.it

Abstract

Grape pathogenesis-related proteins can cause haze in wines that is undesirable for consumers. Bentonite is used to remove these proteins but is non-renewable and reduces wine volume due to poor settling. As a potential bentonite alternative, grape seed powder (GSP, 5-10-20 g/L) was added two grape juice varieties (Semillon and Sauvignon Blanc) prior to fermentation. GSP addition removed haze-forming proteins and produced heat-stable wines without substantial changes to wine phenolics content or colour. For comparison, GSP was also added directly to four heat-unstable wines. This required higher doses of GSP (25–32 g/L) for protein removal and haze prevention and induced substantial colour differences (ΔE) compared to untreated control wines. Moreover, with equal GSP treatment (20 g/L), the impact on the wine matrix after juice treatment were substantially less than after direct wine treatment, especially for phenolic content (A_{280}) and overall colour difference (ΔE) between treated wines and untreated control wines. The results of this study indicate that GSP has potential as a sustainable and economical alternative to bentonite.

This work has been published in the journal *Food Chemistry*:

Romanini, E.; McRae, J.M.; Colangelo, D.; Lambri, M. (2019) First trials to assess the feasibility of grape seed powder (GSP) as a novel and sustainable bentonite alternative. Food Chem. 305: 125484: https://doi.org/10.1016/j.foodchem.2019.125484

Is *Brettanomyces bruxellensis* becoming more SO_2 tolerant in industry?

C.E. Bartel[1], A.R. Borneman[1], C.D. Curtin[1,2], C.A. Varela[1]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]Current address: Oregon State University, Corvallis, Oregon, USA

Abstract

Brettanomyces bruxellensis *is a wine spoilage yeast that is commonly controlled through the addition of SO_2, among other measures. Current winemaking trends, including minimising SO_2 additions, may provide levels that are insufficient to kill* B. bruxellensis. *These sub-lethal concentrations may give rise to strains with increased tolerance to SO_2. We investigated if* B. bruxellensis *was capable of developing tolerance to SO_2 when exposed to increasing, sub-lethal levels of SO_2, via an adaptive evolution experiment. Two strains of* B. bruxellensis *(AWRI 1499 and AWRI 1613) were grown in increasing concentrations of SO_2 for up to 100 generations (up to 283 days). Single isolates from these populations were evaluated for SO_2 tolerance. Simultaneously, we investigated if Australian wine isolates of* B. bruxellensis *were increasing their tolerance to SO_2 over time. More than 200 Australian* B. bruxellensis *isolates were acquired from three time periods (2000-2004, 2010–2014 and 2016–2018) and screened for SO_2 tolerance. Results from the adaptive evolution experiment demonstrated that* B. bruxellensis *is capable of developing tolerance to SO_2, with single isolates from tolerant populations showing between three and five times greater tolerance than parental strains. The analysis of Australian isolates indicated that the mean maximum SO_2 tolerance remained largely unchanged from 2000–2004 to 2010–2014 but had greatly increased for the period of 2016–2018. These results combined demonstrate that* B. bruxellensis *has the potential to develop SO_2 tolerance in industry and suggests that the Australian wine industry should manage SO_2 additions carefully and consider alternative strategies for controlling* B. bruxellensis.

Introduction

Brettanomyces bruxellensis is a wine spoilage yeast that produces the volatile phenols 4-ethyl phenol (4-EP) and 4-ethyl guaiacol (4-EG), which contribute to wine characteristics often described as 'barnyard' and 'Band-Aid'. Commonly, *B. bruxellensis* is controlled through a range of winemaking practices including the addition of SO_2, with the current recommendation being a single addition, post-malolactic fermentation of 80 mg/L total SO_2. While total SO_2 is a convenient measure of the amount of this preservative in wine, SO_2 is highly reactive and upon addition to wine 60 to 65% of the added SO_2 binds to wine compounds (AWRI 2020), with the remaining components ('free' SO_2) made up of bisulfite ions (HSO_3-) and molecular SO_2 (mSO_2). Molecular SO_2 is the actual antimicrobial form and its concentration in wine is highly dependent on wine pH (Figure 1). An mSO_2 concentration above 0.8 mg/L for white wines and above 0.6 mg/L for red wines is recommended for sufficient protection against undesirable microorganisms (Coulter 2017).

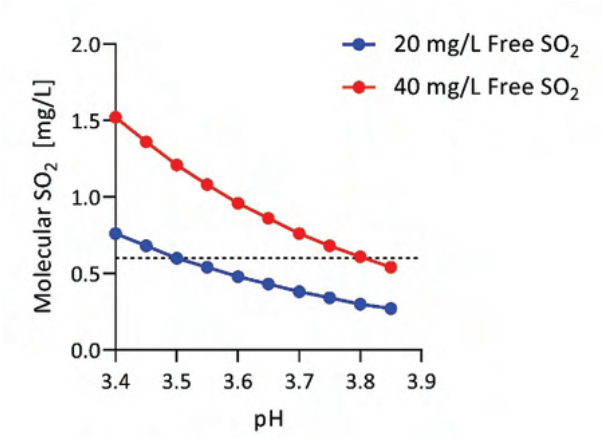


Figure 1. The amount of molecular SO_2 in wine is dependent on wine pH. A wine with 20 mg/L free SO_2 (blue curve) at pH 3.5 has a concentration of 0.6 mg/L mSO_2, which is at the level recommended for red wine (black dashed line). The same wine at pH 3.8 has levels below recommendations and requires 40 mg/L free SO_2 (red curve) to provide the recommended level of mSO_2. Calculations are based on a wine with 13% ethanol, at 20°C (IFV 2019).

Currently the Australian wine industry is seeing a trend of increasing pH in red wines (Godden et al. 2015). At higher pH careful calculation is required to ensure sufficient SO_2 is present in wine to provide adequate protection against microbial growth. Insufficient SO_2 addition provides sub-lethal concentrations of mSO_2 and may give rise to *B. bruxellensis* strains with increased tolerance to SO_2. This study investigated if it was possible to increase SO_2 tolerance in *B. bruxellensis* through exposure to increasing, sub-lethal concentrations of SO_2. It also investigated whether Australian isolates of *B. bruxellensis* are increasing their tolerance to SO_2 over time.

Methodology

Adaptive evolution

Two *Brettanomyces bruxellensis* strains with different levels of SO_2 tolerance, AWRI 1499 (high tolerance) and AWRI 1613 (low tolerance), were subjected to an adaptive evolution experiment in laboratory media with increasing, sub-lethal concentrations of SO_2. Single isolates were taken at 50 and 100 generations and screened for SO_2 tolerance in a 96-well plate in laboratory media spiked with SO_2. Growth curves of tolerant isolates were obtained at different SO_2 concentrations.

SO_2 tolerance of industry isolates

To investigate if Australian isolates of *Brettanomyces bruxellensis* are increasing their tolerance to SO_2 over time, 247 isolates were acquired from three time periods: 2000-2004 (38 isolates), 2010-2014 (42 isolates) and 2016-2018 (167 isolates). Isolates from 2000-2004 and 2010-2014 were obtained from the AWRI Wine Microorganism Culture Collection, while the 2016-2018 isolates were isolated from 24 wine samples from 10 different wineries. Isolates were analysed for SO_2 tolerance in a 96-well plate in laboratory media spiked with SO_2.

Results

Adaptive evolution

Both *Brettanomyces bruxellensis* strains (AWRI 1499 and AWRI 1613) were able to increase their tolerance to SO_2 following adaptive evolution experiments. Within 250 days *B. bruxellensis* populations

were growing in SO_2 concentrations three and four times higher than the initial concentrations tolerated by AWRI 1499 and AWRI 1613, respectively.

Growth kinetics of tolerant isolates showed an increase in tolerance to SO_2, with AWRI 1499 increasing its tolerance from 0.6 mg/L to 1 mg/L mSO_2, and AWRI 1613 from 0.4 mg/L to 1 mg/L mSO_2. Genome sequencing of these isolates is currently underway to determine genetic alterations that may give rise to the observed increase in SO_2 tolerance.

SO_2 tolerance of industry isolates

An analysis of the mean maximum SO_2 tolerance of isolates collected from three time periods is summarised in Table 1. These results demonstrate that while there are still isolates in industry with low SO_2 tolerance, more isolates with a greater tolerance to SO_2 are being seen than in the past 20 years.

Table 1. A summary of SO_2 tolerances of *B. brettanomyces* isolates

Cohort	Mean maximum SO_2 tolerance (mg/L mSO_2)	Range of SO_2 tolerance (mg/L mSO_2)
2000–04	0.61	0.27 – 0.82
2010–14	0.52	0.14 – 0.96
2016–18	0.86	0.14 – 1.1

Conclusions

This work has shown that *Brettanomyces bruxellensis* has the ability to develop SO_2 tolerance when exposed to sub-lethal levels of SO_2. An analysis of the SO_2 tolerances of *B. bruxellensis* isolates obtained from industry suggests that SO_2 tolerance is potentially emerging in industry. Given the small winery sample size, these findings need to be verified. Further sampling and collection of *B. bruxellensis* isolates will help to determine if tolerance is emerging and how widespread it is in Australia. To minimise the risk of emergence of SO_2-tolerant strains of *B. bruxellensis*, wineries should focus on all of the factors that contribute to controlling *B. bruxellensis*, including maintaining winery cleanliness, adhering to barrel sanitation protocols and carefully considering wine pH when calculating SO_2 additions to ensure they are sufficient.

References

AWRI (The Australian Wine Research Institute) (2020) Controlling *Brettanomyces* during winemaking. AWRI fact sheet: https://www.awri.com.au/wp-content/uploads/2014/05/Brett-fact-sheet.pdf

Coulter, A. (2017) Ask the AWRI: Understanding molecular SO_2. Aust. N.Z. Grapegrower Winemaker 636: 76.

Godden, P.; Wilkes, E.; Johnson, D. (2015) Trends in the composition of Australian wine 1984-2014. Aust. J. Grape Wine Res. 21: 741–753.

IFV (Institut Francais de la Vigne et du Vin) (2019) Molecular SO_2 calculator: http://www.vignevin-occitanie.com/outils-en-ligne/so2-actif-ou-moleculaire/

Is it the age or the autolysis? Pulling apart where sparkling wine character comes from

S. Sawyer[1], R. Longo[1], M. Solomon[2], L. Nicolotti[2,3], H. Westmore[1], A. Merry[1], G. Gnoinski[1], A. Ylia[2,3], R. Dambergs[1,4,5], F. Kerslake[1]

[1]Tasmanian Institute of Agriculture, University of Tasmania, Prospect and Sandy Bay, Tas.
[2]The Australian Wine Research Institute, Urrbrae, SA
[3]Metabolomics Australia, Urrbrae, SA
[4]WineTQ, Monash, SA
[5] National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW
fiona.kerslake@utas.edu.au

Abstract

Sparkling wines made by the traditional method (méthode traditionnelle) undergo an in-bottle secondary fermentation, which is initiated by the addition of yeast and sugar, and usually followed by several months or years of ageing on lees (sur lie). This study found that wine age was the main determinant of compositional changes in yeast-derived and oxidation-associated compounds such as esters, carboxylic acids, higher alcohols and aldehydes. Ageing base wines off or on lees produced similar aroma profiles to tiraged wine based on the six sensory characters assessed in this study, irrespective of variety. For Chardonnay, the profile was more developed in the base wine aged off lees than the tirage or base wine aged on lees and in Pinot Noir, the base wine aged on lees was more developed. The results of this study suggest time on lees post-secondary fermentation has less of an impact on aroma development than the length of the ageing period.

Introduction

During the making of méthode traditionnelle sparkling wine, sur lie ageing is attributed to sensorial changes, enhanced foaming properties and the development of a character winemakers refer to as 'autolytic' or 'autolysis' (Mafata et al. 2018; Jolly et al. 1993; Charpentier et al. 2005). Longer sur lie ageing has been attributed to the development of the desirable characteristics of a premium sparkling wine (Pérez-Magariño et al. 2015; Riu-Aumatell et al. 2006; Torrens et al. 2010). The products of yeast autolysis (cell death) and the gradual release of these compounds into wine during ageing on lees have been reported to be the origin of precursor and sensory-active compounds that lead to the development of this 'autolytic' character (Mafata et al. 2018; Jolly et al. 1993; Charpentier et al. 2005). However, the description of this character is problematic because unlike other wine character descriptors, it does not relate to a common or readily available sensory experience and is therefore difficult to understand. 'Autolytic' character has been variously described as the aroma/flavour of bread, dough, pastry, biscuits, toast, yeast, butter and butterscotch (Vannier et al. 1999; Mafata et al. 2018).

Products of yeast autolysis, including potential precursors to sensory-active compounds such as amino acids, nucleotides, fatty acids and mannoproteins are considered responsible for the sensory changes that accompany ageing sur lie along with changes in fermentation-derived aroma compounds such as esters, carboxylic acids and higher alcohols (Feuillat and Charpentier 1982; Leroy et al. 1990; Pueyo et al. 2000; Charpentier et al. 2005; Nunez et al. 2005; Alexandre and Guilloux-Benatier 2006). However, although mannoproteins and ageing on lees have been demonstrated to contribute positively to foaming (Pérez-Magariño et al. 2015; Nunez et al. 2005, 2006), the evidence for the contribution of other compounds to changes during ageing on lees is sparse and often contradictory (Leroy et al. 1990; Alexandre 2019). Thus, this study was designed to gain a better understanding of whether flavour development in sparkling wines is due to yeast autolysis and the associated release of compounds, or due to the developmental changes that occur over time.

Winemaking

Commercial, single-variety base wines (*Vitis vinifera* L. cvs. Pinot Noir and Chardonnay) were used for this trial. A control wine for each variety was prepared in triplicate by tiraging the base wine with a commercial liqueur de tirage consisting of yeast (*Saccharomyces cerevisiae*, IOC 18-2007, Lallemand), sucrose (24 g/L) and bentonite (0.1 g/L) and then storing at 15°C until disgorgement (6-, 12- and 24-months post-tirage). An additional wine was tiraged and aged at 25°C, for use as a benchmark for development of each character. Each variety of base wine was also bottled in triplicate without any lees, or with primary ferment lees added back, and then stored at 15°C until disgorgement (6-, 12- and 24-months post-tirage). The base wine with no lees added back was still run through 'disgorgement' for consistency of air exposure during this process. Forty-six analytes were identified and quantified from bottled wines as described by Siebert et al. (2005) and Mayr et al. (2015) with some modifications. For the Siebert et al. (2005) method, the injector temperature was set at 260°C and the oven temperature started at 40°C, then increased to 60°C at 20°C/min (held for 14 min), followed by a series of temperature ramps. The first ramp was to 70°C at 10°C/min, the second ramp was to 80°C at 10°C/min, the third ramp was to 160°C at 20°C/min, and the final ramp was to 260°C at 10°C/min and held for 2 min. The total run time was 45.5 min. The vial and contents were heated to 40°C for 5 min with agitation. 2-Methylbutanal was also identified and quantified by the method described in Mayr et al. (2015). All statistical analyses were performed using JMP v14.0 (SAS Institute, Cary, NC). Principle component analysis (PCA) included all 46 analytes. Statistical differences in the sensory comparison and compositional data were determined using Student's t-test (sensory only) and analysis of variance (ANOVA).

Yeast-derived and oxidation-associated compounds in the wines

A total of 46 analytes were identified and quantified in Chardonnay and Pinot Noir base and sparkling wines. In order to understand the role of yeast in the secondary fermentation, these analytes included important yeast-derived compounds (such as ethyl and acetate esters, carboxylic acids and higher alcohols) and compounds associated with ageing and oxidation (such as furanones and aldehydes). Principal component analysis (PCA) across the time points revealed that regardless of variety, age was the main determinant and treatment effects had to be considered in the context of age (Figures 1, 2). There was little separation between the treatments based on yeast-derived and oxidation-associated compounds. Variability was more promi-

nent in Chardonnay (Figure 1) than Pinot Noir (Figure 2). Close clustering of the two base wines and the tiraged wine at 6 and 12 months indicated they were developing at a similar pace. Since 24 months' in-bottle ageing showed the strongest separation by treatment, this time point was investigated more closely.

Sensory evaluation of the wines

Comparative sensory analysis was conducted using an expert tasting panel of Australian sparkling winemakers. Each of the base wines was compared to the control (tiraged) wine after 24 months' in-bottle ageing with respect to six sensory characters mutually agreed to be associated with sparkling wine development (Figure 3). In this study, ageing at a higher sur lie temperature (25°C) was used as a benchmark for development of each character. In Chardonnay, the base wine aged off lees was perceived as significantly more intense than the control for two characters, 'nutty' and 'honey', and the base wine aged on lees was perceived to have a more intense 'honey' character compared to the control (Figure 3a). The Pinot Noir base wine aged on lees was also perceived to have a more intense 'honey' character (Figure 3b). However, the base wine aged off lees was perceived as the same as the control. Regardless of variety, it is interesting to note that the base wines were not perceived as being lower in intensity than the control for any of the characters assessed in this study.

Where does the 'autolytic' or developed character come from?

Of the 46 analytes quantified, compounds that were significantly different by ANOVA in the base and tiraged wines at 24 months' in-bottle ageing are presented in Table 1 and 2 for Chardonnay and Pinot Noir, respectively. Generally, in Chardonnay, the esters, acids and alcohols developed similarly in both base wines, but the most prominent changes were observed in the Strecker aldehydes (Table 1). For all four aldehydes analysed (2-methylpropanal, 2-methylbutanal, methional, phenylacetaldehyde), the concentrations were more than 99% higher in the base wine aged off lees than both the control wine and base wine aged on lees and above their respective odour thresholds. These compounds are associated with 'malty', 'cooked potato-like', 'honey' and 'floral' characters (Culleré et al. 2007; Czerny et al. 2008; Mayr et al. 2015) and their formation is dependent on the nitrogen content and oxidative potential of the wine (Bueno et al. 2016). In Pinot Noir, the Strecker aldehydes again feature prominently (Table 2) in the base wines above their respective odour thresholds (with only three aldehydes showing treatment effects) but the higher concentrations of octanoic and decanoic acids (associated with 'sweaty', 'cheese' and 'rancid' notes (Francis and Newton 2005)) are likely contributing the flavour perception and complexity in the base wine aged on lees.

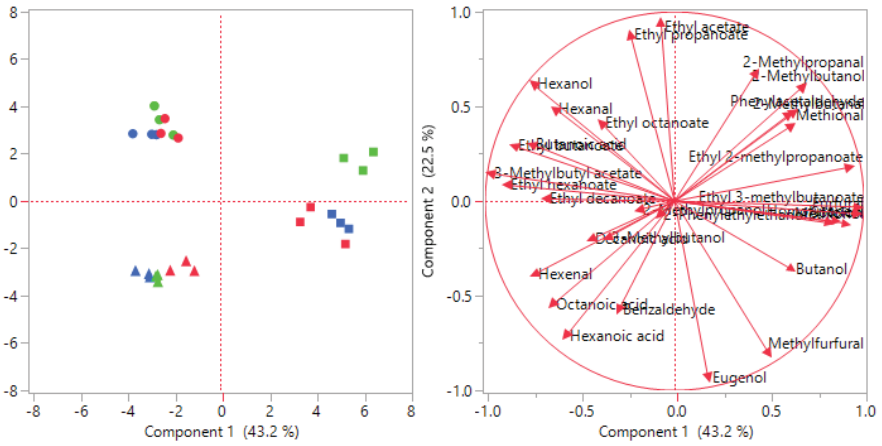


Figure 1. PCA biplots of volatile compounds in Chardonnay aged for 6 (circle), 12 (triangle) and 24 months (square). Green, blue and red symbols represent the base wines aged off lees, base wine aged on lees and the tiraged wines, respectively.

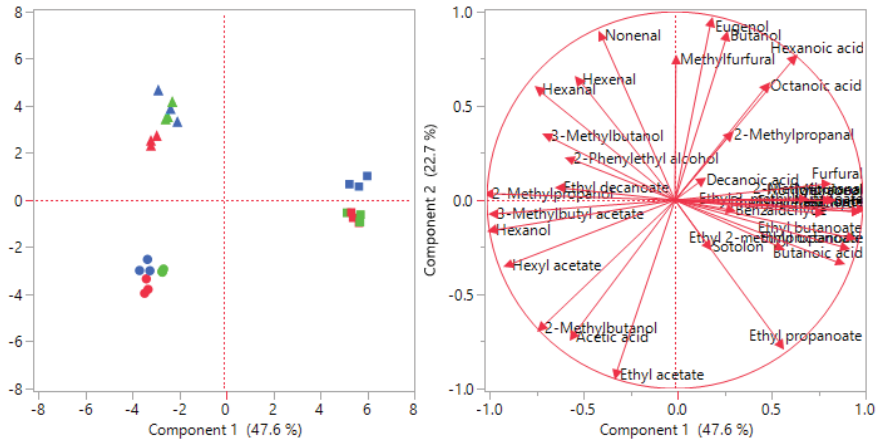


Figure 2. PCA biplots of volatile compounds in Pinot Noir aged for 6 (circle), 12 (triangle) and 24 months (square). Green, blue and red symbols represent the base wines aged off lees, base wine aged on lees and the standard tirage wines, respectively.

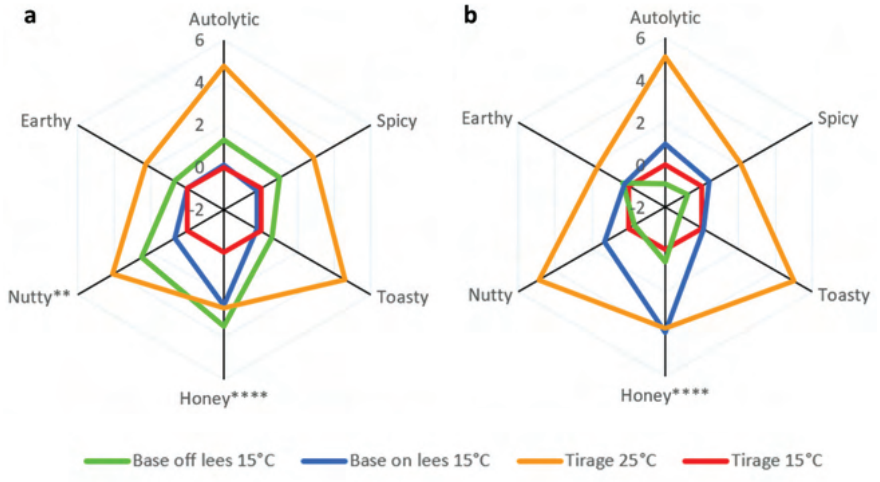


Figure 3. Comparative sensory analysis of Chardonnay (a) and Pinot Noir (b) at 24 months' in-bottle ageing. Asterisks indicate statistical significance of treatments compared to the control (tiraged wine aged at 25°C was used as a benchmark for visualisation purposes only). *: P<0.05; **: P<0.01; ***: P<0.001; ****: P<0.0001

Table 1. Percentage change of chemical compounds in Chardonnay compared with the control tiraged wine after 24 months' in-bottle ageing

Compound	Base wine off lees	Base wine on lees	Tiraged wine	Relative odour threshold*	Odour descriptor*
Esters					
Ethyl acetate	+20^{A}	+24^{A}	0^{B}	-12	Ethereal, fruity
Ethyl propanoate	+16^{A}	+12^{A}	0^{B}	>200	Ethereal, fruity, rum
Ethyl octanoate	-9^{AB}	-12^{A}	0^{B}	-100	Fruity, fat
Ethyl decanoate	-61^{A}	-61^{A}	0^{B}	-37	Fruity (grape)
Ethyl 2-methylpropanoate	+28^{A}	+25^{A}	0^{B}	-72	Sweet, rubber
3-Methylbutyl acetate	-12^{A}	-13^{A}	0^{B}	-76	Fruity (banana)
Carboxylic acids					
Octanoic acid	-22^{A}	-29^{A}	0^{B}	-96	Sweaty, cheese
Decanoic acid	-66^{A}	-63^{A}	0^{B}	-66	Rancid, fat
Higher alcohols					
2-Phenylethanol	+15^{A}	+10^{AB}	0^{B}	+46	Honey, spice, rose, lilac
2-Methylbutanol	+20^{A}	+8^{AB}	0^{B}	-95	Malty, solvent-like
3-Methylbutanol	+9^{A}	+4^{AB}	0^{B}	-74	Whiskey, malt, burnt
Methionol	-35^{C}	-18^{B}	0^{A}	-30	Cooked potato-like
Eugenol	-8^{A}	+2^{B}	0^{B}	+88	Clove-like
Furanones					
Homofuraneol	-60^{A}	-33^{B}	0^{C}	-100	Caramel
Aldehydes					
2-Methylpropanal	+99^{A}	+2^{B}	0^{B}	-58	Malty
2-Methylbutanal	+122^{A}	-66^{B}	0^{AB}	-96	Malty
Methional	+345^{A}	-20^{B}	0^{B}	-87	Cooked potato-like
Phenylacetaldehyde	+174^{A}	-17^{B}	0^{B}	-91	Honey, floral

*Relative odour threshold is presented as percentage change against control. Percentage changes above the odour threshold value indicate concentrations are above the empirical odour threshold. Odour thresholds and descriptors were obtained from Mayr et al. (2015), Francis and Newton (2005), Culleré et al. (2007), Czerny et al. (2008) and Guth (1997).
^{ABC}Different superscript letters across a row indicate significant differences among treatments ($p < 0.05$) by ANOVA and Tukey HSD.

Table 2. Percentage change of chemical compounds in Pinot Noir compared with the control tiraged wine after 24 months' in-bottle ageing

Compound	Base wine off lees	Base wine on lees	Tiraged wine	Relative odour threshold*	Odour descriptor*
Esters					
Ethyl acetate	+3^{A}	-18^{B}	0^{A}	-29	Ethereal, fruity
Ethyl propanoate	+2^{A}	-11^{B}	0^{A}	>200	Ethereal, fruity, rum
Ethyl butanoate	-3^{AB}	-7^{B}	0^{A}	-94	Fruity (apple)
Ethyl decanoate	-9^{B}	+30^{A}	0^{B}	+46	Fruity (grape)
Ethyl 2-methylpropanoate	+18^{A}	-3^{B}	0^{B}	-74	Sweet, rubber
3-Methylbutyl acetate	+7^{AB}	+11^{A}	0^{B}	-72	Fruity (banana)
Carboxylic acids					
Hexanoic acid	-3^{B}	+6^{A}	0^{AB}	-95	Sweaty
Octanoic acid	-5^{B}	+30^{A}	0^{B}	-94	Sweaty, cheese
Decanoic acid	-10^{B}	+94^{A}	0^{B}	-10	Rancid, fat
Higher alcohols					
2-Phenylethanol	+1^{A}	-13^{B}	0^{A}	+280	Honey, spice, rose, lilac
2-Methylpropanol	+3^{A}	-4^{B}	0^{AB}	+129	Solvent, wine, bitter
2-Methylbutanol	-2^{AB}	-8^{B}	0^{A}	-96	Malty, solvent-like
3-Methylbutanol	+1^{A}	-6^{B}	0^{AB}	-76	Whiskey, malt, burnt
Methionol	-21^{B}	-17^{B}	0^{A}	-30	Cooked potato-like
Furanones & lactones					
Homofuraneol	-64^{B}	-57^{B}	0^{A}	-99	Caramel
Sotolon	-43^{B}	-37^{B}	0^{A}	+29	Curry, seasoning
Aldehydes					
2-Methylbutanal	+171^{A}	+23^{B}	0^{B}	-94	Malty
Methional	+210^{A}	+97^{B}	0^{C}	-83	Cooked potato-like
Phenylacetaldehyde	+113^{A}	+40^{B}	0^{C}	-89	Honey, floral

*Odour threshold is presented as percentage change against control. Percentage changes above the odour threshold value indicate concentrations are above the empirical odour threshold. Odour thresholds and descriptors were obtained from Culleré et al. (2007), Czerny et al. (2008), Francis and Newton (2005), Guth (1997) and Mayr et al. (2015).
^{ABC}Different superscript letters across a row indicate significant differences among treatments ($p < 0.05$) by ANOVA and Tukey HSD.

Favourable aromatic development, and not just organoleptic properties, in champagne and sparkling wines are thought to only occur in the presence of yeast lees (Feuillat and Charpentier 1982). Development has been reported to be the result of the release of aroma precursors and compounds during yeast autolysis as well as the activity of enzymes released during this time (Feuillat and Charpentier 1982; Leroy et al. 1990; Pueyo et al. 2000; Charpentier et al. 2005; Nunez et al. 2005; Alexandre and Guilloux-Benatier 2006; Kemp et al. 2014). The close development of the base and tiraged wines throughout the ageing period of the current study suggests factors determining aroma and flavour development in sparkling wines are predominantly determined by the content of the base wine. Fruit-derived volatiles were not analysed in this study as the literature focused on the contribution from yeast-derived volatiles, but their release into the wine matrix and effect on flavour perception during the ageing process should be assessed in further studies. While the secondary fermentation and subsequent ageing on lees is important for the development of carbon dioxide and other organoleptic properties in sparkling wine, the results of the current study suggest the impact of the secondary fermentation on the aroma and flavour compounds analysed herein is limited and wine age has a greater impact on the development of characteristic flavours in premium sparkling wines.

Acknowledgements

This work was supported by Wine Australia (grant UT1502), with support from the Tasmanian Institute of Agriculture, Wine TQ, Hill-Smith Family Vineyards and The Australian Wine Research Institute (AWRI). We also thank Metabolomics South Australia which is funded through Bioplatforms Australia Pty Ltd (BPA), a National Collaborative Research Infrastructure Strategy (NCRIS), and investment from the South Australian State Government and the AWRI. The cooperation and time donated by various Australian winemakers at the tasting sessions is gratefully acknowledged.

References

Alexandre, H. (2019) Yeasts and Sparkling Wine Production. Romano, P.; Ciani, M.; Fleet, G.H. (eds.) Yeasts in the Production of Wine. New York: Springer.

Alexandre, H.; Guilloux-Benatier, M. (2006) Yeast autolysis in sparkling wine - a review. Aust. J. Grape Wine Res. 12: 119–127.

Bueno, M.; Carrascón, V.; Ferreira, V. (2016) Release and Formation of Oxidation-Related Aldehydes during Wine Oxidation. J. Agric. Food Chem. 64: 608–617.

Charpentier, C.; Aussenac, J.; Charpentier, M.; Prome, J.C.; Duteurtre, B.; Feuillat, M. (2005) Release of nucleotides and nucleosides during yeast autolysis: Kinetics and potential impact on flavor. J. Agric. Food Chem. 53: 3000–3007.

Culleré, L.; Cacho, J.; Ferreira, V. (2007) An assessment of the role played by some oxidation-related aldehydes in wine aroma. J. Agric. Food Chem. 55: 876–881.

Czerny, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.M.; Schieberle, P. (2008) Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. Eur. Food Res. Technol. 228: 265–273.

Feuillat, M.; Charpentier, C. (1982) Autolysis of Yeasts in Champagne. Am. J. Enol. Vitic. 33: 6–13.

Francis, I.L.; Newton, J.L. (2005) Determining wine aroma from compositional data. Aust. J. Grape Wine Res. 11: 114–126.

Guth, H. (1997) Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. J. Agric. Food Chem. 45: 3027–3032.

Jolly, N.P.; Janse, B.J.H.; Rooyen, T.J.V.; Louw, J.H. (1993) Hybridization and typing of yeasts used in sparkling wine fermentations. Am. J. Enol. Vitic. 44: 217–226.

Kemp, B.; Alexandre, H.; Robillard, B.; Marchal, R. (2014) Effect of Production Phase on Bottle-Fermented Sparkling Wine Quality. J. Agric. Food Chem.63: 19–38.

Leroy, M.J.; Charpentier, M.; Duteurtre, B.; Feuillat, M.; Charpentier, C. (1990) Yeast Autolysis During Champagne Aging. Am. J. Enol. Vitic. 41: 21–28.

Mafata, M.; Buica, A.; Du Toit, W.; Panzeri, V.; Van Jaarsveld, F.P. (2018) The effect of grape temperature on the sensory perception of Méthode Cap Classique wines. S. Afr. J. Enol. Vitic. 39**:** 132-140.

Mayr, C.M.; Capone, D.L.; Pardon, K.H.; Black, C.A.; Pomeroy, D.; Francis, I.L. (2015) Quantitative analysis by GC-MS/MS of 18 aroma compounds related to oxidative off-flavor in wines. J. Agric. Food Chem. 63: 3394–3401.

Nunez, Y.P.; Carrascosa, A.V.; Gonzalez, R.; Polo, M.C.; Martinez-Rodriguez, A. (2006) Isolation and characterization of a thermally extracted yeast cell wall fraction potentially useful for improving the foaming properties of sparkling wines. J. Agric. Food Chem.54: 7898–7903.

Nunez, Y.P.; Carrascosa, A.V.; Gonzalez, R.; Polo, M.C.; Martinez-Rodriguez, A.J. (2005) Effect of accelerated autolysis of yeast on the composition and foaming properties of sparkling wines elaborated by a champenoise method. J. Agric. Food Chem. 53: 7232–7237.

Pérez-Magariño, S.; Ortega-Heras, M.; Bueno-Herrera, M.; Martínez-Lapuente, L.; Guadalupe, Z.; Ayestarán, B. (2015) Grape variety, aging on lees and aging in bottle after disgorging influence on volatile composition and foamability of sparkling wines. LWT Food Sci. Technol. 61: 47–55.

Pueyo, E.; Martinez-Rodriguez, A.; Polo, M.C.; Santa-Maria, G.; Bartolome, B. (2000) Release of lipids during yeast autolysis in a model wine system. J. Agric. Food Chem. 48: 116–122.

Riu-Aumatell, M.; Bosch-Fuste, J.; Lopez-Tamames, E.; Buxaderas, S. (2006) Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees. Food Chem. 95: 237–242.

Siebert, T.E.; Smyth, H.E.; Capone, D.L.; Neuwohner, C.; Pardon, K.H.; Skouroumounis, G.K.; Herderich, M.; Sefton, M.A.; Pollnitz, A.P. (2005) Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Anal. Bioanal. Chem. 381: 937–947.

Torrens, J.; Riu-Aumatell, M.; Vichi, S.; López-Tamames, E.; Buxaderas, S. (2010) Assessment of volatile and sensory profiles between base and sparkling wines. J. Agric. Food Chem. 58: 2455–2461.

Vannier, A.; Brun, O.X.; Feinberg, M.H. (1999) Application of sensory analysis to champagne wine characterisation and discrimination. Food Qual. Pref. 10: 101–107.

Australian wine biosecurity: are we keeping up?

J. Luck[1], C. Elliott[2], I. Lee[3]

[1]Plant Biosecurity Research Initiative, Hort Innovation, Melbourne, Vic.
[2]Wine Australia, Adelaide, SA
[3]Vinehealth Australia, Adelaide, SA
jo.luck@horticulture.com.au

Abstract

There are a number of changing features of the Australian wine industry which may increase its exposure to biosecurity risk, such as the consolidation of processing facilities with more movement of grapes across regional and state boundaries, increased contract vineyard management, increased wine tourism and ongoing tight margins for grapegrowers. This may mean that biosecurity is a lower priority for many. Each of these factors increases the risk of the introduction and spread of pests and diseases. In recent times, several Australian agricultural industries have experienced the negative economic and social impacts of biosecurity incursions with 'ripple' effects on employees and surrounding communities. Good vineyard biosecurity practices support a strong and sustainable wine industry, aiming to reduce the threat of pests and diseases such as Xylella*, brown marmorated stink bug and phylloxera. With a multitude of biosecurity threats facing the sector, understanding the risks and quickly detecting and responding to these threats, if they arrive, is critical and cross-sectoral collaboration and learning is an important part of managing those risks. Access to innovation and technology to prevent new incursions, or the spread of existing pests and diseases, is also vital. In 2017, a novel partnership between Wine Australia, six plant research and development corporations, Plant Health Australia and the Department of Agriculture was formed – the Plant Biosecurity Research Initiative (PBRI). The PBRI aims to avoid duplication of research efforts across industries and leverages investment to support larger cross-sectoral projects, to contribute to better biosecurity for Australian growers.*

Biosecurity in Australia

Australia has a reputation of being free of many of the pests and diseases that affect crops in other countries. There is, however, increased movement of people and commodities around the world, thereby increasing the likelihood of a pest or disease entering and establishing in Australia. With more than 60,000 km of coastline, Australia provides extensive pathways for exotic pests and diseases to enter the country. The Department of Agriculture plays an important role in the biosecurity system by screening millions of passengers at airports, international mail, seaports, ships and cargo containers by using x-ray machines, surveillance and detector dogs and maintaining a pre-border treatment and certification system (Department of Agriculture 2019a). State biosecurity agencies also play a role in biosecurity by implementing emergency response efforts during an incursion and managing the interstate movement of goods that present a biosecurity risk.

Australia's biosecurity risks are growing in scale and complexity due to changing global demands, growing passenger and trade volumes, the rise of online shopping, population expansion and climate change. The responsibility of biosecurity is shared between government, industry and the community. Landowners, producers and businesses along the supply chain have a responsibility to prevent and manage pests and diseases on their own land but, in some situations, state governments may need to intervene to protect public or industry interests from the uncontrolled spread of some established pests.

Australian wine industry biosecurity

Protecting vineyards against pests and diseases is an increasing challenge with many of the global trends (above) presenting greater risks to the wine industry. Pests and diseases can be introduced and spread through the movement of soil, vine material (such as leaves, stems, roots, cuttings, rootlings, potted vines, grapes, grape juice, grape marc), clothing, footwear, machinery, equipment, vehicles and cargo (Vinehealth Australia 2018). There are some specific features of the industry that also present a risk to its biosecurity, namely:

- a move to larger and fewer processing facilities, which means more movement of fruit between properties
- an increase in contract labour, vineyard management and wine tourism resulting in the movement of people and equipment between properties
- pests harbouring in imported machinery, equipment and general materials (e.g. wine barrels) increasing the risk of exotic incursions such as the brown marmorated stink bug.

If an incursion occurs, affected vineyards may show a decline in vine health, yield and quality will reduce and ultimately vine death can occur, leading to decreasing property value—at least in the short term—and placing the sustainability of individual enterprises at risk. Vineyards affected by damaging pests or diseases may need to be replanted. For those infested with phylloxera, tolerant or resistant rootstocks will be required which costs approximately five times more than own-rooted vine material (Vinehealth Australia 2018). Wine businesses will be subject to increased biosecurity compliance and regulatory requirements such as workplace health and safety and Agvet chemical regulation. This will increase the costs of doing business; for example, the purchase, installation and maintenance of equipment, as well as indirect costs for training activities. Regulation may also restrict movement of grapes from a vineyard, resulting in them not being processed or limiting options for processing.

Further restrictions may be placed on the movement of grape products between wineries and the movement of grapevine planting material from a vineyard or nursery. This would be expected to increase during a biosecurity incident where stricter movement controls are introduced to minimise the risk of the further spread of a pest or disease. Premium wine brands relying on the marketing of their heritage vines may be negatively impacted during an incursion. Infected vines or those at risk may need to be removed and destroyed as part of the containment or eradication strategy. Many aspects of vineyard operations will need to be managed carefully during an incursion. A quarantined property will potentially create major disruption to the business and its reputation through the restriction of movement, and compulsory decontamination or treatment of people, plants and products and vehicles, machinery and equipment on and off the property.

Industry biosecurity

Some of the key features of good biosecurity for the wine industry are:

- being familiar with the existing and emerging biosecurity threats that may create a serious impact on business (e.g. *Xylella* or phylloxera)
- developing and implementing measures to minimise the risk of pests or diseases establishing, such as regular monitoring for symptoms of pests or diseases and practising good vineyard hygiene
- developing good relationships with nearby growers, industry bodies and government agencies to remain aware of new and emerging threats, share information and learn from the experiences of others
- complying with legislation, especially when moving pest vectors such as grapes, machinery, equipment and vine material between states and regions and when importing new varieties from overseas
- using accredited nurseries which propagate high-health material and monitoring new material on arrival at the property and after planting
- monitoring for symptoms or pests in the vineyard and reporting anything unusual to the relevant state department (Plant Health Australia 2009).

Partnerships between the wine industry, government and industry bodies are important during a biosecurity incursion. Organisations like Plant Health Australia (PHA) play an important role in developing those partnerships. Industry membership of PHA provides the option of being a signatory to the Emergency Plant Pest Response Deed, which enables an avenue for protection in the event of a plant pest incursion. PHA also provides material and expertise on biosecurity awareness and has developed detailed industry biosecurity plans, including a Viticulture Industry Biosecurity Plan. It curates the Biosecurity Portal which provides access to information about biosecurity surveillance, diagnostics, training, technical information, tools, national policies and strategies, and legislation to plant health professionals and stakeholders across government, industry and the community.

High-priority biosecurity threats

Xylella

The Department of Agriculture has prepared a top 40 list of unwanted pests and diseases which threaten $30 billion worth of agricultural industries (Department of Agriculture 2019d). The number one pest on this list is from the genus *Xylella*, a group of bacteria with a host list of more than 560 different species, many of them occurring in Australia and including *Vitis* species. Originally identified in California in the 1880s, it subsequently spread to South America and was also detected in Central America. In 2013, the CoDIRO strain of *Xylella fastidiosa* (subsp. *pauca*) was detected in olive trees in southern Apulia, Italy—this was the first detection outside of the Americas. In 2013, olive trees in Apulia, which produces 40% of Italy's olive oil, started declining and dying. Of the 60 million olive trees in this region, approximately 21 million trees are regarded as at risk with the infected area approaching one million hectares (Saponari et al. 2019). It was discovered that a small insect, the meadow spittlebug, transmitted the *Xylella* bacteria to the olive trees (Figure 1).

Genetic analyses suggest that this strain was accidentally introduced to Italy from Costa Rica or Honduras via infected ornamental coffee plants (Godefroid et al. 2019). The regions at high risk of *Xylella* encompass the Mediterranean coastal areas of Spain, Greece, Italy and France, the Atlantic coastal areas of France, Portugal and Spain as well as the south western regions of Spain and the lowlands in southern Italy (Figure 2) (Godefroid et al. 2019). The bacterium has been detected in Spain, Portugal, France (Corsica and some regions of the mainland) and in the Middle East (EPPO 2019). If *Xylella* becomes established in Australia, it is estimated that it will cost the grape and wine sector up to $7.9 billion (Hafi et al. 2017). There is no cure, so preparedness for Australian vineyards and other susceptible crops is vital. If *Xylella* was introduced into Australia, the regulations imposed on an infected vineyard would be very strict and affected businesses would experience serious disruption to movement into and out of the affected property, until the disease was delimited and contained.

Grapevine viruses

There are more than 60 recognised virus and virus-like diseases of grapevines worldwide (Wine Australia 2019). Many cause losses in yield and quality of fruit, reduced vine growth, graft incompatibility and vine decline or death. Grapevine viruses can be spread by insect vectors, soil-borne nematode vectors or by using infected cuttings during standard grafting and propagation practices. Symptomless infections often occur and infected grapevines may act as a reservoir for the virus. In 2016 and 2017, the first detections of Grapevine Pinot Gris Virus (GPGV) occurred in vineyards across New South Wales, Victoria and South Australia (Figure 3). Unfortunately, due to the established nature of this virus, eradication was not feasible and GPGV is now considered established in NSW, Vic. and SA (Victorian Viticulture Biosecurity Committee 2018).

Grapevine red blotch-associated virus (GRBaV) is a recently described virus which was first reported on Cabernet Sauvignon in the Napa Valley (California, USA) in 2008. The symptoms of GRBaV generally start appearing in autumn as irregular blotches on leaf blades and the basal portions of shoots. Primary and secondary veins on leaves turn red, as well as red blotch symptoms on leaves, also causing a significant reduction in sugar accumulation in grapes (PHA 2013). This virus is related to leafroll-associated viruses but has not been detected in Australia.

Figure 1. The meadow spittlebug *Philaneus spumarius*. Photo credit: Kevin Hall

Figure 2. Olive trees in Puglia infected with *Xylella*. Photo credit: PanareoFotografia

Brown marmorated stink bug

The brown marmorated stink bug (BMSB) has become a major pest risk for both Australia and New Zealand in recent years, with increasing numbers of detections at borders (Figure 4). Native to China, this pest is able to survive on machinery and equipment being shipped from overseas and has been found on tractors, cars and containers of electrical cabling recently entering Australia. Several interceptions have been made close to shipping ports where the insect has been fumigated and controlled. This pest has not been found on any crops in Australia yet and early detection is vital to avoid establishment. Like *Xylella*, BMSB has a large host range in the vicinity of more than 300 plant species. It is a major problem in East Asia, North America and Europe. Physical damage to grape berries can occur leading to bunch rots and yield loss. Stink bugs have a foul-smelling odour when disturbed and the volatile trans-2 decenal may cause wine taint, imparting 'green' or 'coriander-like' aromas (Mohekar et al. 2017).

The Department of Agriculture has a webpage devoted to BMSB, including the high-risk countries of origin and a list of regulated goods and treatment (Department of Agriculture 2019b). The pesticides effective in controlling this insect are from broad-spectrum groups such as pyrethroid or neonicotinoids, which are are not compatible with integrated pest management (IPM) programs or approved for organic viticulture. Significant resources are required to physically inspect for BMSB in all shipping containers arriving at Australian ports. New techniques for more efficient and sensitive detection are required and a focus for future research and development.

Figure 3. Symptoms of Grapevine Pinot Gris Virus. Photo credit: Dr Pasquale Saldarelli, Senior Scientist/Virologist, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy

Figure 4. Adult brown marmorated stink bug. Photo credit: Daniel D. Dye II

Phylloxera

One pest that is already causing disruption to vineyards in some parts of Australia is phylloxera, which is confined to Victoria and NSW (Figure 5). Infestation by this pest leads to the decline and death of the vine and unfortunately most vineyards in Australia are not planted on phylloxera-resistant rootstocks and are thus susceptible to attack (Vinehealth Australia 2018). Phylloxera is managed using three Phylloxera Management Zones, where each winegrowing region is classified according to whether it has been found to have phylloxera or not. These zones are: Phylloxera Infested Zones, Phylloxera Exclusion Zones and Phylloxera Risk Zones. The National Phylloxera Management Protocol outlines the movement restrictions for phylloxera risk vectors such as grapevine material, machinery, equipment, grape-related material and people into and between these three zones. Phylloxera 'crawlers' can be easily picked up by clothing, footwear, equipment and vehicles, potentially leading to movement of the pest to other vineyards and regions. Infested soil, leaves, planting material, diagnostic samples, grapes and grape marc are also vectors.

States or areas that are free of this pest enforce strict legislative requirements on the movement of phylloxera vectors to reduce the risk of the introduction of phylloxera (National Vine Health Steering Committee 2009). In addition, the adoption of simple farm-gate hygiene activities, such as visitor log books to check where visitors, contractors, sales representatives and other visitors have been earlier; ensuring compliance with state biosecurity regulations with respect to phylloxera vectors that are being brought onto the property (e.g. machinery); and following a 'clean in, clean out' policy, will also reduce the risk of a phylloxera incursion.

Recent incursions in other crops in Australia

The tomato potato psyllid, Western Australia

Tomato potato psyllid (TPP) is a tiny (3 mm) sap-sucking insect that was detected in Western Australia (WA) in February 2017, prompting a comprehensive biosecurity response. This was the first time TPP had been found in Australia (Department of Agriculture 2019c). The psyllid feeds on tomato, potato, capsicum, chilli, eggplant and sweet potato, causing stunting, wilting, yellowing and curling of leaves. Within days of the pest's discovery, movement of plants and produce thought to be hosts of the psyllid were restricted within WA and to interstate markets. This insect has now established in WA and is under active control. So far, the organism that causes zebra chip disease in potatoes, *Candidatus* Liberibacter solanacearum, has not been detected in the insects.

Figure 5. Phylloxera adults and nymphs. Photo credit: Agriculture Victoria, Rutherglen

Panama disease, Queensland

Panama disease was first detected in Queensland on Cavendish banana plants on a farm in the Tully Valley in March 2015. Unfortunately, more than 80 per cent of Australia's bananas are grown in the region. The disease is caused by *Fusarium oxysporum* Tropical Race 4 (TR4), a fungus in the soil that affects the plant rather than the fruit. There has been widespread damage from this disease overseas, one estimate puts the damage for Indonesian farmers at $120 million annually and for farmers in Taiwan at double that amount (Stokstad 2019). In south-east China, over 100,000 ha of Cavendish bananas had been destroyed by TR4 by 2017 and at current market prices that represents a loss of over 1.4 billion US dollars (Drenth 2019).

Australia has mounted a significant effort to contain TR4 and prevent its spread. It has been calculated that the potential impact of unlimited spread of TR4 in Australia would cost $138 million per annum. The impact on regional and rural communities would be significant (Drenth 2019). Fortunately, due to strict biosecurity measures, the rate of spread has been slow thus far compared with worldwide experience. In order to continue to trade, the farmers affected must abide by biosecurity protocols. Being soil-borne, TR4 is considered impossible to eradicate and this has seen the unprecedented decision by industry to purchase an infected farm in North Queensland to destroy the plantation and ensure replanting and regrowth does not occur to protect the region from further spread.

Citrus canker

Citrus canker is threatening Australia's $700 million citrus industry. It was detected in Darwin, Northern Territory (NT) in April 2018, and subsequently in northern WA in the Ord region. Citrus canker (*Xanthomonas citri* subsp. *citri*) is a serious bacterial disease of commercial varieties of citrus. The disease affects the leaves, twigs and fruit, causing the leaves to drop and fruit to fall to the ground before ripening. In severe cases, it can even cause tree death—a major impact, given that citrus can take five years from planting to production. It had been previously detected in Central Queensland in 2004 and subsequently eradicated at significant cost and effort, including the destruction of more than 600,000 citrus trees (Department of Agriculture 2019c). Citrus canker is extremely persistent and plant destruction is the only way to stop the spread. Movement controls and quarantine measures to contain the disease remain in place in both NT and WA.

Responding to these threats – the Plant Biosecurity Research Initiative

Wine Australia is a member of the Plant Biosecurity Research Initiative (PBRI), a partnership between the nation's plant research and development corporations (RDCs), working collaboratively with Plant Health Australia (PHA), the Department of Agriculture, industry, state and federal biosecurity stakeholders. In 2017-2018, the plant RDCs invested $118 million into single-sector biosecurity research; however, the main goal of the PBRI is to ensure that this effort is aligned with broader national goals and delivered with increased efficiency to avoid duplication of effort between Australia's plant-based industries. Since June 2017, the PBRI has coordinated $46 million cross-sectoral biosecurity research investment. Included is the recently funded national Rural R&D for Profit project ($15.7 million) focused on boosting plant diagnostic capability for Australia. This project includes all PBRI members, each state and territory and New Zealand research agencies. Wine Australia has prioritised capability building for grapevine virus diagnostics as an important part of this project.

The iMapPESTS project is a $21 million mobile cross-industry plant pest surveillance network, which will provide information to primary producers and government on endemic, established, trade-sensitive or exotic pests. The project will work towards enhanced pest management and biosecurity. The iMapPESTS project will validate a system that can rapidly monitor and report the presence of high-priority pests and diseases such as airborne fungal spores and insects.

In January 2019, a three-year project was funded to support a *Xylella* program manager to ensure national awareness and coordination of RD&E and to prevent arrival and establishment of the pest in Australia. The *Xylella* program manager will work closely with Australian researchers and grapevine and horticulture industry members, focusing on *Xylella* preparedness. This will include communicating results from research such as the large *Xylella* diagnostics project ($2 million) supported by Hort Innovation and being led by Agriculture Victoria.

As part of the PBRI collaboration, Hort Innovation funded the simulation 'Exercise Fastidious' which was delivered by PHA in November 2018. To improve preparedness for responding to a detection of *X. fastidiosa*, Exercise Fastidious brought together a broad range of industry and government stakeholders to investigate aspects of decision-making and response strategy development under a scenario where *X. fastidiosa* is hypothetically detected in production horticulture and nursery settings (Plant Health Australia 2019).

Summary

With increasing trade and people movement adding to the risk of pests and diseases entering Australia, the focus on shared biosecurity responsibility between industry and government will be critical to maintaining Australia's strong biosecurity reputation. As part of this shared responsibility, on-farm biosecurity is increasingly important to protect the Australian wine industry. Continued access to innovation and technology is also fundamental to Australia remaining free of many of the world's pests and diseases. The PBRI provides an exciting new funding model for Australian plant industries to invest in research and development that supports wine with co-investment from other industries. In response to the question 'are we keeping up?', the answer is 'just'. With the pressure of new and emerging biosecurity threats and increased trade and travel increasing Australia's biosecurity risk profile, there is significant ongoing work needed to protect our industries and communities.

References

Department of Agriculture (2019a) Biosecurity in Australia: http://www.agriculture.gov.au/biosecurity/australia

Department of Agriculture (2019b) Brown marmorated stink bug (*Halyomorpha halys*): http://www.agriculture.gov.au/pests-diseases-weeds/plant/brown-marmorated-stink-bug

Department of Agriculture (2019c) National pest and disease outbreaks – citrus canker: https://www.outbreak.gov.au/current-responses-to-outbreaks/citrus-canker

Department of Agriculture (2019d) Plant pests and diseases: http://www.agriculture.gov.au/pests-diseases-weeds/plant

Drenth, A. (2019) TR4 Around the World: Ongoing spread and impact. Australian Banana Grower's Council: 26 April: https://abgc.org.au/2019/04/26/tr4-around-the-world-ongoing-spread-and-impact/

EPPO (2019) Global Database, *Xylella fastidiosa*: https://gd.eppo.int/taxon/XYLEFA

Godefroid, M.; Cruaud, A.; Streito, J.-C.; Rasplus, J.-Y.; Rossi, J.-P. (2019) *Xylella fastidiosa*: climate suitability of European Continent. Sci. Rep. 9: 8844.

Hafi, A.; Randall, L.; Arthur, T.; Addai, D.; Tennant, P.; Gomboso, J. (2017) Economic impacts of *Xylella fastidiosa* on the Australian wine grape and wine-making industries. ABARES Canberra.

Mohekar, P.; Lapis, T.; Wiman, N.; Lim, J.; Tomasino, E. (2017) Brown marmorated stink bug taint in Pinot noir: Detection and consumer rejection thresholds of trans-2-decenal. Am. J. Enol. Vitic. 68: 120–127.

National Vine Health Steering Committee (2009) National phylloxera management protocol: October: https://vinehealth.com.au/media/National-Phylloxera-Management-Protocol.pdf

Plant Health Australia (2009) Industry Biosecurity Plan for the Viticulture Industry: August: https://www.wfa.org.au/assets/environment-biosecurity/Biosecurity-Plan.pdf

Plant Health Australia (2019) Exercise Fastidious Report. PHA: 14-15 November 2018: http://www.planthealthaustralia.com.au/wp-content/uploads/2019/06/Exercise-Fastidious-Report.pdf

Saponari, M.; Giampetruzzi, A.; Loconsole, G.; Boscia, D.; Saldarelli, P. (2019) *Xylella fastidiosa* in olives in Apulia: Where We Stand. Phytopathology 109: 175–186.

Stokstad, E. (2019) Devastating banana disease may have reached Latin America, could drive up global prices. Science: 17 July: https://www.sciencemag.org/news/2019/07/devastating-banana-disease-may-have-reached-latin-america-could-drive-global-prices

Victorian Viticulture Biosecurity Committee (2018) Grapevine Pinot Gris Virus. 19 February: https://www.vvbc.org.au/grapevine-pinot-gris-virus

Vinehealth Australia (2018) What is biosecurity?: https://vinehealth.com.au/regulation/what-is-biosecurity/

Wine Australia (2019) Viruses of grapevines: https://www.wineaustralia.com/growing-making/pest-and-disease-management/viruses-of-grapevines

Managing Pierce's disease and red blotch disease

M.L. Cooper[1], R.P.P. Almeida[2], M.P. Daugherty[3], L.G. Varela[4], R.J. Smith[4], M.F. Fuchs[5], K.M. Daane[2], H. Wilson[3]

[1]Division of Agriculture and Natural Resources, University of California, Cooperative Extension, Napa, USA
[2]Environmental Science, Policy and Management, University of California, Berkeley, USA
[3]Department of Entomology, University of California, Riverside, USA
[4]Division of Agriculture and Natural Resources, University of California, Cooperative Extension, Santa Rosa, USA
[5]Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, Ithaca, USA
mlycooper@ucanr.edu

Abstract

Management of bacterial and viral diseases of grapevine is challenging, due mainly to the absence of curative practices. Therefore, as a first line of defence, detection and exclusion protocols are often implemented to identify invasive species and screen plant material. In affected vineyards, management programs are driven by the timely identification and removal of diseased vines to reduce pathogen inoculum. The challenge of properly identifying diseased vines based on visual symptoms can limit the effectiveness of vine removal and requires an investment in trained personnel to lead the identification efforts. Technological advances that would partially or fully automate the process could improve the reliability and efficiency. In the case of insect-vectored diseases, strategies aimed at vector management have shown mixed results, and are often dependent on the ecology of the disease system and the greater landscape context. Studies that elucidate disease ecology are therefore foundational to the development of data-driven disease management programs. The availability of educational resources is critical to train workers and develop management programs. Adoption of a particular management strategy is dependent upon verifying that it works in practice. Grower networks that support the uptake of data-driven control practices and provide independent verification of their usefulness can increase positive pest management outcomes and advance short- and long-term change.

Introduction

Successful disease management outcomes are dependent on the widespread adoption of targeted management practices, driven by a comprehensive understanding of disease ecology. Insect-vectored and incurable bacterial and viral diseases can be the most complicated to manage, given their often complex epidemiology.

The bacterium *Xylella fastidiosa* is the causal agent of Pierce's disease (PD), a lethal, vector-borne disease of grapevines (Davis et al. 1978) with an average annual cost of $92 million to California grapegrowers (Alston et al. 2013). Following inoculation, the bacterium multiplies and moves through the xylem network, resulting in symptoms of reduced water flow (Newman et al. 2003) that include: desiccation of clusters, marginal leaf scorch, uneven shoot maturation, stunted growth and eventual death (Figure 1). There are many factors that affect PD incidence in vineyards including: pathogen, vector, host, alternate host and climate. Epidemics of PD in southern California, USA are driven by the invasive glassy-winged sharpshooter (*Homalodisca vitripennis*) (Blua et al. 1999), whereas in northern California they are driven by the blue-green sharpshooter (*Graphocephala atropunctata*) (Purcell 1975). A range of other sharpshooters and spittlebugs are confirmed vectors of *X. fastidiosa* and may contribute to disease outbreaks under various circumstances.

Within the last decade, red blotch disease has emerged as a consequential viral disease of grapevines in North America. Grapevine red blotch virus (GRBV), the causal agent of red blotch disease (Yepes et al. 2018), has been documented in vineyards throughout the United States (Krenz et al. 2014; Sudarshana et al. 2015; Cieniewicz et al. 2017a). The disease affects vineyard profitability by interfering with water/sugar transport in the vine (Blanco-Ulate et al. 2017; Martínez-Lüscher et al. 2019), thereby reducing fruit quality and ripening (Girardello et al. 2019) and resulting in losses of up to $170,000 per acre over the lifespan of a vineyard, depending on various factors (Ricketts et al. 2017). Although a putative vector has been identified under greenhouse conditions (Bahder et al. 2016), transmission has not been documented under field conditions.

Figure 1. Symptoms of Pierce's disease of grapevines. Photo credits: M.B. Hobbs and M.L. Cooper

Pierce's disease management in California, USA

Xylella fastidiosa is the xylem-limited bacterium that is the causal agent of PD of grapevines. Different strains of *X. fastidiosa* also cause disease in multiple other crops, including almond, citrus, coffee and olive, as well as infecting ornamental hosts such as oleander, oak, elm and sycamore. Its current host list includes more than 300 plant species (EFSA 2015), many of which support bacterial populations without expressing symptoms, making elimination of *X. fastidiosa* from the landscape a challenging endeavour. Transmission of *X. fastidiosa* occurs via multiple xylem-feeding insects that include sharpshooters (Hemiptera: Cicadellidae) and spittlebugs (Hemiptera: Cercopidae). The known sharpshooter vectors include blue-green (*Graphocephala atropunctata*), glassy-winged (*Homalodisca vitripennis*), green (*Draeculacephala minerva)*, red-headed (*Xyphon fulgida*), willow (*Graphocephala confluens*), *Neokolla severini* and *Pagaronia* spp.; and the spittlebug vectors include meadow (*Philaenus spumaris*), *Aphrophora* spp. and *Clastoptera* sp. Most of the vector species have wide host ranges, both for feeding and reproduction.

Management of PD in vineyards is complicated by many factors including the diversity and occurrence of insect vectors, the wide pathogen and vector host ranges, climatic factors that affect transmission and the development of chronic infections. Severe pruning and retraining of diseased vines has limited efficacy, mainly because the bacterium is likely to be systemic before obvious visual symptoms are present (Daugherty et al. 2018). Timely identification and removal of diseased vines can reduce pathogen inoculum in the vineyard, although the impact of this activity on subsequent disease infections remains to be explored.

In areas of California where *G. atropunctata* is the dominant vector species, the elimination of host plant species from riparian corridors can significantly lower PD prevalence to negligible rates (Purcell 1975). However, host plant removal requires permits to work in sensitive riparian habitats; and is often costly due to the working conditions, the difficulty in eliminating many of the key plant species (particularly Himalayan blackberry, *Rubus discolor* and periwinkle, *Vinca major*) and the ongoing maintenance requirements. Regional, coordinated efforts that secure outside funding, such as the restoration efforts in the Napa River watershed in northern California, have successfully addressed the multiple goals of habitat restoration, erosion control and elimination of invasive plant species, while simultaneously reducing PD incidence in the surrounding region.

Activities such as planting and maintaining green barriers between riparian habitats and vineyards to limit the ingress of vectors have limited efficacy—the benefits were demonstrated mainly in years with large vector populations, and subsequent effects on disease prevalence have not been documented (Daugherty et al. 2012). Ongoing studies are exploring the contribution of the various vector species and the role of vineyard pathogen reservoirs in disease epidemiology. The results of such studies have the potential to improve management outcomes. Long-term studies on the environmental conditions of disease outbreaks are also warranted, particularly in areas of California where *G. atropunctata* is the dominant vector species.

In southern California, *H. vitripennis* is the dominant vector species. Although within-vineyard chemical controls may have some value in reducing *H. vitripennis* populations, a long-term, regional, cooperative management program has kept populations relatively low within the region (Daugherty et al. 2015). Regulatory protocols (origin and destination plant inspections and treatments at commercial nurseries) have to date limited the incursion of *H. vitripennis* into northern California.

Red blotch disease management in California, USA

Although likely present in California vineyards for decades (Al Rwahnih et al. 2015), grapevine red blotch virus (GRBV) was recently confirmed as the causal agent of grapevine red blotch disease (Yepes et al. 2018). With widespread distribution in North America (Krenz et al. 2014; Sudarshana et al. 2015; Cieniewicz et al. 2017a), red blotch disease has economic consequences resulting from the detrimental impacts on vegetative growth, yield and fruit quality (Calvi 2011; Girardello et al. 2019; Martínez-Lüscher et al. 2019), as well as the costs associated with management activities aimed at reducing disease spread and excluding the pathogen from commercial vineyards (Ricketts et al. 2017). The most prominent visual symptoms include interveinal reddening of the leaf blade and leaf veins in black-fruited cultivars, and interveinal chlorosis (resembling potassium deficiency symptoms) in white-fruited cultivars (Figure 2).

Red blotch disease management decisions are confounded by the evolving body of knowledge of disease ecology. They are further complicated by the varied timing and severity of symptom development that limits efforts to identify and remove diseased vines. The virus has been isolated from non-cultivated grapevines near vineyards (Perry et al. 2016; Bahder et al. 2016). Although the epidemiological contribution of these infections has not been fully determined, removal of these vines may be considered if future studies are conclusive.

A putative vector, three-cornered alfalfa hopper (*Spissistilus festinus*) has been determined from glasshouse studies (Bahder et al. 2016). This insect is relatively infrequent in vineyard surveys and grapevines are an opportunistic rather than a preferred host (Preto et al. 2019). Surveys also indicate regional differences in insect occurrence. Secondary disease spread may occur in areas populated by *S. festinus* (select vineyard blocks in California) whereas secondary spread has not been conclusively documented when *S. festinus* does not occur (i.e. New York, CA) (Cieniewicz et al. 2017b, 2018). Because grapevines are not a preferred host, insecticide applications in vineyards targeting *S. festinus* are not likely to produce significant reductions in insect populations and could result in detrimental secondary effects. Vegetation management practices (such as cultivation or low-mowing) that reduce the season-long incidence of the preferred leguminous breeding hosts could reduce *S. festinus* populations, but this has not been demonstrated in replicated studies; nor is

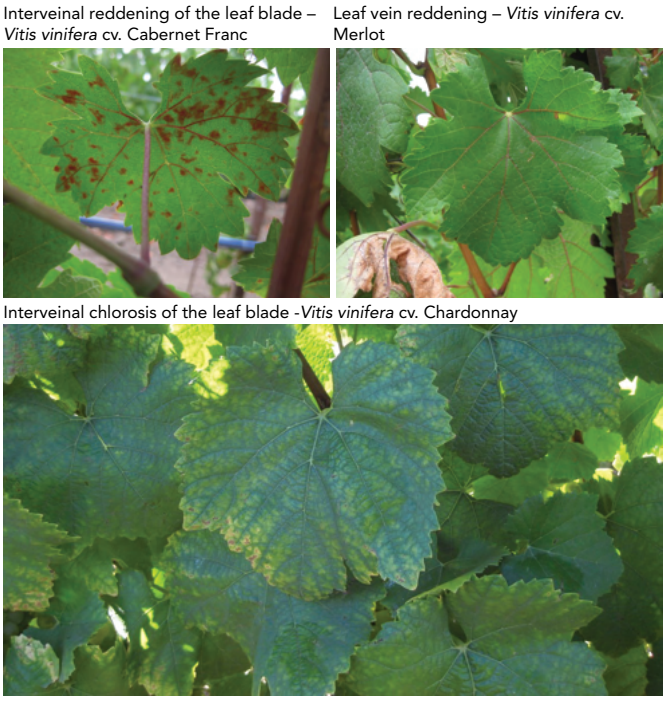


Figure 2. Symptoms of grapevine red blotch disease. Photo credit: M.L. Cooper

it clear what effect this would have on disease spread. Ongoing studies are also evaluating other insect species as potential vectors.

Despite the many unknowns and evolving body of knowledge, activities aimed at reducing disease inoculum have emerged as critical practices. Planting virus-tested nursery stock and removing diseased vines can limit disease spread in production vineyards (Cieniewicz et al. 2019). Technological advances that facilitate the efficient, rapid identification and removal of diseased vines have the potential to advance disease management outcomes. Furthermore, regional efforts to support the uptake of data-driven control practices can increase positive pest management outcomes and advance short- and long-term change.

Conclusions

Grapegrowers in California face the ongoing challenges of Pierce's and red blotch diseases. The many factors affecting PD incidence in vineyards complicate management programs. Although multiple studies have outlined management practices, growers continue to struggle with periodic and severe disease outbreaks. Researchers have outstanding questions such as the contribution of the many vectors and the role of vineyard pathogen reservoirs in disease epidemiology. As red blotch disease is more recently discovered, key epidemiological questions remain, particularly related to field transmission and vector management, to solidify strategies to reduce disease incidence. An improved understanding of Pierce's and red blotch disease systems is critical to inform the selection of management practices, support adoption of management programs and promote successful outcomes.

Acknowledgements

This work was supported by the American Vineyard Foundation, the PD/GWSS Program of the California Department of Food and Agriculture and the Napa County Winegrape Pest and Disease Control District. Technical assistance was provided by A. Adams, M. Hobbs, E. Kuhn, A. Linnett and S. MacDonald. This work would not be possible without the ongoing support of the winegrape industry in Napa County, California, USA.

References

Al Rwahnih, M.; Rowhani, A.; Golino, D. (2015) First report of grapevine red blotch-associated virus in archival grapevine material from Sonoma County, California. Plant Dis. 99: 895.

Alston, J.M.; Fuller, K.R.; Kaplan, J.D.; Tumber, K.P. (2013) Economic consequences of Pierce's Disease and related policy in the California wine grape industry. J. Agric. Resour. Econ. 38: 269–297.

Bahder, B.W.; Zalom, F.G.; Jayanth, M.; Sudarshana, M.R. (2016) Phylogeny of geminivirus coat protein sequences and digital PCR aid in identifying *Spissistilus festinus* as a vector of grapevine red blotch-associated virus. Phytopathology 106: 1223–1230.

Blanco-Ulate, B.; Hopfer, H.; Figueroa-Balderas, R.; Ye, Z.; Rivero, R.M.; Albacete, A.; Pérez-Alfocea, F.; Koyama, R.; Anderson, M.M.; Smith, R.J.; Ebeler. S.E.; Cantu, D. (2017) Red blotch disease alters grape berry development and metabolism by interfering with the transcriptional and hormonal regulation of ripening. J. Exp. Bot. 68: 1225–1238.

Blua, M.J.; Phillips, P.A.; Redak, R.A. (1999) A new sharpshooter threatens both crops and ornamentals. Calif. Agric. 53: 22–25.

Calvi, B. (2011) Effects of red-leaf disease on Cabernet sauvignon at the Oakville Experimental Vineyard and mitigation by harvest delay and crop adjustment. MS thesis. UC Davis, CA.

Cieniewicz, E.J; Perry, K.L.; Fuchs, M.F. (2017a) Grapevine red blotch: molecular biology of the virus and management of the disease. Meng, B.; Martelli, G.P.; Golino, D.A.; Fuchs, M.F. (eds) Grapevine viruses: molecular biology, diagnostics and management. Springer-Verlag Berlin: 303–314.

Cieniewicz, E.J; Pethybridge, S.J.; Gorny, A.; Madden, L.V.; McLane, H.; Perry, K.L.; Fuchs, M.F. (2017b) Spatiotemporal spread of grapevine red blotch-associated virus in a California vineyard. Virus Res. 241: 156–162.

Cieniewicz, E.J; Loeb, G.; Pethybridge, S.; Perry, K.L.; Fuchs, M.F. (2018) Insights into the ecology of grapevine red blotch virus in a diseased vineyard. Phytopathology 108: 94–102.

Cieniewicz, E.; Wise, A.; Smith, R.; Cooper, M.; Martinson, T.; Fuchs, M. (2019) Studies on red blotch ecology inform disease management recommendations. March; Wine Business Monthly: 92–102.

Daugherty, M.P.; Almeida, R.P.P.; Smith, R.J.; Weber, E.A.; Purcell, A.H. (2018) Severe pruning has limited efficacy for managing Pierce's disease. Am. J. Enol. Vitic. 69: 289–294.

Daugherty, M.P.; Gruber, B.R.; Almeida, R.P.P.; Anderson, M.M.; Cooper, M.L.; Rasmussen, Y.D.; Weber, E.A. (2012) Testing the efficacy of barrier plantings for limiting sharpshooter spread. Am. J. Enol. Vitic. 63: 139–143.

Daugherty, M.P.; O'Neill, S.; Byrne, F.; Zeilinger, A. (2015) Is vector control sufficient to limit pathogen spread in vineyards? Environ. Entomol. 44: 789–797.

Davis, M.J.; Purcell, A.H.; Thomson, S.V. (1978) Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199: 75–77.

EFSA PLH Panel (EFSA Panel on Plant Health) (2015) Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. EFSA J. 13(1): 3989.

Girardello, R.C.; Cooper, M.L.; Smith, R.J.; Lerno, L.; Bruce, R.C.; Eridon, S.S.; Oberholster, A. (2019) Impact of grapevine red blotch disease on grape composition of *Vitis vinifera* Cabernet Sauvignon, Merlot and Chardonnay. J. Agric. Food Chem. 67: 5496–5511.

Krenz, B.; Thompson, J.; McLane, H.; Fuchs, M.; Perry, K.L. (2014) Grapevine red blotch-associated virus is widespread in the United States. Phytopathology 102: 232–1240.

Martínez-Lüscher, J.; Plank, C.M.; Brillante, L.; Runze, Y.; Al Rwahnih, M.; Cooper, M.L.; Smith, R.J.; Girardello, R.C.; Oberholster, A.; Kurtural, S.K. (2019) Grapevine red blotch virus may reduce carbon translocation leading to impaired grape berry ripening. J. Agric. Food Chem. 67: 2437–2448.

Newman, K.L.; Almeida, R.P.P.; Purcell, A.H.; Lindow, S.E. (2003) Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. Appl. Environ. Microbiol. 69: 7319–7327.

Perry, K.L.; McLane, H.; Hyder, M.Z.; Dangl, G.S.; Thompson, J.R.; Fuchs, M.F. (2016) Grapevine red blotch-associated virus is present in free-living *Vitis* sp. Proximal to cultivated grapevines. Phytopathology 106: 663–670.

Preto, C.R.; Bahder, B.W.; Bick, E.N.; Sudarshana, M.R.; Zalom, F.G. (2019) Seasonal dynamics of *Spissistilus festinus* (Hemiptera: Membracidae) in a California vineyard. J. Econ. Entomol. 112: 1138–1144.

Purcell, A.H. (1975) Role of the blue-green sharpshooter, *Hordnia circellata*, in the epidemiology of Pierce's disease of grapevines. Environ. Entomol. 4: 745–752.

Ricketts, K.; Gómez, M; Fuchs, M; Martinson, T.; Smith, R.; Cooper, M.; Moyer, M.; Wise, A. (2017) Mitigating the economic impact of grapevine red blotch: optimizing disease management strategies in U.S. vineyards. Am. J. Enol. Vitic. 68: 127–135.

Sudarshana, M.R.; Perry, K.L.; Fuchs, M.F. (2015) Grapevine red blotch-associated virus, an emerging threat to the grapevine industry. Phytopathology 105: 1026–1032.

Yepes, L.M.; Cieniewicz, E.; Krenz, B.; McLane, H.; Thompson, J.R.; Perry, K.L.; Fuchs, M.F. (2018) Causative role of grapevine red blotch virus in red blotch disease. Phytopathology 108: 902–909.

Sins of the past, opportunities for the future: future-proofing vineyards

R. Hamilton

Hamilton Viticulture, Ashton, SA
hamilton.viticulture@bigpond.com

Abstract

In 2000 Australia had just under 120,000 ha of bearing vines. Approximately half of these plantings were established in the previous ten years. The main driver for this rapid expansion was a focus on export markets and this, combined with corporate wine company mergers, created an urgent requirement for development of vineyards.

Australian researchers and viticulturists developed visionary schemes in the 1970s for establishment of vineyards using improved propagation material. This included focus on vine selection and source area multiplication as well as implementation of vine health and quarantine standards. However, the unprecedented demand for planting material in the 1990s overwhelmed these schemes as demand outstripped supply.

The development of a vineyard with the capacity for long-term productivity (40 years plus) is reliant on ensuring that the planting material has known varietal provenance, proven performance and meets health standards. Although planting material comprises a small proportion (approximately 5% on own roots and 15% for grafted vines) of the total cost of vineyard establishment, it plays a key role in determining the profitability of a vineyard.

Strategy 2025, released in 1996, recognised the need for research, development and extension in support of the expanding grape and wine industry. This facilitated investment in research and development although focus on research relating to vine propagation material and vine health was low on the list of industry priorities. Despite this, there were several critical papers developed by researchers and industry working groups that provide excellent reference for the future and remain relevant to the present. Review of these papers, together with documentation of the experiences of key nursery operators, propagation specialists and researchers active during this period, is recommended for industry long-term sustainability. Consolidation and understanding of this material, together with focus on vine health issues, will be key steps towards ensuring that new and re-worked vineyards provide long-term profitability.

Introduction

Since 1843, the Australian wine industry has experienced five periods of rapid growth (Anderson and Aryal 2015; Figure 1). During the period from 1986 to 1995, Australian vineyard plantings averaged just under 60,000 ha. In the following 10 years, as part of the fifth cycle of growth to 2005, just under 94,000 ha of vineyard were planted, an increase of 250%. This unprecedented and sustained rate of vineyard development occurred as a result of several factors including opportunities for wine exports, significant taxation advantages and a weak Australian dollar.

The key elements for vineyard longevity and profitability include the variety planted as well as its location, as these are essential requirements for meeting market demands for wine type and style. The location determines the soil types available, the (expected) climate and the potential for available supplementary water supplies. The choice of varieties, based on the suitability to the location, provides opportunity to maximise returns based on the location. Profitability of any vineyard is inextricably linked to market demand for the wine type and style that may be produced by its grape varieties. Any changes in demand for these varieties will clearly affect vineyard profitability in the long term. Another looming challenge is the impact of climate change (Labbé et al. 2019) which, based on the current rate of change, may impact within the nominal 40-year life of a vineyard.

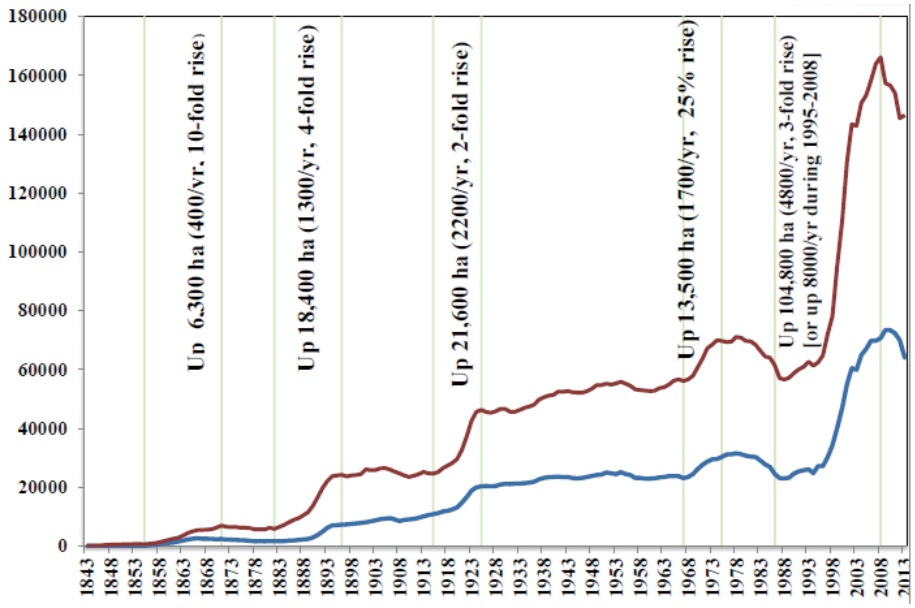


Figure 1. Bearing area of vineyards, wine production, and wine exports, 1986 to 2013. Data extracted from Anderson and Aryal (2015). The data shown in red depicts vineyard planting in Australia and the blue shows data for South Australia.

Improved propagation material

Regardless of these factors, the pedigree of propagation material is the major factor determining vineyard longevity. Any impediments in relation to the variety/clone, vine health and uniformity of the material will erode the performance potential of that material. This, combined with the management of the vineyard both at planting (rate and uniformity of establishment) and long term (predictability of performance), may affect both productivity and longevity of the vineyard.

To explain this further, the concept of improved vine material helps ensure the requirements that enable vineyards to meet production and quality targets and have an extended life span. The first requirement is genetic uniformity. This means that there is a need for a disciplined approach to be taken in management of any propagation source from its initial selection to ensure that there is no opportunity for contamination with other material, whether that be varietal or clonal. The discipline in relation to maintaining propagation material uniformity then ensures that future efforts to confirm identity and maintain freedom from, or knowledge of the make-up of, pathogens are assured.

Examples of past problems in relation to varietal identity include varietal contamination (Cabernet Franc and Merlot, where the more vigorous Cabernet Franc in a Merlot source block increased the likelihood of cuttings being taken over Merlot) and misidentification (Albariño and Savagnin Blanc). The availability of DNA testing has greatly benefited ampelographic methods for varietal identification but adds costs to the process, making the discipline of ensuring genetic uniformity more important in managing propagation material. Mixed clones are more difficult to manage as there are currently no markers that may be reliably used to define clonal material.

Pathogen status and its ongoing management are critical factors for vineyard longevity. This begins with establishment of the cuttings in the nursery and continues with the vines' ability to establish in the vineyard in the year of planting with minimal growth impediment. If reworking is ever to be considered, detailed knowledge of the vineyard's virus status at planting is critical to ensure that a known virus was not present at planting. However, due to the potential for vectoring (by scale or mealybug) of some viruses (e.g. leafroll 1 and 3, GVA), vineyards should be visually checked for virus symptoms with targeted sampling where symptoms are evident or random sampling if not.

The use of hot water treatment (HWT) through a certified facility is a critical final step to ensure that there is minimal risk of surface contamination of propagation material (e.g. fungal and bacterial pathogens, Waite and May (2005)). This service is offered by nursery operators and is essential to manage risk of crown gall and root/trunk disease contamination of propagation material.

Origins of vine improvement groups in Australia

Vine improvement selections were first actioned in the late 1950s (Harry Tulloch in 1957, South Australian Department of Agriculture, Nuriootpa Research and Advisory Centre) and gained momentum across Australia through research, development and extension activities including state government (e.g. Richard Cirami and Michael McCarthy, South Australian Department of Agriculture and John Whiting, Victorian Department of Agriculture) and Australian Government activities (e.g. Alan Antcliff, CSIRO; Ward 2014).

These activities were supported by emerging industry groups, the first being the Barossa Grapegrowers' Vine Selection Society Inc. (BGVSS), which was formed in 1967. The driving force behind this group was a close relationship between local growers and viticultural researchers. The focus was on identifying and developing new vine selections and establishing and maintaining high quality *vinifera* source blocks in the Barossa. This concept rapidly spread through the main grapegrowing areas in South Australia (including the Adelaide Hills, Barossa, Clare, Langhorne Creek, McLaren Vale, Riverland and South East). With time the South Australian state organisations developed overarching bodies including the Vine Variety Trust Fund (1969) as an activity of the then Phylloxera Board of South Australia and the South Australian Vine Improvement Committee (SAVIC) in 1974.

Momentum built in the late 1960s, at the time of the importation of key Chardonnay clones from UC Davis in 1969. At that time interest in white wines was emerging from an essentially red wine focus with a declining fortified wine market. With quarantine restrictions, these UC Davis Chardonnay selections did not begin to be released to industry until the mid to late 1970s. Until then there was little material apart from Penfolds Bin 58 (1958 selection) and the limited Marble Hill selection brought in from France, which ended up at the Governor's residence in the Adelaide Hills, despite phylloxera regulations in SA.

In the meantime, similar state-based organisations were formed including the Victorian and Murray Valley Vine Improvement Association, Murray Irrigation Area Vine Improvement Society, Western Australian Vine Improvement Association as well as groups in Tasmania and Queensland.

In 1988 the Australian Vine Improvement Association (AVIA) was formed in response to the need for coordination of vine improvement groups across Australia with the following objectives:

- Promote the development of Australian viticultural industries by coordinating grapevine improvement activities throughout Australia
- Establish and maintain a national germplasm collection for the benefit of the Australian grape industry
- Manage a National Vine Accreditation Scheme – on behalf of vine improvement groups
- Negotiate and enter into agreements with grapevine breeders for the appointment of the Association as the head licensee for the production and marketing of grapevine varieties in Australia
- Facilitate the equitable distribution of high-quality propagation grapevine and rootstock material to all producing areas in Australia.

In 1997 the Vine Industry Nursery Association (VINA) was incorporated with the following objectives:

- To represent and promote the interests of vine nurseries in matters of general interest that may affect their well-being and viability
- To coordinate the efforts of vine nurseries in order to give unity of purpose and strength in the best interests of vine nursery development
- To provide a channel for communication and dissemination of information between vine nursery operators and with other sectors of the grape and wine industry.

Funding grapevine propagation material programs

In South Australia there was strong support from the State Government for vine improvement activities in terms of research and advisory staff as well as research stations (Nuriootpa Research and Advisory Centre and the Loxton Research Centre in South Australia), particularly from the 1980s to the early 2000s. This activity provided the basis for maintaining vine variety and clonal collections, propagation of clonal and rootstock trials and development of vine increase rows to provide cuttings for establishing source blocks for industry.

Concomitant with this was strong interest in regional vine improvement committees where funding for their activities was based on levies on cutting material. In the planting boom in the 1990s these levies covered the cost of expansion of regional vine improve-

ment programs. During peak cutting activity in South Australia there were levies for regional vine improvement groups, as well as for the state and national bodies (AVIA). However, these businesses were not sustainable in the recessionary environment after the planting boom and, despite minimal support from industry bodies, they have managed to survive on behalf of the grape and wine industry, albeit under difficult circumstances.

Of note is the fact that viticulture was hailed as having an ideal crop improvement program in a national meeting of the Horticultural Propagation Working Group in 1990. This related to two key factors. The first was the strength of government and industry partnerships and the second was the development of grower-owned source areas for distribution of planting material following multiplication of selected material with known provenance and health status.

Sharing of this information galvanised the major horticultural fruit crops (citrus, stone fruit, pome fruit, almonds and strawberries) to establish industry-funded crop improvement programs through levies with matching Australian Government funding. The importance of this focus for several horticultural industries was regarded as fundamental to the extent that the pome fruit (APAL 2020) and citrus industries (Pat Barkley, pers. comm.) allocated 5–10% of federally matched research levy funds for a period of over ten years to support propagation resource material programs whilst they established. In 1991, the Riverland Vine Improvement Committee opened the Monash Horticultural Improvement Centre on its Monash property. This enabled the establishment of source blocks for the national almond, and state-based citrus and pome fruit industries.

Although the concept of funding of vine improvement activities through levies based on cutting material seemed appropriate during the boom in plantings, this approach left a major problem as vineyard development waned. Currently plantings are largely limited to re-establishment of older vineyards and development of vineyards with emerging varieties, albeit from a small base. This, combined with substantial withdrawal of state government support has resulted in state-based collections and activities languishing with minimal support since the mid-2000s. Several regional vine improvement groups (including Adelaide Hills, Barossa, Riverland and the Victorian and Murray Valley Vine Improvement Association) have maintained activity, striving to maintain the integrity of germplasm plantings as well as establishing new source area blocks where suitable material was available. The funding for this activity is largely self-generated by the vine improvement groups and relies on the vision, entrepreneurship and enthusiasm of devoted industry personnel.

At this time the Australian grape and wine industry has not addressed the issue of the importance of propagation material to the long-term future of the industry (Hamilton 1993). The loss of state government support and limited federal funding (through matching of grape levies) means that the industry is reliant on the work and support for vine improvement that was in place in the late 1990s. This legacy is being eroded and requires industry commitment, in terms of appropriate and adequate funding and supporting industry committees, to ensure that the core elements are maintained with capacity to continue access to improved vine material in the foreseeable future.

In recognition of the need for oversight of the grape and wine industry vine health issues, the National Vine Health Steering Committee (NVHSC) commenced activities in the late 1990s. Membership of the NVHSC included chief quarantine officers from the three major grapegrowing states (NSW, South Australia and Victoria), with invitations to their equivalents from other grapegrowing states (Queensland, Tasmania and WA). Industry members were nominated from industry bodies in each state. The NVHSC was chaired by the Federal Chief Quarantine Officer, with secretarial support funded by the then Grape and Wine Research and Development Corporation (GWRDC, now Wine Australia).

The NVHSC considered matters of importance to vine health in Australia and directed the formation of working groups to provide advice to the NVHSC to inform decision-making on industry endorsed protocols for vine health issues. Working groups were charged with reviewing key issues and providing recommendations to NVHSC for consideration. Working groups included the Phylloxera Technical Reference Group (2001–2010), Grapevine Yellows Technical Reference Group (2001–2005), Scientific Advisory Panel for Grapevine Leaf Rust (2004–2007) and the Variety Collection and Propagation Technical Reference Group (VCPTRG, 2005–2008).

The formation of the VCPTRG arose from recommendations from a GWRDC commissioned report entitled 'Review of Vine health parameters, implementation priorities and capabilities for Vine Improvement Groups and Accredited Nurseries. Grape and Wine Research and Development Corporation' (Constable and Drew 2004). The report recommended that '…Australian Grapevine Foundation Planting Scheme (AGFPS) is required to ensure planting material of the required health status and provenance is available to meet the needs of the wine grape, dried vine fruit and table grape as well as the vine nursery industries'. The report's final recommendation was as follows:

> Success of the proposed AGFPS depends on the establishment and effective operation of a 'driver', preferably responsible to the NVHSC through a relevant TRG and funded by industry and Government through both the GWRDC and HAL. The model enabling and supporting the 'driver' should draw on features of the AusCitrus Scheme and APFIP. The support for the coordinator should be commensurate with the national responsibility of the position.

The work of the VCPTRG and the Constable and Drew (2004) report provide the basis for the maintenance of the remnants of the vine collections that have some certification as to identity and vine health status, which provide the basis for vine improvement distribution of propagation material. Despite limited funds and minimal support from the main industry bodies, South Australian Vine Improvement are working to maintain the core elements of industry-funded collection material in a planting at Monash, in the South Australian Riverland.

Review of the activities of the NVHSC in the late 2000s resulted in withdrawal of levy-based funding in support of the committee, even though the main costs were covered by participating organisations. This, combined with a downturn in the grape industry at that time, meant that the peak industry bodies regarded issues relating to plant propagation material as being low on the list of priorities for funding.

The main government collections (CSIRO, Sunraysia and SARDI, Nuriootpa) continue to have limited access (McMichael et al. 2013) but remain as a potential resource for material that is not in the vine improvement collection.

The Vine Industry Nursery Association (VINA) is also limited in resources and yet continues planning to develop a program for certification of propagation material, with plans to have this finalised in the early 2020s.

The reference documentation for all these activities (Constable and Drew 2004) remains relevant (see Plant Health Australia (2009) 'Industry Biosecurity Plan for the Viticulture Industry') and is based on considerable input from key industry bodies as well as investment from government-matched industry funds. Furthermore, a large working group assisted a project to write the Australian Standard AS5588 -2013 (2013) entitled 'Grapevine Propagation Material'.

The future

However, to capitalise on these past investments the Australian grape and wine industry must revisit the issue of the importance of propagation material to the future of the wine industry. The focus at present includes strong investment in marketing Australian wine and creating increasing demand for the future. Despite this, the grape and wine industry does not have an assured means for provision of certified propagation material. Furthermore, it is reliant on programs that have had minimal support and currently limited capacity to supply propagation material that has the capability for producing winegrapes that will meet future market demands.

A comparison of the main varieties established in South Australia in 1995, 2006 and currently (2018) is presented in Figure 2. In 1995 the three major varieties were Shiraz, Cabernet Sauvignon and Chardonnay, comprising 58.8% of total plantings. In 2018 the top three varieties comprised 70.8% of established plantings. This demonstrates that despite increasing interest in new and emerging varieties, other varieties have decreased in planted area by approximately 25% in the last 23 years. Recent activity may well redress this trend.

The original aim for vine improvement organisations was to hold collections which retain all varieties and clones known to be in Australia which is estimated as '…close to 900, with multiple clones of some varieties' (McMichael et al. 2013). A driver for this is the realisation that the collection includes unique pre-phylloxera selections that would otherwise to be lost to viticulture.

Another critical issue lies in the static nature of the collections. Clonal development work has all but ceased since the late 1990s. Private importers (who have no obligation to share material) and dedicated nurseries (with long-term vision and underpinning businesses) have driven importations. However, there remains a lack of clonal diversity with emerging varieties and indeed some well-established varieties with current renewed interest (e.g. Grenache). A further issue is that new varieties and clones (both scion and relatively recent rootstock selections, e.g. CSIRO) are not being evaluated as to their compatibility in grafted vineyards (Whiting 2012).

The enormity of this task has frustrated attempts to underwrite this material and has dissuaded industry bodies from contributing to support for the maintenance of propagation source material. A focus on the main varieties (both scion and rootstock) would provide an underpinning for industry. Increasingly, emerging varieties are, and will continue to be, self-funded and may be added to the program later. Once a program is in place to manage a basic repository of certified material (provenance and health status), consideration could be given to inclusion of other material to ensure preservation of Australia's unique vine varietal collection.

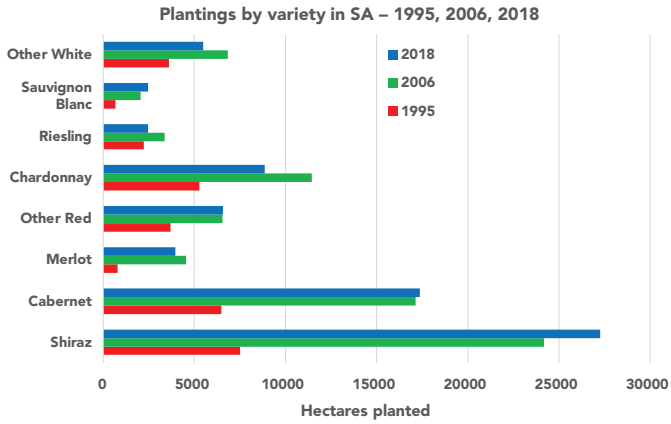


Figure 2. Comparison of planting area (ha) by variety in South Australia for 1995, 2006 and 2018. Data from the annual Winegrape Crush produced by Wine Australia on behalf of the Winegrape Council of SA, the South Australian Wine Industry Association Incorporated and Primary Industries and Regions SA.

Industry must also support germplasm establishment and maintenance as it is at risk with the paucity of source material. This will require more widespread (to reduce biosecurity risks) and diverse multiplication blocks (in commercial vineyards) of key varieties and clones.

There have been considerable changes in resources in terms of knowledge relating to propagation, planting and vine establishment for establishing new vineyards in the last 30 years. Until 1990, Departments of Agriculture had considerable resources in both research and extension for grapegrowing. In South Australia alone there were more than 12 full-time equivalents (FTEs) in extension and vine research station management focusing on vine improvement resources in the 1980s. All these extension resources have gone and scientific support for viticultural pathology has halved, with limited capability remaining in entomology and no capacity in virology and nematology. Most state Departments of Agriculture have followed this trend, the exceptions being NSW, Tasmania and WA with several FTEs dedicated to viticulture. During this time, the major corporate wineries have also considerably reduced their viticultural and grower liaison FTEs.

Fortunately, there were publications made available prior to the loss of key personnel which give insight into early clonal selection activities. These include an ASVO seminar held in 1986 entitled 'Aspects of grapevine improvement in Australia' (Lee 1987) where there was discussion on future directions proposed by industry following review of the early stages of vine selection. These issues were revisited ten years later in a seminar entitled 'Quality management in viticulture' (Hamilton and Hayes 1996). Details of the established clonal selection trials are listed in a technical report (Cirami 1980). Two publications by Phil Nicholas (2006a, b) detail all material then available in Australia and planted in commercial vineyards.

Similar documentation was published in relation to rootstock research. Peter May's 1994 review continues to be an essential reference in relation to rootstock use in Australia. More recently, John Whiting's 2012 review provides understanding of the way in which rootstocks perform in Australian vineyards. These resources have been summarised into a 'Grapevine rootstock selector tool' (Wine Australia 2019) which is based on an original concept developed by Phil Nicholas (1997) and subsequently updated as new data on rootstock performance became available.

Review of resources in support of vineyard establishment show that there are several documents available, with two key items published as the planting boom began to gain momentum in the early 1990s. The first was the proceedings from an ASVO seminar (Hayes 1993). This seminar reviewed key elements in developing and redeveloping vineyards. The second was the proceedings from an ASVO seminar (Hamilton 1995). This seminar included reports from vineyard managers and nursery operators detailing their experiences in vineyard development as well as input from researchers on best practice management for vineyard development.

Conclusion

The Australian grape and wine industry was fortunate in having strong government support and visionary government viticulturists in the 1970s and 1980s. These viticulturists, together with progressive industry grapegrowers, built the foundations of a culture that understood the need for vine selection to ensure sustainable grape yield and quality of production for the future. As knowledge of the importance of vine health evolved, practices were developed to allow grapegrowers to access propagation material of known provenance and health status.

However, the grape and wine industry failed to recognise and prioritise support for a program for the supply of vineyard propagation

material of known provenance and health status and relied on vine improvement organisations to maintain these standards on behalf of the industry. This was manageable during the unprecedented boom in vineyard plantings from the mid-1990s until the peak of vineyard plantings in 2008 as levies (price-premium on cutting material, not genuinely a levy or tax) on planting material were the basis for funding of vine improvement programs. During this period the industry chose to ignore the long-term needs for maintaining the supply of certified propagation material for vineyard establishment, failed to pay an appropriate price to ensure sustainability of vine improvement schemes and allowed government funding of governmental viticulturists and supporting scientists to erode to minimal support levels.

Since 2008, vineyard plantings in Australia have fallen to a current area of approximately 135,000 ha, a net reduction of 30,000 ha or 20% of plantings that were established in 2008. At a conservative value of $25,000 per ha this is a net loss of $0.75 billion invested in the grape and wine industry.

During the 17th Australian Wine Industry Technical Conference there was continued reference to the opportunity for future marketing of Australian wine in the international wine market and the likely need for increases in planted areas to realise this opportunity. In order to achieve this aim sustainably, the grape and wine industry must urgently review its support for improved propagation material. This includes the plantings that supply material of known provenance and health status as well as ensuring the final implementation of certification systems from primary source collections through source areas to the final propagation material, ready for establishing in vineyards. In addition, as personnel with direct experience in the development of broad scale new vineyards are reducing in number, review of past literature should be completed to ensure that previous successful experience and learning is captured and made available. This will help ensure establishment of sustainable vineyards with long-term prospects for future marketing of Australian wine.

Acknowledgement

My thanks to Peter Hayes and Chris Rogers (Rogers Viticulture) for review and comments on this paper.

References

Anderson, K.; Aryal, N. (2015) Growth and cycles in Australia's wine industry: A statistical compendium, 1843 to 2013. University of Adelaide Press: https://www.adelaide.edu.au/press/system/files/media/documents/2019-04/uap-austwine-ebook.pdf

APAL (Apple and Pear Australia Ltd) (2020) Australian Pome Fruit Improvement Program, Variety evaluation and tree certification services for the apple and pear industry: https://apal.org.au/programs/more-industry-programs/apfip/

Australian Standard AS5588-2013 (2013) Grapevine Propagation Material.

Cirami, R.M. (1980) Vine Improvement in South Australia. Result from Clonal and Rootstock Trials 1966 – 1980. Depart. of Agriculture, South Australia: Technical Report 171.

Constable, F.; Drew, C. (2004) Review of vine health parameters, implementation priorities and capabilities for Vine Improvement Groups and Accredited Nurseries. Grape and Wine Research and Development Corporation. Project NVH 03/01: https://www.wineaustralia.com/getmedia/0807646b-1fb9-4cfd-80df-61b96d76809d/NVH-03-01

Hamilton, R. (1993) Improved vine material – Its role in vineyard profitability. In: Vineyard Development and Redevelopment. Hayes, P.F. (ed.) Proceedings of a seminar held on 23 July: Australian Society of Viticulture and Oenology, Inc.

Hamilton, R. (ed.) (1995) Optimising vineyard establishment – development practices. Proceedings of a seminar held on 17 May: Australian Society of Viticulture and Oenology, Inc.

Hamilton, R.; Hayes, P. (eds) (1996) Quality management in viticulture. Proceedings of a seminar held on 2 August: Australian Society of Viticulture and Oenology, Inc.

Hayes, P.F. (ed.) (1993) Vineyard development and redevelopment. Proceedings of a seminar held on 23 July: Australian Society of Viticulture and Oenology, Inc.

Labbé, T.; Pfister, C.; Brönnimann, S.; Rousseau, D.; Jörg Franke, J.; Bois, B. (2019) The longest homogeneous series of grape harvest dates, Beaune 1354–2018, and its significance for the understanding of past and present climate. Clim. Past 15: 1485–1501.

Lee, T.H. (ed.) (1987) Aspects of grapevine improvement in Australia. Proceedings of a seminar held on 20 November: Australian Society of Viticulture and Oenology, Inc.

May, P. (1994) Using grapevine rootstocks: the Australian perspective. Grape and Wine Research and Development Corporation. Adelaide, SA: Winetitles: 62 p.

McMichael, P.; Hamilton, R.; Tassie, E. (2013) Summary of a review of grapevine germplasm collections in Australia. Grape and Wine Research and Development Corporation. Project GWR 1112: https://www.wineaustralia.com/getmedia/b30b1a55-8431-4aa0-b4db-baf3ed18b2fa/GWR-1112-Review

Nicholas, P. (1997) Rootstock characteristics. Aust. Grapegrower Winemaker 400: 30.

Nicholas, P. (2006a) Grapevine clones used in Australia. South Australian Research and Development Institute: http://www.graftedvines.com.au/images/Grapevine%20Clones%202006.pdf

Nicholas, P. (ed.) (2006b) National register of grapevine varieties and clones. Mildura, Vic.: Australian Vine Improvement Association.

Plant Health Australia (2009) Industry Biosecurity Plan for the Viticulture Industry: https://www.wfa.org.au/assets/environment-biosecurity/Biosecurity-Plan.pdf

Waite, H.; May, P. (2005) The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. Phytopathol. Mediterr. 44: 144–152.

Ward, C. (2014) Transforming the Australian Wine Industry. CSIROpedia, 5 September: https://csiropedia.csiro.au/transforming-the-australian-wine-industry/

Whiting, J. (2004) Grapevine rootstocks. In: Dry, P.R.; Coombe, B.G. (eds) Viticulture Volume 1 – Resources 2nd ed. Adelaide, SA: Winetitles: 167–188.

Whiting, J (2012) Rootstock breeding and associated R&D in the viticulture and wine industry. Grape and Wine Research and Development Corporation. Project GWR 1009: http://www.mvwi.com.au/items/517/Rootstock_Review_-_John_Whiting_FINAL_(email).pdf

Wine Australia (2019) Grapevine rootstock selector tool: http://grapevinerootstock.com/

Exploring and exploiting the natural genetic diversity of *Vitis vinifera*

A.R. Graça

PORVID – Associação Portuguesa para a Diversidade da Videira, Lisboa, Portugal
antonio.graca@sogrape.pt

Abstract

Vineyards across the globe face increasing environment and market pressures. Climate change induces higher variability than ever before. This variability affects value in the wine business from deviant grapevine phenology, extreme climatic events, emerging diseases and changes to the sensory expression of regional terroir. This scenario—already a reality—is likely to worsen, according to forecasts, over the next decades. Resilience of vineyards and the production systems they support becomes critical. Vineyard resilience is supported by the inherent genetic diversity of grapevines. Yet the monoclonal system of sanitary certification, created in Europe by the mid-20th century and copied globally, focuses on efficiency while reducing diversity and therefore resilience. Today, a very limited number of genotypes are cloned globally to plant single-clone blocks, which are eugenic and susceptible to new challenges. Since the 1970s in Portugal, a polyclonal selection method has been developed, tested and deployed across the industry. This method allows prior estimation of gains and their stability across different environments, while conserving genetic diversity untouched for future needs. The project became mainstream in 2009 when the largest wine companies in cooperation with state institutions created PORVID, an organisation determined to increase value from exploring the autochthonous grapevine varieties of Iberia. Its mission: conservation of 50,000 genotypes from 250 varieties by 2050—making them and knowledge of them publicly available. Ten years on: 30,000 genotypes of 200 varieties have been conserved, the diversity of 63 varieties fully characterised, 20,000 hectares planted with polyclonal selections and this new method has become an OIV resolution. Although not considered a centre of diversity for the European grapevine, Australia has a number of vineyards with grapevines that pre-date the onset of phylloxera in Europe and may, arguably, hold more diversity in those varieties than anything since planted in Europe. The new method created in Portugal offers a conservation and evaluation solution that will support the resilience of Australian vineyards. PORVID's governance model (where large companies cooperate with the state for the benefit of the whole cluster) may inspire, with appropriate adaptations, a pathway for future sustainable exploitation of grapevine germplasm in Australia.

Introduction

Climate change will be a major challenge for viticulture in the coming decades. Growers need to deploy adaptive strategies if production of quality wines is to continue profitably under a changed climate characterised by warmer temperatures, higher frequency and severity of extreme climate events and intra-annual variability. An effective way to support this adaptation is to tap into the natural diversity of grapevines and obtain selections that will contribute to an environmentally friendly, cost-effective solution for vineyards (van Leeuwen and Darriet 2016). This paper details how the solution of polyclonal selections developed in Portugal could be applied to support the resilience of the Australian wine industry.

Climate effects in vineyards

In many wine regions across the world, effects of the changing climate are being felt in grapevine phenology, yield reduction and grape quality (Schultz 2014; Fraga et al. 2016; van Leeuwen and Darriet 2016; Cook and Wolkovich 2016). However, an increase in variability is also expected, mostly at the inter-annual level but also on intra-annual time-scales (Figure 1). The same is true in terms of the periodicity and severity of extreme events that can seriously impact vineyards' operational costs and profitability (Fraga et al. 2016; Cook and Wolkovich 2016; Viceto et al. 2017).

Figure 1. Intra-annual climate variability: effect of the 2018 summer heatwave on grapevines that grew vigorously with absence of water stress in a dry-farmed vineyard in the Setúbal wine region of Portugal. Temperatures reached 46°C

Resilience or lack thereof

Resilience is the capacity of a system to absorb disturbance and reorganise itself while undergoing change so as to still retain essentially the same function, structure, feedback and therefore identity. Resilience is a dynamic concept focusing on how to persist with change (Walker et al. 2004; Folke et al. 2010; Folke 2016) and how to evolve with change. According to Goerner et al. (2009), sustainability of an organisation as a function of diversity is achieved when that organisation has sufficient diversity to achieve resilience but not so much that it becomes inefficient. In this way, sustainability would follow a parabolic curve, slightly skewed to the right side (Figure 2), because in natural systems resilience seems to play a greater role in sustainability than does efficiency.

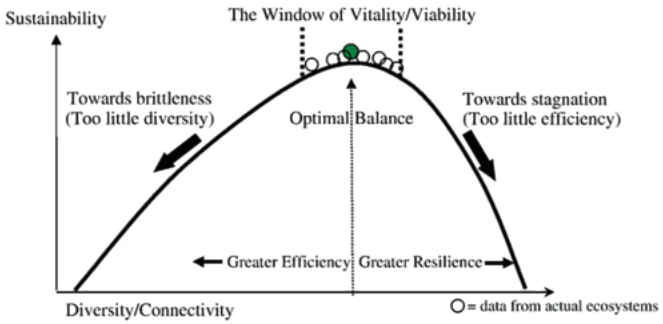


Figure 2. Sustainability as a function of efficiency and resilience (Goerner et al. 2009)

Using this resilience thinking, one may argue that pre-WWII vineyards may have been stagnating outside the window of viability (to the right) and that all modernisation implemented since has pushed those production systems to the left side of the curve, ensuring the sustainability needed to underpin the global success of the wine sector in the second half of the 20th century. Yet, the progressive reduction of genetic diversity in grapevines (replacing field blends with single varieties and later, mass selections with single-genotype clonal selections) (Luján 1984; Martins and Eiras-Dias 2008) and of general biodiversity in vineyards (eliminating most other organisms through chemical protection) has arguably gone too far. Modern production systems have potentially moved outside the window of viability again, this time on the left side, making them brittle and unable to cope with new pressures, climatic, sanitary or otherwise (Barnes et al. 2010). The situation was further worsened because of the industrialisation of nurseries and the drive to eradicate viruses (yet another decrease in biodiversity) resulting in new plantations being made mostly from the same mother plants instead of the more diverse local stock as was usual practice in the past. This has decreased the capacity of grapevines to resist disease and has also reduced their viability and lifespan (Waite et al. 2015; Mondello et al. 2017; Grohs et al. 2017; Waite et al. 2018), namely by increasing susceptibility to grapevine trunk diseases, confirming the brittleness of the modern system.

Using diversity as a driver for innovative selection

Portugal was a relative latecomer in terms of grapevine breeding and selection. When most other nations in Europe started breeding programs as early as the mid-19th century, Portugal only deployed such a program in the 1940s. In the same way, clonal selection in Portugal started about 10 to 20 years later than most other European wine-producing nations and 100 years later than Germany, which pioneered clonal selection in the 19th century (Reynolds 2015). However, this late start in Portugal worked to its advantage as it coincided with the availability of personal computers, thus making it possible to use processing power to solve complicated statistical problems involving complex equations and huge datasets—previously unmanageable using human brain power alone.

Trying to address clonal selection in the classical way, since 1978, did not produce relevant results and acquisitions were affected heavily by genotype x environment interaction which did not make genetic gains stable across different regions (Martins et al. 1998; Martins and Gonçalves 2015). This problem rallied academic researchers and technical staff from state and private companies, around whom an informal network grew with the sharing of information and the creation of a linked system of trial vineyards (Figure 3), mostly set in commercial acreage. By 2009, 163 trial vineyards existed for 61 varieties, producing data which year after year became integrated in a huge database. Statistical analyses were performed to extract information relevant to each variety's range of variation for traits such as yield, fertility and berry composition, to decide which varieties offered greater potential for selection gain and to support efforts to improve the profitability of commercial vineyards (Gonçalves et al. 2019). Some unexpected benefits of this work include: the capacity to pinpoint the origin of each variety by comparing the diversity of its populations across different regions (Martins et al. 2006); the ability to establish relative ancestrality between varieties (Martins 2009); and, most importantly, conservation of their diversity to restart selections on different traits or directions whenever necessary. In this way, what started as the development of a classical grapevine improvement methodology specific to Portuguese viticulture veered away in spectacular fashion, opening the path for a fundamental shift in the way grapevine selection could be better understood and used to improve the sustainability of vineyards and their inherent business models (Gonçalves and Martins 2012).

By 2009, the system of linked trial vineyards outgrew the management capacity of the informal network and an association was created by private companies, state institutions and universities: PORVID – Portuguese Association for Grapevine Diversity. One year later, PORVID signed a protocol with the Portuguese Ministry of Agriculture by which the latter placed a 600-acre state-owned estate under the management of PORVID for a period of 50 years. The objective was to create a Conservation Centre for Autochthonous Grapevine Varieties holding a minimum of 50,000 genotypes from 250 grape varieties. In 2011, a nationwide project was launched to recover intra-varietal diversity from old vineyards, abandoned or marked for grubbing up under the financing aids for vineyard reconversion of the European Union. In 2018, this effort resulted in the conservation of 30,000 genotypes belonging to a presumed 233 varieties, of which 167 have already been confirmed (Figure 4) (Gonçalves et al. 2019).

Polyclonal selection – an innovation with consequence

Grapevine selection in the European Union is regulated by an intricate set of legal documents, the fundamental one (Council Directive 68/193/EEC) dating back to 1968 and still largely in force, despite several amendments made at later times. The document treats varieties as homogeneous entities (art. 5b, 2 and 3) ignoring the well-demonstrated existence of intra-varietal diversity and confusing the

Figure 3. Trial and conservation vineyard in the Douro Valley of Portugal holding 197 genotypes of Touriga Nacional (the flagship red variety of the country) under an experimental design allowing for diversity assessment and polyclonal selection. Labels on vine posts signal the different experimental units (plots) in the trial.

Figure 4. Aspect of diversity conservation in pots at the Conservation Centre for Autochthonous Grapevine Varieties in Portugal (233 varieties so far conserved with more than 30,000 genotypes). For each variety, when a sufficient sample of genotypical diversity is achieved (70 genotypes) a field trial vineyard is set up to supply data for diversity assessment and polyclonal selection. Sixty-three varieties have already undergone this process.

notions of variety (art. 2-1, AA) and clone (art. 2-1, AB). It is, in fact, the clone that can be considered as a stable entity, as it replicates the same genotype for a limited number of vegetative multiplications. In any case, therefore, only the single clone can be 'certified' (art. 2-1, F) grapevine material, all others being considered under the 'standard' category (art. 2-1, G) of lesser quality.

Yet, the work with intra-varietal diversity performed in Portugal since 1978 has provided methodological tools to objectively quantify the effect of genotype × environment interaction (G×E) for every selected clone. With time, differences in stability between clones towards that effect were clear and it also became clear that a balanced mix of selected clones would always be less sensitive than any single clone (Figure 5). This phenomenon, anticipated from theoretical analysis and verified experimentally in field trials, led to the realisation that the application of appropriate tools (mixed models and EBLUPs—Estimated Best Linear Unbiased Predictors—of genotypical effects) could in fact indicate a mix of genotypes from the initial field trial containing a representative sample of diversity within the variety. Such a mix would perform in the same way or even better towards G×E as the balanced mix of selected clones, thus saving the time needed to assess each clone's environmental stability, typically 3 to 5 years. In this way, polyclonal selection cuts the usual selection process duration (8 to 12 years) to about half (3 to 5 years) further allowing for subsequent selections to improve the same variety in different traits, if the initial field trial is not removed. Also, polyclonal selection and diversity conservation become inextricably linked as conservation supports commercial value from grapevine selection gains that can be made to trickle downstream along the value chain (profits from increased yield or heat resistance), while commercial value from selection gains renders conservation essential for higher profitability, sustaining businesses (Martins and Eiras-Dias 2008).

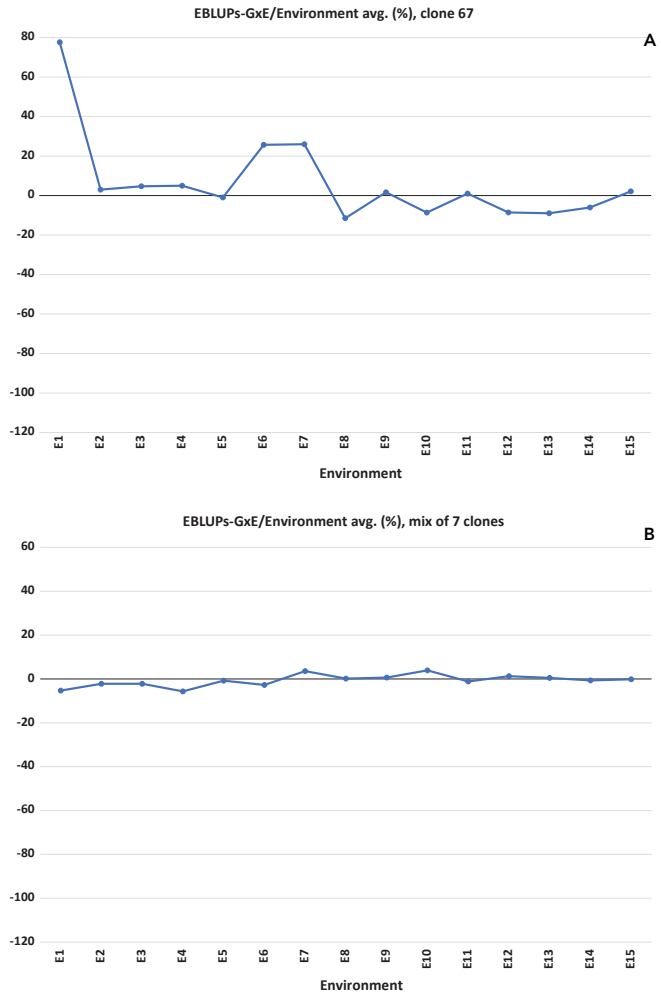


Figure 5. Comparison of stability towards GxE of a single clone (A - Vinhão clone 67) against a balanced mix of seven clones (B) of the same variety including clone 67, across 15 different environments (pairs of site and year) (PORVID 2008)

Today in Portugal, 500 hectares of polyclonal multiplication fields have produced plants to establish 20,000 hectares of polyclonal commercial vineyards (10.4% of total national vineyard acreage) representing an estimated annual added value in excess of 10 million euros for growers.

To make this approach usable and replicable in other countries, Portugal has proposed in the OIV's viticulture commission the approval of a resolution detailing all aspects of this innovative selection methodology. This document was voted for unanimously at the 2019 General Assembly of that organisation, of which Australia is also one of its 47 members.

The case for Australian grapevine diversity

Australia is home to an interesting set of vineyards planted before 1900, whose original grapevines still survive today (Figure 6). Data collected in the Hunter Valley of New South Wales (McIntyre 2015; Johnston 2019) and the Barossa Valley of South Australia (Barossa Grape & Wine Association 2019) show a significant number of these 'heritage' or 'centenarian'/'ancestor' vineyards, in the latter case amounting to over 160 hectares of varieties: Cabernet Sauvignon (4.44), Grenache (31.04), Shiraz (113.16), Mataro (8.02), Riesling (6.00) and Semillon (5.73). As the replanting of French vineyards with grafted plants following the phylloxera blight only ended in 1914 (Boehm 1996), and most of the plants that provided the source of those ancient Australian vineyards were brought mostly before the 1880s, it seems highly likely they represent a sample of diversity from European pre-phylloxera vineyards. Those vineyards were usually planted in a very different way from today's vineyards. Grown on their own roots, vineyards were planted using locally available plants. In this way, intra-varietal diversity accumulated in vineyards as plants were multiplied successively, micromutations being responsible for this slow shift from the original genotype. At the same time, inter-varietal diversity also accumulated as spontaneous crossings of pollen between different varieties would produce grapes whose pips, if successfully germinated, would give origin to a new variety with diverging traits from its parents. If they yielded well and tasted good, it is highly likely that growers would keep and multiply them.

One must not forget that in those days the layering technique of burying a live cane from one plant to produce a new root system and a new plant was quite widely used all over Europe, only stopping because of the phylloxera threat (Boehm 1996). Furthermore, during the long grafting campaign in Europe, it is possible that empirical selection was performed as growers tried to graft better performing plants in the new vineyards. Even if that attempt failed because of the environmental effect on phenotypical expression, it may have

Figure 6. Heritage vineyard in the Barossa, Australia

contributed to a mild reduction of diversity in the new 20th century vineyards when compared to their predecessors. That reduction at the single-vineyard scale might have been negligible, if noticeable at all. But in the framework of a massive replanting campaign that spanned all European winegrowing areas, this reduction must have made a dent in the original diversity, until then untouched.

The formulated hypothesis, then, is that Australia possesses today high intra-varietal diversity in its centenary/ancestor vineyards, allowing for high gains from selection, those gains becoming stable if selections are polyclonal. A further, even if less likely, hypothesis is that diversity is greater than anything found in Europe today for the same variety, as very few vineyards planted before 1900 remain and those that do were almost all completely grafted to protect from phylloxera (except for the ones planted in sandy soils) (Boehm 1996).

To verify the hypothesis, an experiment is needed. This would involve the recovery of the most diverse possible set of plants from one variety in as many centenary/ancestor vineyards as possible. The clear candidate for the experiment is Shiraz, owing to the large acreage of that variety in vineyards and the flagship status this variety enjoys in Australia. Plant material recovery should be performed in as many vineyards as possible using no more than two to three grapevines per vineyard. In this way, considering a minimal number of 70 and ideal number of 100 plants (Martins and Gonçalves 2015) for the diversity sample, 35 vineyards of Shiraz should be identified that, because of their current ownership and history, are likely to have been planted by different people and in different times. For each vineyard, chosen plants to mark should be separated from each other by at least five rows and more than 20 metres in the row. Since the main concern at this stage is to collect a representative sample of intra-varietal diversity of the ancient variety with wide genetic variability, choice of plants for specific traits should be avoided. However, the rejection of markedly undesirable plants is advised (symptomatic of viral or trunk disease infection, abnormal phenotypic aspects, malformations, etc.) and/or plants that do not correspond morphologically to the selected variety. A simple reference system, as universally understandable as possible, for locations of chosen plants and their vineyards of origin must be created and maintained, to allow subsequent verification of the representativeness of sampling and for traceability from the final selected genotypes back to the original mother plant or its location. It is appropriate that harmful viruses be tested at this stage by ELISA, considering compliance with the legal framework for certification of vine plants in each country. However, viruses with recognised low frequency of natural occurrence in the region concerned may not be tested in this phase.

Figure 7. Field-grafting a trial vineyard at the Conservation Centre

The sampled canes (assumed to be different genotypes of the target variety) will be used to create a trial vineyard where each genotype will be replicated several times. Those replicates will be placed in locations across the vineyard to maximise spatial randomisation and control environmental deviation. If desired, it is possible to enrich the samples with clones of commercial genotypes from nurseries: this will allow understanding of where those clones sit in the range of variation of the variety for any specific trait. Using a randomised complete block design, each genotype should produce material for six plots, each plot consisting of three replicates of the original plant in the sample. Each plot is replicated in each of the six blocks defined within the trial vineyard, its position relative to other plots being different in all six blocks. Eventually, in the finished trial vineyard, there will be 18 replicates of each of the 100 original plants in the sample, organised in six plots of three plants each, one for each block (see Figure 8 for an example with just 30 plants, which is insufficient for diversity assessment in an ancient variety but makes for easier visualisation of the trial design).

In general, the trial vineyard is managed according to standard agronomic techniques in the relevant region (plant density, training system, pruning methods, etc.), making sure that agronomic operations are applied in a strictly homogeneous way. However, the large number of genotypes and respective replicates requires unusually strict control during planting. This control is easier when planting on own roots. If grafting is needed, using field-grafting becomes more efficient (planting the rootstock and grafting buds from samples the following year). If using bench-grafted plants, they should preferably be rooted in labelled pots to minimise error. Rootstocks should be chosen according to soil characteristics that will host them; the same rootstock must be used for all the plants in the trial.

With this experimental set-up it becomes possible to obtain data that will provide information on the true genotypic value of each genotype in the sample, as randomly distributed replicated plots cancel each other's environmental deviation when averaging observed values of any quantitative trait (yield, fertility, berry sugar content, pH, colour, leaf surface temperature, etc.). By having the data for the whole genotype sample of the variety it is possible to calculate the range of variation for each trait which, in turn, informs the potential gain to be obtained from selection.

For data analysis, mixed models are fitted. The final objective is to estimate variance components, to find empirical best linear unbiased predictors (EBLUPs) of genotypic effects and calculate genetic gain. Selection can be made in favour of one evaluated trait, or several, considered either individually or under the form of a selection index. The number of genotypes selected which will constitute the polyclonal material is the result of a compromise between desired gain (increases when the number of selected genotypes decreases) and stability of behaviour of the selected set of genotypes in different environments; that is, low G×E interaction. This stability increases with the number of selected genotypes. Experimental results in the literature (Martins and Gonçalves 2015) show that the group stability grows sharply from one to seven genotypes and more moderately above that number. Based on these results, obtained polyclonal material should consist of balanced mixtures of 7 to 20 genotypes, depending on the specific conditions of each selection. However, even though that number may rise above 20, it should never be less than seven. The balance of the mixture implies that each genotype is represented in the group with a frequency of 1/n, where n is the total number of genotypes in the mixture. Due to feasibility reasons, some tolerance for those limits must be accepted. In any case, the frequency of any single genotype must never exceed twice that of the least frequent genotype.

Future outlook

The experience gained in Portugal from this line of work demonstrates that it requires collaboration and the sharing of work for a common good (Martins 2011).

An Australian-based conservation vineyard for Shiraz having a representative sample of diversity for that variety in Australian vineyards would be a major asset for the whole industry. If the sample reveals high enough diversity, potential gains from selection can be calculated for any trait deemed relevant. Examples could include yield, grape composition and resistance or tolerance against biotic and abiotic pressures, among others. Polyclonal selections would sustain those selection gains across multiple geographical areas and environmental conditions. Maintenance of the trial vineyard in good conditions would allow for different selection goals to be addressed at any time, supporting innovation and identity for wines to be produced. Options could include higher acid selections for sparkling wine, lower sugar selections for low-alcohol wines, late ripening selections for warmer regions and so on.

If it becomes proven that Australian Shiraz vineyards hold higher diversity than any other country in the world, it will become a landmark for the Australian wine industry and a tribute for a country that has made Shiraz a household name across the world.

The same approach can be used for any other variety that is planted in at least 25 vineyards.

PORVID is willing and able to support any such work if necessary. This support can be delivered through remote consulting with the technical staff in charge of developing the sample survey, trial vineyard plantation and data analysis for polyclonal selection. Australian staff could also be trained in Portugal with hands-on practice and immersion in ongoing work alongside theoretical training in all methods

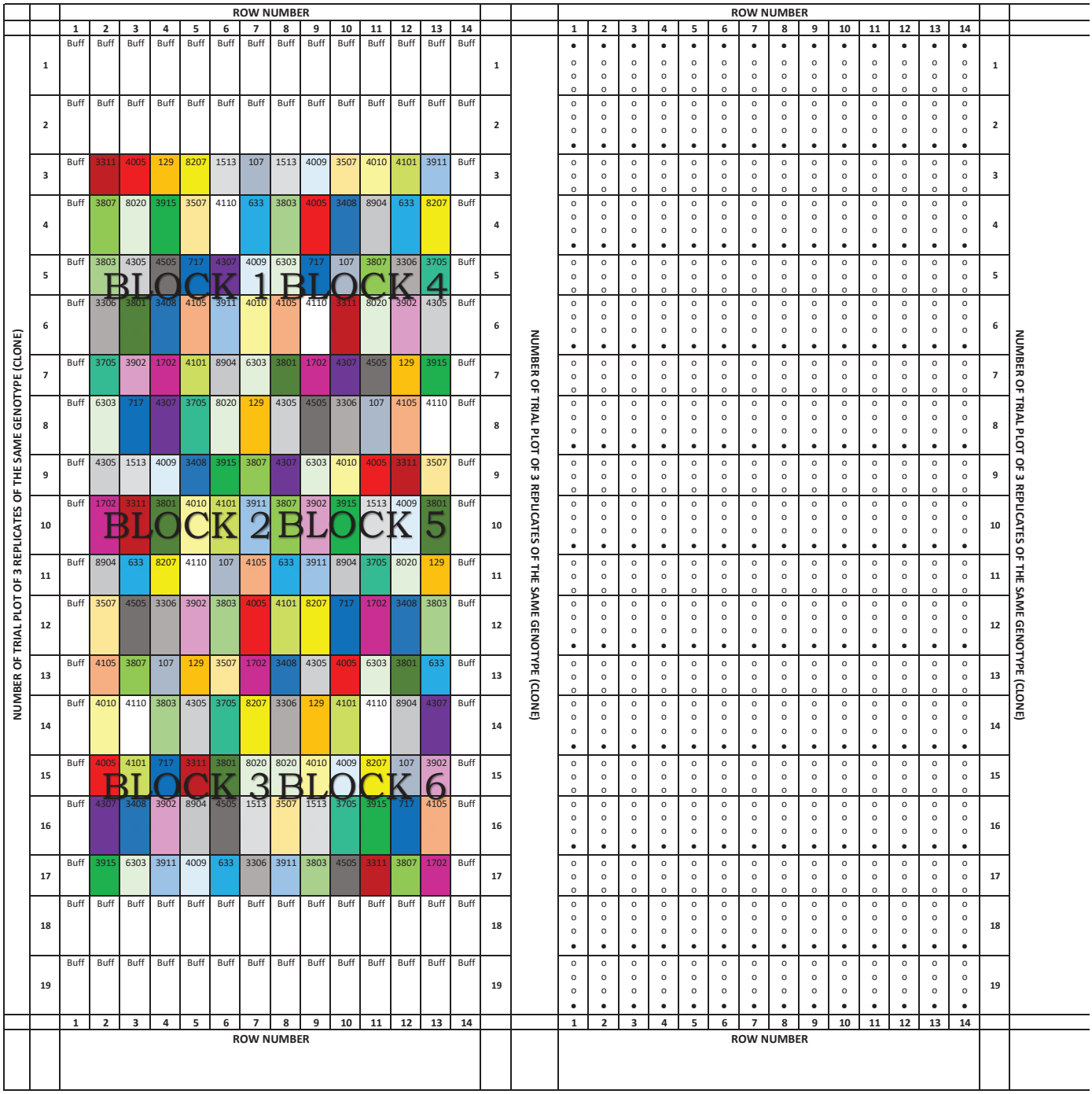


Figure 8. Experimental design of a trial vineyard using a small sample of 30 genotypes (note quantity is inadequate for diversity assessment of an ancient variety. It is included here for easier visualisation of the organisation of replicates, plots and blocks within the trial vineyard). Left side details randomisation of 30 plots each repeated in each of the six blocks. Each cell represents three plants; numbers and colours designate different original plants in the sample. Buffer plants encircling the trial area reduce borderline heterogeneities. Right side shows the actual grapevines (o) and trellis posts (•) inside the plots (lighter lines) and blocks (darker lines). In this small experimental plan there are 18 plants of each genotype, organised in three-plant plots replicated six times, one for each block. Note how each plot location in each block is randomly different to control environmental deviation.

used. Visitors can also be hosted at trial vineyard sites and at the Conservation Centre to experience direct contact with the process and its outcomes for growers and winemakers. Sporadically, it may be feasible to have PORVID staff visit Australia for short-stay on-site guidance.

Having benefited greatly from bilateral interaction between both countries in terms of both oenology and viticulture, this frontier of development opened in Portugal could also serve to strengthen the technical bond between both countries.

References

Barnes, A.M.; Wratten, S.D.; Sandhu, H.S. (2010) Biodiversity in vineyards: worth the bother? Aust. N.Z. Grapegrower Winemaker 560: 25–33.

Barossa Grape & Wine Association (2019) Barossa Chapters: Old Vines. July: https://www.barossawine.com/wp-content/uploads/2017/12/Barossa-Chapters-Old-Vines.pdf

Boehm, E.W. (1996) The Phylloxera Fight: Protecting South Australia from the phylloxera threat. Adelaide, SA: Winetitles: 90 p.

Cook, B.I.; Wolkovich, E.M. (2016) Climate change decouples drought from early wine grape harvests in France. Nat. Clim. Change 6(7): 715.

Folke, C. (2016). Resilience (republished). Ecol. Soc. 21(4): 44.

Folke, C.; Carpenter, S.R.; Walker, B.H.; Scheffer, M.; Chapin III, F.S.; Rockström, J. (2010) Resilience thinking: integrating resilience, adaptability and transformability. Ecol. Soc. 15(4): 20.

Fraga, H.; Santos, J.A.; Moutinho-Pereira, J.; Carlos, C.; Silvestre, J.; Eiras-Dias, J.; Mota, T.; Malheiro, A.C. (2016) Statistical modelling of grapevine phenology in Portuguese wine regions: observed trends and climate change projections. J. Agric. Sci. 154(5): 795–811.

Goerner, S.J.; Lietaer, B.; Ulanowicz, R.E. (2009) Quantifying economic sustainability: Implications for free-enterprise theory, policy and practice. Ecol. Econ. 69(1): 76–81.

Gonçalves, E.; Martins, A. (2012) Genetic Variability Evaluation and Selection in Ancient Grapevine Varieties. Abdurakhmonov, I.Y. (ed.) Plant Breeding. Rijeka, Croatia: Intech: 333–352.

Gonçalves E.; Graça A.; Martins A. (2019) Grapevine clonal selection in Portugal: A different approach. BIO Web Conf. 12 (01003): 2–6.

Grohs, D.S.; Almança, M.A.K.; Fajardo, T.V.; Halleen, F.; Miele, A. (2017) Advances in propagation of grapevine in the world. Revista Brasileira de Fruticultura 39(4): e-760.

Johnston, S. (2019) Hunter Valley Heritage Vineyards Strategic Study. Hunter Valley Wine & Tourism Association (unpublished data): 11.

Luján, A.G. (1984) La erosión genética en viticultura. Agrishell: 7–11.

Martins, A. (2009) Variabilidad genética de las variedades de cepas portuguesas: métodos y estrategias para su conservación, evaluación y uso. ACENOLOGIA. 23 December: http://www.acenologia.com/cienciay-tecnologia/variedades_portuguesas_cien1209.htm

Martins, A. (2011) Selecting grapevine varieties, a history with its roots in the regions of the Douro and Vinho Verde. Magalhães, N. (coord.) Francisco Girão, an innovator in vitiviniculture in the north of Portugal, Vol II. Porto: Fundação Francisco Girão: 206–229.

Martins, A.; Carneiro, L.; Mestre, S.; Gonçalves, E.; Neves-Martins, J.; Almeida, C.; Ramadas, I.; Eiras-Dias, J.E.; Madeira, D.; Magalhães, N. (1998) Facteurs d'instabilité du rendement de clones de vigne. Proceedings of the 23rd world congress of vine and wine (OIV). 22-27 June: Lisboa, Portugal: 169–174.

Martins, A.; Carneiro, L.C.; Gonçalves, E.; Eiras-Dias, J.E. (2006) Methodologie pour l'analyse et conservation de la variabilite genetique des cepages: exemple du Aragonez. Proc. XXIX Congrès Mondial de la Vigne et du Vin (OIV). 26–30 June: Logroño.

Martins, A.; Eiras-Dias, J.E. (2008) The role of polyclonal selected material for sustainable viticulture. Second International Congress on Mountain and Steep Slope Viticulture (CERVIM). 13–15 May: Monforte de Lemos, Galicia, Spain.

Martins, A.; Gonçalves, E. (2015) Grapevine breeding programmes in Portugal. Reynolds, A. (ed.) Grapevine Breeding Programs for the Wine Industry. Cambridge: Woodhead Publishing: 159–182.

McIntyre, J. (2015) Finding Irrawang: James King, scientific transnationalism and colonial wine heritage. Worlds in a wine glass. 11 December: https://juliemcintyrewinehistory.wordpress.com/2015/12/11/finding-irrawang-james-king-scientific-transnationalism-and-colonial-wine-heritage/

Mondello, V.; Armengol, J.; Mugnai, L.; Rego, C.; Vaczy, K.; Kaliterna, J.; Larignon, P.; Kortekamp, A.; Fontaine, F. (2017) The scientific basis for a more efficient control of GTDs from nursery to vineyard. Winetwork project: http://www.winetwork-data.eu/intranet/libretti/0/libretto16271-01-1.pdf

Schultz, H. (2014) Global change, sustainability and challenges for grape and wine production. J. Wine Econ. 11(1): 181–200.

PORVID (2008) Catálogo de clones seleccionados. PORVID. 20 April: 165–171: https://bit.ly/2XWnzQr

Reynolds, A. (ed) (2015) Grapevine Breeding Programs for the Wine Industry. Cambridge: Woodhead Publishing: 466 p.

van Leeuwen, C.; Darriet, P. (2016) The impact of climate change on viticulture and wine quality. J. Wine Econ. 11(1): 150–167.

Viceto, C.; Cardoso, S.; Marta-Almeida, M.; Gorodetskaya, I.; Rocha, A. (2017) Assessment of future extreme climate events over the Porto wine Region. In: EGU General Assembly Conference Abstracts (19): 950.

Waite, H.; Whitelaw-Weckert, M.; Torley, P. (2015) Grapevine propagation: principles and methods for the production of high-quality grapevine planting material. N.Z. J. Crop Hortic. Sci. 43(2): 144–161.

Waite, H.; Armengol, J.; Billones-Baaijens, R.; Gramaje, D.; Hallen, F.; Di Marco, S.; Smart, R. (2018) A protocol for the management of grapevine rootstock mother vines to reduce latent infections by grapevine trunk pathogens in cuttings. Phytopathol. Mediterr. 57(3): 384–398.

Walker, B.; Holling, C.S.; Carpenter, S.R.; Kinzig, A. (2004) Resilience, adaptability and transformability in social-ecological systems. Ecol. Soc. 9(2): 5.

Understanding the role of regionality in Shiraz – sensory and chemical profiles of Shiraz wines from six different regions

W. Pearson[1,2], L. Schmidtke[2], L. Francis[1], S. Li[2], J. Blackman[2], T. Carr[2]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Science, Wagga Wagga, NSW

Abstract

Terroir can be broadly considered to be the contributions and interactions of environmental, human and cultural factors that impart unique characteristics to a product. In this study regional differences in sensory characteristics of Shiraz wines were explored in an Australian context, evaluating the sensory profiles of premium wines from six well-regarded Shiraz-producing regions. Initially, large sets of regionally produced wines were evaluated by groups of winemakers from each specific region, using a newly described, rapid sensory method called Pivot© Profile, in order to obtain an overall regional sensory snapshot of the wines produced in that region. A subset of wines from each region was then carefully selected using the Pivot© Profile data and further evaluated using traditional sensory descriptive analysis along with comprehensive chemical analysis. This work has given detailed quantitative information of the sensory properties that can be expected from each of the regions, along with their associated chemical pattern, and provided links between regional sensory attributes and chemical profiles. The sensory fingerprints that differentiate the regions can help winemakers, wine trade and consumers to appreciate what sensory attributes can be expected when producing/selling/purchasing a wine from one of the regions studied here, and points of difference compared to other wines.

Introduction

Terroir (from the French for land – terre) is an idea that connects an agricultural product to a particular region or site. The unique characteristics of a product associated with a region or area can be influenced by factors such as climate, topography, geology, geography and human interactions. In simpler terms, it is the impact of place on the appearance and flavour of a product.

Australia has the world's second largest area of Shiraz vineyards (Robinson et al. 2012), with Shiraz vines accounting for nearly 30% of all vineyard area in Australia (Wine Australia 2019). There have been very few studies focusing on sensory differences related to the regionality of Shiraz, with a relatively recent investigation (Johnson et al. 2013) providing data on several wines selected from each of 10 Australian regions. A challenge for research studies is the ability to assess large enough numbers of wines to make meaningful comparisons across regions, given the variability within a region and the complexity of premium Shiraz sensory profiles. With advances in rapid sensory descriptive methods (Varela and Ares 2012), evaluating many wines to assess sensory differences between regions is now a reasonable proposition.

The aim in the present study was to evaluate the sensory profile of Shiraz wines from well-known Shiraz-producing regions in Australia to determine in detail which sensory properties could be related to the places the wines came from. In order to achieve this, a large number of samples from each of six regions were characterised using the rapid sensory methodology Pivot© Profile (Thuillier et al. 2015) using groups of local winemakers.

Regional Pivot© Profile evaluations

Winemakers from each of the six regions (Hunter Valley, Heathcote, Yarra Valley, Canberra District, Barossa Valley and McLaren Vale) were used as judges, as they are very familiar with the wine characteristics and styles of their regions and would be expected to be able to describe and discern small differences. These assessments were conducted to show the sensory differences within each region, and to provide information that could be used to select representative wines from each region for a subsequent formal descriptive analysis study. Between 22 and 28 wines were chosen from each region, with all wines being commercially available and selected to represent the diversity of wine styles within the region. Wines which exhibited an obvious fault were excluded. Wines from the 2015 and 2016 vintages were chosen to incorporate some vintage variation.

An example of the Pivot© Profile results is shown in Figure 1 for the 23 Yarra Valley wines assessed by the winemaker panel. The results are displayed as a correspondence analysis map to visualise the sensory differences between the samples and the attributes that were most related to the groups of wines.

This biplot represents a sensory 'fingerprint' of the region. Statistical cluster analysis was completed to separate the samples into groups based on their sensory attributes. The method separated the Yarra Valley wines into four clusters, with the largest cluster comprising 11 wines: this cluster thus represents the more common Shiraz wines found from this region. Generally, the sensory characteristics of the Yarra Valley clusters ranged from higher tannin, high purple colour,

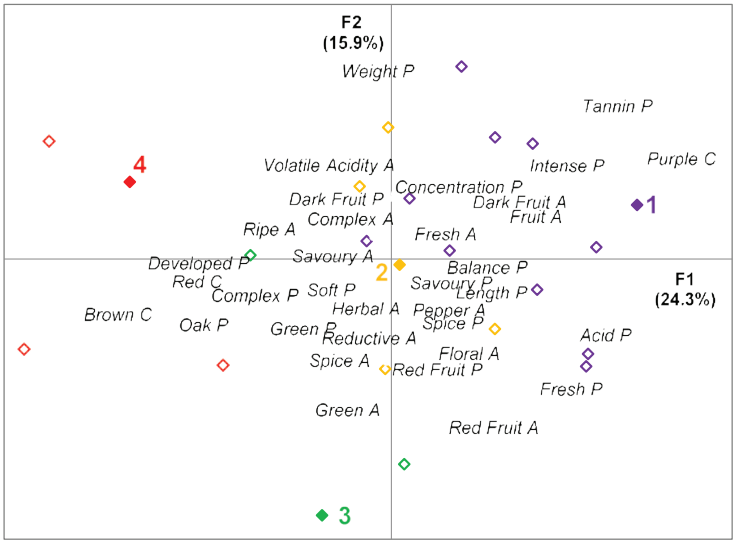


Figure 1. Results of the Pivot© Profile assessment summarising the sensory properties for the 23 Yarra Valley wines. The four clusters identified are shown in different colours. Wines with solid symbols were chosen for the multi-regional descriptive analysis evaluation. C = Colour; A = Aroma; P = Palate

concentrated and 'dark fruit' driven (the largest cluster 1) to more 'red fruit' and 'dark fruit', 'spice' and 'pepper' aromas, with more palate weight and some slightly faulty wines (cluster 2, five wines), 'red fruit', 'green' and 'spicy' wines (cluster 3, three wines), and 'oak-driven', 'brown', 'developed' and 'complex' wines (cluster 4, four wines). A similar approach was used for each of the six regions to identify wines that represented the range of sensory properties of the regions.

Quantitative sensory descriptive analysis: comparing wines across regions

After the Pivot© Profile evaluations, wines carefully selected from the clusters identified from each region were included in a comparative sensory descriptive analysis study using a trained AWRI panel. Twenty-two wines were selected, with four from each of Yarra Valley, Canberra District, McLaren Vale and Barossa Valley, and three wines from the Hunter Valley (upper and lower) and Heathcote. These wines ranged in price from $27 to $92 (median $35), with alcohol levels from 13.1 to 15.8% v/v (median 14.1%). All wines except two were from single vineyards.

Figure 2 shows the results of the quantitative sensory descriptive analysis study in the form of a Principal Component Analysis (PCA) map. The separation of the wines shows that along PC1 from left to right there is a general tendency of warm regions to cool regions. However, situated to the right are the Hunter Valley wines, which would certainly be considered a warm region (Iland et al. 2017). This is likely a result of the fact that the two years chosen for this study (2015 and 2016) were both high rainfall vintages which led to earlier harvests.

The four Barossa Valley wines located along PC1 were grouped fairly tightly together, indicating a comparable sensory profile for the attributes best defined along PC1. These were high in 'opacity' C, 'dark fruit' A/P, 'viscosity' P, 'woody' A/P, 'dried fruit' A and 'astringent' P, and low in 'stalky' A/P, 'red fruit' A/P, 'floral' A and 'confection' A.

Three of the McLaren Vale wines also show a relatively tight grouping, slightly to the left of the Barossa samples. One wine is to the right of Figure 2, relating more to the attributes 'red fruit' A/P, 'mint' A/P, overall 'fruit' P, 'floral' A and 'confection' A.

The three wines originating from Heathcote exhibited some of the greatest separation along PC1, with the wine HC1 – from the largest cluster of wines from this region – having especially high scores for the attributes 'opacity' C, 'dark fruit' A/P, 'dried fruit' A, 'beef stock' A, 'umami' P and 'hot' P, and very low scores for the attributes 'red fruit' A/P, 'confection' A, 'floral' A and 'stalky' A/P. This wine varied quite considerably from the other two wines from the region, and therefore indicates a broad regional sensory profile.

The four wines from the Canberra District were also spread out along PC1, with two samples at the far right and the other two situated close to the origin. Interestingly, the two far right wines were from the same subregion (Murrumbateman) with the other two wines from different subregions (Lake George and Majura Valley). The two Murrumbateman wines were rated highly for the attributes 'red fruit' A/P and 'stalky' A/P, and low for the attributes 'opacity' C, 'dark fruit' A/P and 'woody' A/P. The two other wines were separated along PC2. The Lake George Canberra wine CB3 was rated high for the attributes 'dark fruit' A/P, 'brown' C, 'floral' A, 'dried fruit' A, 'spice' A, 'woody' A, 'mint' A/P and 'pepper' P. The Majura Valley wine CB4 was rated high for the attributes 'earthy' A/P, 'cooked veg' A, 'drain' A, 'beef stock' A, 'woody' P, 'brown' C, 'floral' A, 'dried fruit' A and 'pepper' P.

The four Yarra Valley wines were separated by a comparable distance to the Canberra wines along PC1, with two wines located near the centre of the biplot, while the other two are spread out along the right side of PC1. Trends among the Yarra wines include high means for 'stalky' A/P for three of the four wines, and 'mint' A/P, 'red fruit' A/P, 'floral' A and 'pepper' P for two of the four wines. Three of the four wines also scored low for the attributes 'astringency' P and 'dried fruit' A.

The three Hunter Valley wines can all be found right of the origin along PC1. All three wines scored high for the attribute 'red fruit' A/P, and low for the attributes 'dark fruit' A/P, 'viscosity' P, 'pepper' P and 'woody' A/P. Of all the regions, the Hunter Valley wines were most similar.

Links between chemical composition and distinctive regional sensory characteristics

The 22 regional Shiraz wines were analysed for 69 different chemical measures. An analysis of variance was completed, to assess which compounds differed across the regions. A PCA analysis of the chemical data is shown in Figure 3, with the sensory attributes overlaid on the plot.

The Canberra District wines were rated relatively highly for the attribute 'floral' and were high in the monoterpene compounds citronellol, trans-geraniol, linalool and terpinolene, compounds

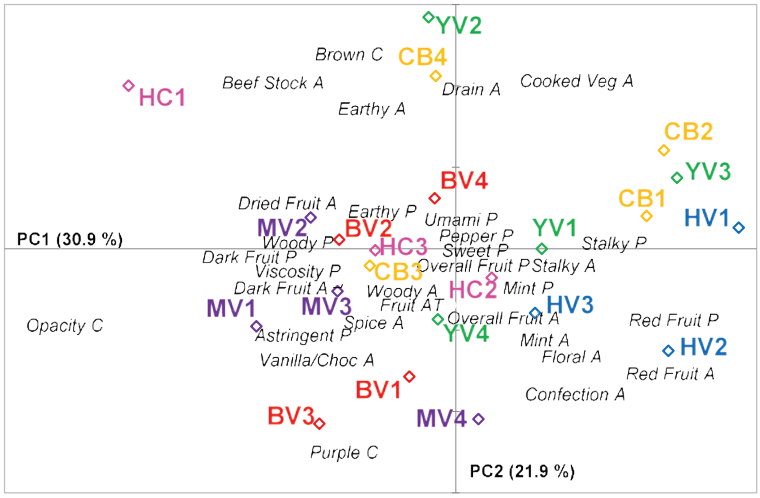


Figure 2. Sensory properties of wines from each of the six regions from the sensory descriptive analysis study. HC: Heathcote; YV: Yarra Valley, HV: Hunter Valley; CB: Canberra; BV: Barossa Valley; MV: McLaren Vale. The letter C after an attribute = Colour, A = Aroma, P = Palate and AT = Aftertaste

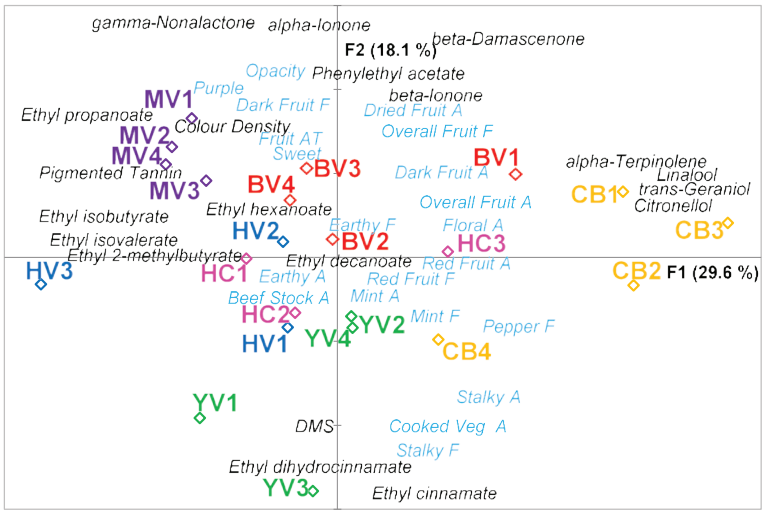


Figure 3. PCA biplot of the chemical measures for the 22 wines with the sensory attributes overlaid. HC: Heathcote; YV: Yarra Valley, HV: Hunter Valley, CB: Canberra; BV: Barossa Valley; MV: McLaren Vale. The letter C after an attribute = Colour, A = Aroma, P = Palate and AT = Aftertaste

noted for their floral aromas. The Yarra Valley wines were higher in ethyl cinnamate, ethyl dihydrocinnamate and dimethyl sulfide, which were related to the 'stalky' and 'cooked veg' attributes. The Barossa Valley wines were separated from wines from the other regions with higher concentrations of beta-damascenone, alpha- and beta-ionone, gamma-decalactone and phenyl ethyl acetate, which were linked to the 'dark fruit'/'fruity' sensory attributes. McLaren Vale wines were also higher in these compounds, together with colour density, pigmented tannin and several fermentation-derived esters, and were also high in the measures pigmented tannin and colour density.

The chemical compounds that were found to be associated with distinctive characteristics, once confirmed, will provide avenues to enhance or otherwise control sensory properties that give rise to regional differences. Viticultural or winemaking techniques are available that alter the concentration of many of the compounds identified, and the compounds can be used as targets for experimental trials.

Conclusion

The investigation allowed an understanding of the range of sensory properties of Australian Shiraz, and what sensory characteristics are related to region of origin. The study showed that Australian Shiraz wines can exhibit sensory profiles that represent the place they come from. Understanding these regional sensory characters assists grapegrowers, winemakers and wine marketers in knowing what sensory attributes are expected from a wine from these regions. Reliable sensory descriptions help in aligning the different sectors of the wine industry to be able to communicate clearly, including between growers and winemakers; within wine companies; amongst wineries within a region; and for websites, retail sales personnel and customers. With a knowledge of an established sensory profile, and with causative chemical compounds known, grapegrowers and winemakers can strive to maintain or enhance the regional characters found in their grapes. Wine marketers and sales professionals can also use the sensory information to help tell the stories of their regional wines to their customers.

References

Iland, P.G.; Gago, P.; Caillard, A.; Dry, P.R. (2017) Australian Wine: styles and tastes – people and places. Adelaide, SA: Patrick Iland Wine Promotions Pty Ltd.

Johnson, T.E.; Hasted, A.; Ristic, R.; Bastian, S.E.P. (2013) Multidimensional scaling (MDS), cluster and descriptive analyses provide preliminary insights into Australian Shiraz wine regional characteristics. Food Qual. Pref. 29: 174–185.

Robinson, J.; Harding, J.; Vouillamoz, J. (2012) Wine Grapes. New York: HarperCollins Publishers. 1241 pp.

Thuillier, B.; Valentin, D.; Marchal, R.; Dacremont, C. (2015) Pivot© Profile: A new descriptive method based on free description. Food Qual. Pref. 42: 66–77.

Varela, P.; Ares, G. (2012) Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterisation. Food Res. Int. 48: 893–908.

Wine Australia (2019) Providing insights on Australian Wine: https://www.wineaustralia.com/getmedia/ebf6c25d-1ad7-4b87-9cf9-181664da91aa/Shiraz-snapshot-2018-19.pdf

'Sweetness' of dry wines: from molecular origin to practical monitoring

A. Marchal[1], B. Cretin[1], S. Fayad[1], M. Gammacurta[1], V. Moine[2], P. Marullo[1,2], D. Dubourdieu[1]

[1]Université de Bordeaux, ISVV, Unité de recherche ŒNOLOGIE, Villenave d'Ornon, France
[2]Biolaffort, Bordeaux, France
axel.marchal@u-bordeaux.fr

Abstract

Although 'sweetness' of dry wines is clearly perceived during tasting, its molecular markers have remained unknown for a long time. To investigate the molecular origins of this 'sweetness', research was carried out on the basis of practical observations. Indeed, some winemakers frequently note a gain in 'sweetness' of dry wines during post-fermentation maceration of red wines and oak ageing. In addition, variations in 'softness' are frequently observed according to grape varieties, terroirs and vintages. These observations suggest three potential origins of 'sweetness': yeast, grapes and oak. Using both analytical chemistry and sensory analysis, these drivers of 'sweetness' have been explored and several new molecular markers have been identified. The results make it possible to envisage oenological applications aimed at better control of winemaking and ageing of dry white and red wines.

Introduction

Wine is a complex matrix containing thousands of compounds, only a small number of which have been identified. Some of them have organoleptic properties. These compounds are likely to contribute to the different flavours of wine and especially the 'soft' component, which plays a major role in the taste balance of dry wines, by reducing their acidity and their bitterness. These taste balances are intimately linked to the composition of the grapes; however, they are modulated during winemaking and by the selective extraction of the constituents of the berry, and they evolve during ageing in barrel and in bottle. They are based on the perception of sapid molecules in wine, a class of molecules of which only a part has been identified. The key compounds driving the perception of 'sourness' are well known—these are organic acids, in particular tartaric acid, but also malic, lactic, citric or succinic acids. Their origin and the factors influencing their content in wines have been the subject of numerous studies (Ribéreau-Gayon et al. 2006). The bitter properties of phenolic compounds have been described for a long time and their presence in white and red wines suggests their gustatory contribution. However, few data linking molecular structure, detection threshold and concentrations in wines are available in the literature. The bitter component of dry wines remains partly unexplained, as does the origin of 'sweet' flavour. It is indeed necessary to distinguish sweet wines, whose sweetness is due to glucose and fructose from the grapes, not transformed by yeasts, from dry wines. The latter have a 'soft' character, which cannot be attributed to carbohydrates, the contents of which are below their detection threshold; its molecular markers remain unknown. In our team, the search for such taste effectors has been guided by empirical observations. According to some winemakers, two factors could contribute to the 'sweetness' gain of dry wines: post-fermentation maceration of red wines and ageing in oak wood. In addition, variations in the 'sweet' character are frequently observed depending on the grape varieties, terroirs and vintages. The work carried out in the laboratory was guided by these observations in an attempt to better understand the molecular origins of the 'sweet' flavour of dry wines.

Contribution of yeast autolysis to 'sweetness' of dry wines

Most fermented drinks, especially wine, undergo a period of contact with the yeast lees at the end of the fermentation process. The lees consist mainly of microorganisms (yeasts from alcoholic fermentation) as well as organic residues from the grapes. During winemaking and ageing, the presence of yeast lees leads to various physico-chemical, aromatic and gustatory modifications. The increase in 'sweetness' during ageing on the lees of white wines, although well observed in the cellar, cannot be easily linked to any chemical knowledge. The same 'sweetening' phenomenon is observed during post-fermentation maceration of red wines. However, these two phases coincide, for each type of winemaking, with the autolysis of yeasts. This post-fermentation process corresponds to the breakdown of yeast cell walls under the effect of endogenous enzymes (Babayan et al. 1981). This results in the solubilisation of cellular substances, in particular polysaccharides, nucleotides, proteins and peptides. The presence of molecules with a 'sweet' flavour among the autolysis products of yeast would make it possible to interpret the observations of the winemakers.

To study the potential effect of yeast lees on wine taste, yeasts were added to dry wines at various concentrations. After autolysis, tasters ranked the treatments according their 'sweetness' intensity. The sensory results showed significant differences: the 'sweetness' intensity increased with the quantity of yeasts added, demonstrating the 'sweetening' effect of yeast lees through the release of 'sweet' compounds (Figure 1).

Then, an approach combining biochemistry, molecular biology and sensory analysis was used to search for compounds involved in this phenomenon. We showed that a yeast protein called HSP12 significantly contributes to the increase in 'sweetness' during autolysis (Marchal et al. 2011a).

To study the effect of biotic factors on HSP12 expression, fermentations were carried out in a synthetic medium from eight yeast strains, including four commercial oenological strains, the Δ°HSP12 strain, which differs from the F×10 strain only by the absence of the HSP12 gene and three strains from other biotopes (distillery, brewery and oak exudates). These last three strains gave rise to sluggish fermentations, which confirms their poor adaptation to oenological conditions. A biomass sample was taken at 46 g/L of released CO_2 in order to measure the expression of the HSP12 gene. At this stage, all the

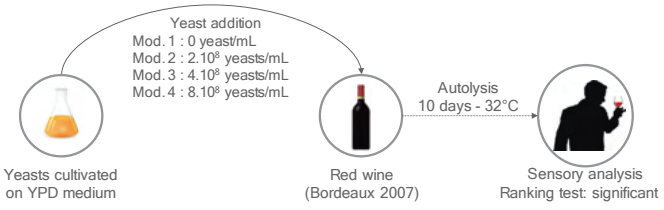


Figure 1. Demonstration of the effect of yeast autolysis to 'sweet' taste of dry wines

fermentations were still active and the yeasts collected had a high rate of viability. Figure 2 shows that the level of HSP12 expression varies significantly according to the yeast strain. Concomitantly, the same strains were added to a dry wine to undergo autolysis. A trained panel tasted the resulting wines and significant differences in 'sweetness' intensity were observed between the treatments (Marchal et al. 2015c). These results demonstrate that wine taste is affected by yeast autolysis and that the yeast strain can modulate the 'sweetness' intensity through the HSP12 protein.

Contribution of grape compounds to 'sweetness' of dry wines

For most red wines, extraction takes place in the fermentation phase by maceration of the grapes in the juice. The leaching of the cap promotes the dissolution of the constituents of the skin, the seed and, in certain types of winemaking, the stem. With the completion of alcoholic fermentation, human action becomes more discreet or even non-existent. After inerting the vats with carbon dioxide to avoid the proliferation of aerobic spoilage microorganisms, the wine is generally left in contact with the marc without any other intervention; this is post-fermentation maceration. During this infusion phase, the temperature of the wine can be increased using a technique known as hot post-fermentation maceration (HPFM), allowing the yeast autolysis described above. This vatting phase seems to play a major organoleptic role in giving more structure, density and 'sweetness' to the wine.

In order to assess the sensory consequences of HPFM, experiments were set up in three estates in Bordeaux, France. Each plot was harvested and vinified according to a classic protocol, in stainless steel tanks of 120 hL. The alcoholic fermentation was carried out with *Saccharomyces cerevisiae* (strain F33, Laffort) at a temperature of 26°C (in the juice). For each tank, a first running-off was performed at the end of fermentation to fill two barrels having previously contained two harvests (2-year old barrel), which led to the treatment denoted in Figure 3 as 'AF'. Then, each tank was inerted with CO_2 and kept at 30°C to carry out the HPFM for 10 days. At the end of this stage, a second running-off into two 2-year-old barrels was performed, which led to the treatment denoted as 'PFM'. The AF and PFM wines underwent malolactic fermentation (MLF) in barrels. The samples used in this study were taken from the barrels after six months of ageing. Basic oenological analyses (ethanol, pH, total acidity, volatile acidity, total polyphenols index) did not show any significant differences between the AF and PFM treatments of each batch. The samples were submitted to a panel of 30 tasters, in the form of a triangular test and a sensory profile.

For the wines made at each estate in this trial, sensory panellists were able to distinguish between the AF and PFM treatments. The PFM wines were perceived to be significantly 'sweeter' and less astringent than the AF wines. However, as previously demonstrated, the autolysis of yeasts releases 'sweet' compounds. It is therefore possible that the taste changes perceived during post-fermentation maceration, as established above, result solely from the biochemical mechanisms linked to autolysis rather than from the diffusion of constituents from the solid parts of the grape. These two phenomena had to be distinguished to assess their respective contributions. In this experiment, the PFM treatment benefited jointly from these two contributions, and the AF treatment experienced neither of them.

Previously, we conducted autolysis in a red wine enriched with yeasts and stored for 10 days at 32°C. This protocol was applied to the AF treatments to obtain AF + Y treatments. Compared to the AF treatment, these wines benefited from the release of yeast peptides, but compared to the PFM treatment, there was no HPFM process involving contact with the grape marc.

The significant difference between the AF and AF + Y methods has already been observed and established the 'sweetness' contribution of yeast lees. Here, a triangular test followed by a sensory profile revealed that taste differences were perceived between AF + Y and PFM treatments, with more 'sweetness' in the latter. These new results demonstrate that the gain in 'sweetness' observed during post-fermentation maceration does not only result from the autolysis of yeasts, but also from the contact of wine with the grape marc. This establishes the presence in dry wines of 'sweet' compounds coming from grapes.

To search for such compounds, a taste-guided purification protocol was developed and two 'sweet' molecules were identified: *epi*-DPA-G and astilbin (Figure 4) (Cretin et al. 2019). Their concentration in wine is affected by various winemaking parameters and in particular the presence of stems (Fayad et al. 2019).

Contribution of oak wood ageing to 'sweetness' of dry wines

Oak ageing is a crucial step of winemaking, during which wine bouquet and taste are deeply modified. These modifications can be due to the moderate oxidation of wine compounds during ageing, or to the release of molecules from wood (Ribéreau-Gayon et al. 2006). Intense research in this field has led to the identification of the key volatile compounds coming from oak wood: vanillin, β-methyl-γ-octalactone (called oak-lactone), eugenol and 2-furanmethanethiol (Chatonnet

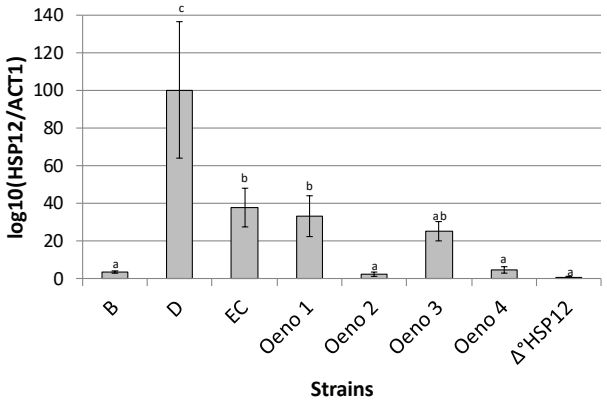


Figure 2. Influence of yeast strain on HSP12 expression

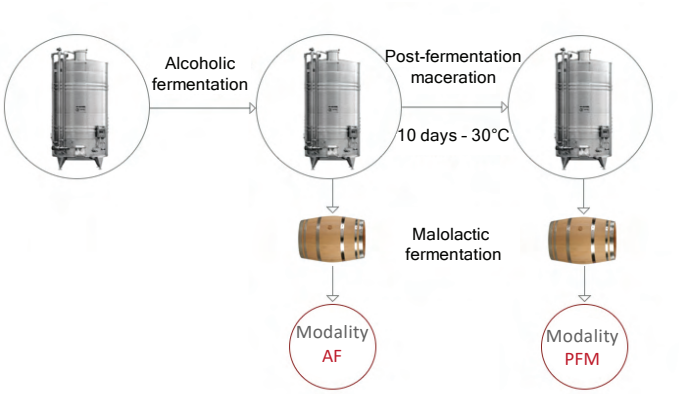


Figure 3. Experimental design used to investigate the sensory role of HPFM

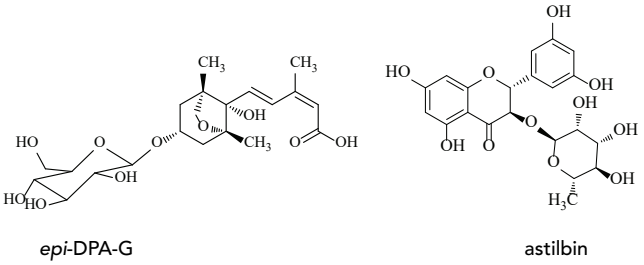


Figure 4. Chemical structures of *epi*-DPA-G and astilbin, two sweet compounds from grapes

1995; Tominaga et al. 2000). To a large extent, these volatiles explain the 'vanilla', 'coconut', 'spicy' and 'roast coffee' aromas typical of oaked wines. Moreover, oak wood releases non-volatile compounds likely to modulate the taste of wine. Practically, winemakers noticed the modification of tannin perception of wine ('structure', 'dryness', 'bitterness' and/or 'sweetness'). Many works have focused on ellagitannins. The sensory properties ('bitterness' and 'astringency') of isolated ellagitannins have been investigated and recent works described the determination of their perception threshold using a half-tongue test (Glabasnia and Hofmann 2006, 2007; Stark et al. 2005). Adducts between ellagitannins and grape flavonoids were also identified in red wine; they could be involved in the colour change of wine during ageing (Chassaing et al. 2010). Beyond ellagitannins, other non-volatile compounds are released from oak wood such as coumarins (Moutounet et al. 1989) or lignans (Cretin et al. 2015; Marchal et al. 2015a). However until now, few research data have been published on the sensory properties of non-volatile compounds and more particularly the 'sweet' component of their taste.

Demonstration of the 'sweetening' effect of oak wood ageing

Experiments were set up in two cellars in order to study the potential 'sweetening' effect of oak ageing (Marchal et al. 2013). A 2007 Bordeaux white wine was produced in four types of containers: a 4 hL stainless steel tank, a 50 hL new oak wood tank, two 225 L 1-year old barrels and two new barrels. The ageing was carried out on total lees for five months. A 2008 red wine from Crozes-Hermitage was aged in four types of containers: a new oak wood tank of 85 hL, two 2-year old barrels, two 1-year old barrels and two new barrels. The ageing lasted 12 months.

At the end of their ageing, the four treatments of each experiment were subjected to a sensory analysis. A panel of 32 tasters was asked to classify the wines according to the intensity of their 'sweetness'. The application of a Friedman test showed significant differences between the wines tasted; in each series, the wine matured in new barrels had the highest level of 'sweetness'. These results confirmed the hypothesis of an increase in the 'sweet' taste of dry wines in contact with oak wood. The same wines were tasted a second time using a nose-clip and the same results were obtained. Consequently, the increase of 'sweetness' in contact with oak wood is not due to volatile compounds. These facts demonstrate the existence of 'sweet' non-volatile compounds present in oak wood and released into wine during ageing. The present study aimed at identifying such compounds.

Development of a taste-guided purification protocol to isolate 'sweet' compounds from oak wood

For this purpose, a novel inductive methodology was developed. It was based on taste-guided fractionations of oak wood extract, to isolate 'sweet' fractions (Marchal et al. 2011b). The method consisted first of a centrifugal partition chromatography experiment, which provided 15 different wood fractions differentiated according to their affinity with the solvents used in the study (n-heptane/ethyl acetate/methanol/water). The collected fractions were freeze-dried and tasted in order to identify those characterised by a 'sweet' taste. The most interesting ones were then purified using preparative HPLC. Finally, purified compounds were analysed using mass spectrometry jointly with two-dimensional nuclear magnetic resonance (2D ^{1}H and ^{13}C NMR), in order to elucidate their chemical structure.

Two oleanane-type triterpenoids substituted with galloyl and glucosyl moieties were identified, both exhibiting a 'sweet' taste. We term these compounds, which have never been reported, Quercotriterpenoside I and II (QTT I and QTT II, respectively) (Figure 5).

Sensory threshold and occurrence of wine

The purified compounds were characterised by a strong 'sweet' taste. They were tasted in real white wine and the sensory thresholds were measured using ISO procedures. The threshold found was 590 µg/L for QTT I, which made this compound a very prominent marker of 'sweet' taste arising from barrel ageing. The analysis of oak barrel wines demonstrated that the amount of QTT in wines varies widely and can reach 1000 µg/L for the sum of QTT I and II, confirming the role of this compound in the perception of 'sweetness' at least for some oak-aged wines.

Isolation of other triterpenosides in oak wood

The QTT I and II chemical family had been poorly studied in cooperages, with the exception of the work of Arramon and co-workers (2002) describing bartogenic acid, 23-hydroxybartogenic acid as well as their mono-glucosylated derivatives (Glu-BA and Glu-HBA) in extracts of oak wood, wines and brandies matured in casks. The natural pathways of biosynthesis in plants generally induce structural diversity, in the form of isomers or derivatives. Moreover, molecules with similar structures may have similar taste properties. Structural analogues to QTT I and II, derived from the same genin (arjungenin) were therefore sought in extracts of oak wood. Various new compounds have been identified, most of them linked to 'sweetness' (Table 1) (Gammacurta et al. 2019; Marchal et al. 2015b).

Besides these 'sweet' compounds, bitter triterpenoids have also been isolated from oak wood. Among them, Glu-BA identified by Arramon et al. (2002) is particularly abundant.

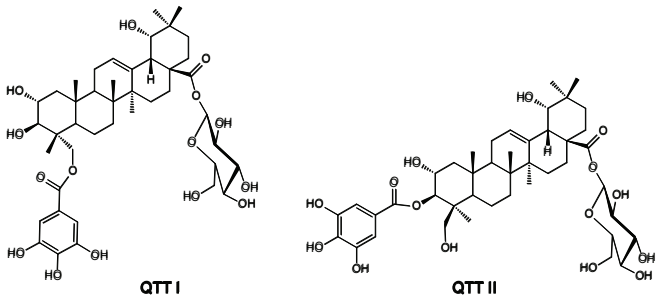
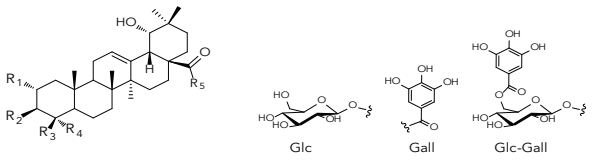


Figure 5. Chemical structures of QTT I and II

Table 1. Names and chemical structures of new triterpenoids identified in oak wood



Compound	R_1	R_2	R_3	R_4	R_5	References
Arjungénin	OH	OH	CH_3	CH_2OH	OH	Honda et al. 1976
Arjunglucoside I	OH	OH	CH_3	CH_2OH	Glc	Honda et al. 1976
Acid 23-*O*-galloylarjunique	OH	OH	CH_3	OGall	OH	Machumi et al. 2013
Acid 3-*O*-galloylarjunique	OH	OGall	CH_3	CH_2OH	OH	Gammacurtaet al. 2019
Acid 24-*O*-galloylsericique	OH	OH	OGall	CH_3	OH	Gammacurtaet al. 2019
Acid 3-*O*-galloylsericique	OH	OGall	CH_2OH	CH_3	OH	Gammacurtaet al. 2019
QTT III	OH	OGall	CH_3	CH_2OH	Glc	Marchal et al. 2015
QTT VI	OGall	OH	CH_3	CH_2OH	Glc	Marchal et al. 2015
QTT VII	OH	OH	OGall	CH_3	Glc	Gammacurtaet al. 2019
QTT VIII	OGall	OH	CH_2OH	CH_3	Glc	Gammacurtaet al. 2019
QTT IX	OH	Glc-Gall	CH_3	CH_2OH	OH	Gammacurtaet al. 2019
QTT X	OH	Glc-Gall	CH_2OH	CH_3	OH	Gammacurtaet al. 2019
QTT IV	OH	Glc-Gall	CH_2OH	CH_3	Glc	Marchal et al. 2015
QTT XI	OH	Glc-Gall	CH_3	CH_2OH	Glc	Gammacurtaet al. 2019
QTT V	OH	OGall	CH_3	CH_2OGall	Glc	Marchal et al. 2015

Influence of botanical species on the concentration of QTTs and Glu-BA in oak wood

There are two major European oak species used in cooperage (*Quercus robur* and *Quercus petraea*) and both are present in all French forests. Previous observations suggested that differences between species are associated with geographical origin, although this has not been supported by known chemical markers. Accordingly, the current research quantified the newly identified compounds QTT I, II, III and Glu-BA in oak woods coming from both species.

Forty-six samples of fresh oak material were collected in eight different French forests: three in the north-east (Saint-Clément, Spincourt, Xures), two in the centre (Tronçais, Chateauroux), one in the north-west (Liffré), one in the south-west (Pierroton) and one in the south-east (Laveyron). Samples from Pierroton and Laveyron were provided by the French National Institute for Agricultural Research and Dr. Erwan Guichoux. The other samples were supplied by Seguin Moreau Napa Cooperage. For each tree, two samples were collected: a sample of leaves to determine its species thanks to a genetic analysis (Guichoux et al. 2011, 2013) and a sample of wood to quantify QTT I, II, III and Glu-BA. The quantification method had previously been validated by studying sensitivity, linearity in working range, intra-day repeatability, inter-day precision, trueness and specificity.

Among the 46 samples analysed in this study, 27 were assigned to *Q. petraea* (sessile oak) and 19 to *Q. robur* (pedunculate oak). As is well known, both species were found in some forests (Tronçais and Liffré) confirming that geographical origin is not by itself a relevant element to discriminate sessile and pedunculate oak. The quantitation method developed in this study was applied to quantify for the first time QTT I, II and III in oak wood. The glucosyl derivative of bartogenic acid (Glu-BA) was also measured (Figure 6).

For QTT I, the mean values were 413.5 ± 96.2 µg/g for sessile oak samples and 6.0 ± 2.7 µg/g for pedunculate oak. Similar results were obtained for QTT II and QTT III, demonstrating that sessile oak was richer in QTTs than pedunculate oak. In contrast, Glu-BA mean concentration was higher in pedunculate oak (795.3 ± 271.3 µg/g) than in sessile oak (24.4 ± 10.7 µg/g). Application of 1-way ANOVA revealed significant differences between species for all compounds (p-value < 0.1 %). This trend was similar for samples of different species coming from the same forests, suggesting that the botanical species had a predominant influence on triterpenoid composition of oak wood in comparison with geographical location. These results could be of particular interest regarding the organoleptic effect of oak ageing on wine taste. Indeed, QTT I, II and III develop a 'sweet' taste whereas Glu-BA has been described as 'bitter'. So, the present study highlighted that sessile oak contained more 'sweet' triterpenoids whereas pedunculate oak was richer in 'bitter' triterpene (Marchal et al. 2016).

Although statistical tests revealed significant differences for mean concentrations in QTTs and Glu-BA, some extreme values of individual triterpenes were very close for sessile and pedunculate oak. For instance, the minimum concentration of QTT II measured in sessile oak samples was 23.6 µg/g whereas the maximum value in pedunculate oak samples was 44.1 µg/g. For Glu-BA, the maximum concentration in sessile samples was 105.5 µg/g and the minimum concentration was 36.0 µg/g in pedunculate samples. So, even though mean amounts of QTTs and Glu-BA were respectively higher and lower in sessile oak than in pedunculate oak, high inter-individual variations were observed within species for each triterpenoid as reflected by large confidence intervals. As a consequence, the individual quantification of each triterpenoid did not allow the direct identification of the botanical species. This limitation can be linked with observations concerning other compounds whose concentrations depend on botanical species. Indeed, Prida et al. (2007) showed that a significant number of sessile oak samples contained levels of β-methyl-γ-octalactone similar or even lower than pedunculate oak samples. A similar trend was observed for ellagitannins (Prida et al. 2006). So, none of these compounds (oak-lactone, ellagitannins or triterpenoids) allow an unambiguous discrimination of oak species according to their individual concentration in wood.

Differentiation of sessile and pedunculate oak wood samples according to a triterpenoids index

Beyond absolute concentrations in individual triterpenoids, it seemed that samples could be grouped in two categories according to their relative amounts of QTTs and Glu-BA. To express this relative composition, a triterpenoids index (TI) was calculated as base 10 logarithm of the ratio between the sum of concentrations in QTTs and the concentration in Glu-BA (all given in µg/g).

$$TI = \log \frac{[QTT\ I] + [QTT\ II] + [QTT\ III]}{[Glu - BA]}$$

The average values of this index were calculated for sessile and pedunculate oak wood samples (Figure 7).

Mean TI was positive for sessile samples and negative for pedunculate samples (1.9 and -1.5, respectively). The application of a 1-way ANOVA test revealed that these differences were statistically significant (p-value < 0.1%). More interestingly, confidence intervals were much smaller than the absolute concentrations for triterpenes which expresses a less extended range of values. Indeed, all samples of sessile oak had positive TI values (from 1.2 to 2.4) whereas all samples of pedunculate oak exhibited negative TI values (from -2.2 to -0.8). Contrary to absolute concentrations in individual triterpenoids, there was a huge gap (2 log points) between the closest values of both species; that is, between the lowest value of sessile oak (1.2) and the highest value of pedunculate oak (-0.8).

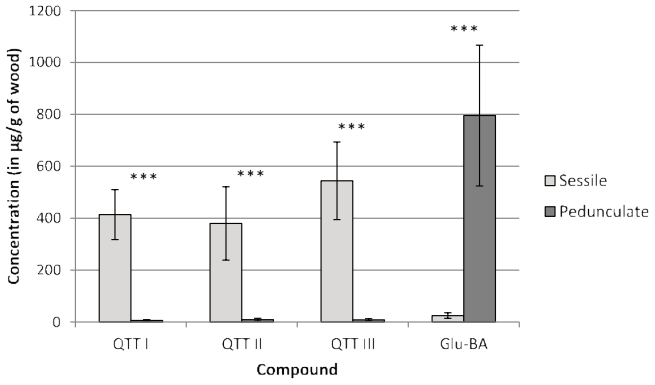


Figure 6. Influence of oak species on QTT and Glu-BA levels in wood

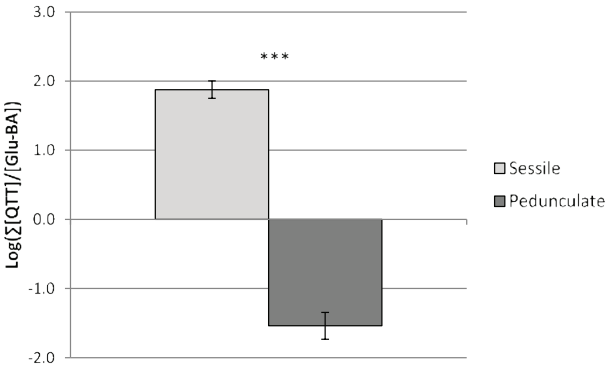


Figure 7. Relationship between triterpenoids index (TI) and oak species. Error bars indicate 95% confidence intervals. *** significant p<0.001

Consequently, the calculation of a TI reflecting the relative composition in triterpenoids of wood appeared to avoid any ambiguity in the assignment of the botanical species. In practice, a positive TI value might indicate that the sample was from sessile oak whereas a negative TI value might correspond to a pedunculate oak sample (Marchal et al. 2016).

This method allows a chemical differentiation of oak species. It has been patented and exploited for the best selection of oak wood to be used for wine ageing.

Conclusions

This paper presents a compilation of studies undertaken at the Université de Bordeaux during the last 10 years dealing with the perception of 'sweetness' in dry wines. On one hand, these studies aimed to provide molecular explanations for this perception by identifying some chemical markers. On the other hand, they sought to highlight some practical parameters likely to affect the taste balance of wine, leading to recommendations for winemakers.

Using an inductive approach based on empirical observations, we demonstrated that 'sweetness' of dry wines comes from yeast lees, grape compounds and contact with oak wood. The molecules responsible are released during the making and ageing of wines. In particular, HSP12 protein is involved in the increase of 'sweetness' observed during yeast autolysis and its expression is affected by the yeast strain. Consequently, the choice of the strain used for alcoholic fermentation can modulate the perception of 'sweetness'. The 'sweetness' can then be modified during the post-fermentation maceration of red wines by the release of grape compounds such as *epi*-DPA-G or astilbin. Their concentrations in wine can vary according to the winemaking conditions. Finally, new 'sweet' compounds have been identified in oak wood: the QTTs. The oak species significantly influences the level of QTT present in wood and their concentration in wine can consequently be increased by using only sessile oak. A new methodology to distinguish oak species has been developed and patented. A better selection of oak wood might lead to a more harmonious integration between wine and barrel.

References

Arramon, G.; Saucier, C.; Colombani, D.; Glories, Y. (2002) Identification of triterpene saponins in *Quercus robur* L. *Q. petraea* Liebl. heartwood by LC-ESI/MS and NMR. Phytochem. Anal. 13: 305–310.

Babayan, T.L.; Bezrukov, M.G.; Latov, V.K.; Belikov, V.M.; Belavtseva, E.M.; Titova, E.F. (1981) Induced autolysis of *Saccharomyces cerevisiae*: Morphological effects, rheological effects, and dynamics of accumulation of extracellular hydrolysis products. Curr. Microbiol. 5: 163–168.

Chassaing, S.; Lefeuvre, D.; Jacquet, R.; Jourdes, M.; Ducasse, L.; Galland, S.; Grelard, A.; Saucier, C.; Teissedre, P.-L.; Dangles, O.; Quideau, S. (2010) Physicochemical studies of new anthocyano-ellagitannin hybrid pigments: about the origin of the influence of oak C-glycosidic ellagitannins on wine color. Eur. J. Org. Chem. 2010(1): 55–63.

Chatonnet, P. (1995) Influence des procédés de tonnellerie et des conditions d'élevage sur la composition et la qualité des vins élevés en fûts de chêne. Thèse pour le doctorat de l'Université de Bordeaux II.

Cretin, B.N.; Sallembien, Q.; Sindt, L.; Daugey, N.; Buffeteau, T.; Waffo-Teguo, P.; Dubourdieu, D.; Marchal, A. (2015). How stereochemistry influences the taste of wine: Isolation, characterization and sensory evaluation of lyoniresinol stereoisomers. Anal. Chim. Acta 888: 191–198.

Cretin, B.N.; Waffo-Teguo, P.; Dubourdieu, D.; Marchal, A. (2019) Taste-guided isolation of sweet-tasting compounds from grape seeds, structural elucidation and identification in wines. Food Chem. 272: 388–395.

Fayad, S.; Cretin, B.N.; Marchal, A. (2019) Development and validation of an LC-FTMS method for quantifying natural sweeteners in wine. Food Chem. 311: 125881.

Gammacurta, M.; Waffo-Teguo, P.; Winstel, D.; Cretin, B.N.; Sindt, L.; Dubourdieu, D.; Marchal, A. (2019) Triterpenoids from *Quercus petraea*: Identification in wines and spirits and sensory assessment. J. Nat. Prod. 82(2): 265–275.

Glabasnia, A.; Hofmann, T. (2006) Sensory-directed identification of taste-active ellagitannins in American (*Quercus alba* L.) and European oak wood (*Quercus robur* L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. J. Agric. Food Chem. 54: 3380–3390.

Glabasnia, A.; Hofmann, T. (2007) Identification and sensory evaluation of dehydro- and deoxy-ellagitannins formed upon toasting of oak wood (*Quercus alba* L.). J. Agric. Food Chem. 55: 4109–4118.

Guichoux, E.; Lagache, L.; Wagner, S.; Léger, P.; Petit, R.J. (2011) Two highly validated multiplexes (12-plex and 8-plex) for species delimitation and parentage analysis in oaks (*Quercus* spp.). Mol. Ecol. Resour. 11: 578–585.

Guichoux, E.; Garnier-Géré, P.; Lagache, L.; Lang, T.; Boury, C.; Petit, R.J. (2013) Outlier loci highlight the direction of introgression in oaks. Mol. Ecol. 22: 450–462.

Marchal, A.; Marullo, P.; Moine, V.; Dubourdieu, D. (2011a) Influence of yeast macromolecules on sweetness in dry wines: Role of the *Saccharomyces cerevisiae* protein Hsp12. J. Agric. Food Chem. 59: 2004–2010.

Marchal, A.; Waffo-Téguo, P.; Génin, E.; Mérillon, J.M.; Dubourdieu, D. (2011b) Identification of new natural sweet compounds in wine using centrifugal partition chromatography-gustatometry and Fourier transform mass spectrometry. Anal. Chem. 83: 9629–9637.

Marchal, A.; Pons, A.; Lavigne, V.; Dubourdieu, D. (2013) Contribution of oak wood ageing to the sweet perception of dry wines. Aust. J. Grape Wine Res. 19: 11–19.

Marchal, A.; Cretin, B.N.; Sindt, L.; Waffo-Téguo, P.; Dubourdieu, D. (2015a) Contribution of oak lignans to wine taste: Chemical identification, sensory characterization and quantification. Tetrahedron 71: 3148–3156.

Marchal, A.; Génin, E.; Waffo-Téguo, P.; Bibès, A.; Da Costa, G.; Mérillon, J.-M.; Dubourdieu, D. (2015b) Development of an analytical methodology using Fourier transform mass spectrometry to discover new structural analogs of wine natural sweeteners. Anal. Chim. Acta 853: 425–434.

Marchal, A.; Marullo, P.; Durand, C.; Moine, V.; Dubourdieu, D. (2015c) Fermentative conditions modulating sweetness in dry wines: Genetics and environmental factors influencing the expression level of the *Saccharomyces cerevisiae* HSP12 gene. J. Agric. Food Chem. 63: 304–311.

Marchal, A.; Prida, A.; Dubourdieu, D. (2016) New approach for differentiating sessile and pedunculate oak: development of a LC-HRMS method to quantitate trichromatism in wood. J. Agric. Food Chem. 64: 618–626.

Moutounet, M.; Rabier, P.H.; Puech, J.L.; Verette, E.; Barillere, J.M. (1989) Analysis by HPLC of extractable substances in oak wood. Application to a Chardonnay wine. Sci. Aliments 9: 35–51.

Prida, A.; Boulet, J.-C.; Ducousso, A.; Nepveu, G.; Puech, J.-L. (2006) Effect of species and ecological conditions on ellagitannin content in oak wood from an even-aged and mixed stand of *Quercus robur* L. and *Quercus petraea* Liebl. Ann. For. Sci. 63: 415–424.

Prida, A.; Ducousso, A.; Petit, R.J.; Nepveu, G.; Puech, J.L. (2007) Variation in wood volatile compounds in a mixed oak stand: Strong species and spatial differentiation in whisky-lactone content. Ann. For. Sci. 64: 313–320.

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. (2006) Handbook of Enology. Vol. 2. The Chemistry of Wine Stabilization and Treatments. Chichester, UK: John Wiley & Sons, Ltd.

Stark, T.; Bareuther, S.; Hofmann, T. (2005) Sensory-guided decomposition of roasted cocoa nibs (*Theobroma cacao*) and structure determination of taste-active polyphenols. J. Agric. Food Chem. 53: 5407–5418.

Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. (2000) A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. J. Agric. Food Chem. 48: 1799–1802.

Receptomics and its potential for wine analysis

M.A. Jongsma, M. Roelse

Business Unit Bioscience, Wageningen University and Research, Wageningen, The Netherlands
maarten.jongsma@wur.nl

Abstract

Receptomics is a novel bio-analytical approach based on parallel screening of large numbers of biological receptors to evaluate potential bioactives, such as aroma and taste compounds. It also holds promise to augment or replace human sensory evaluation of food and beverages. This paper describes a novel microfluidic technique developed in Wageningen for analysis of complex liquid food samples against large arrays of human sensory and health-related receptors—expressed in a human cell line, inside a flow cell. A small pre-study on the analysis of red and white wine against a nearly complete set of bitter receptors is also reported. To ensure the cells would tolerate undiluted wine, it was necessary to first neutralise the wine pH and remove the alcohol. To observe specific activation of receptors, the 16-times diluted sample was contrasted with the 2-,4- and 8-times diluted samples. Surprisingly, it was found that both Shiraz and Gewürztraminer wines induced at higher concentrations a negative signal with some of the receptors that were expected to give positive signals (TAS2-R4, -R7, -R39 and -38PAV) in these two wines. This is somewhat unexpected in light of pure compound assays and observations in other bitter drinks such as beer and coffee. The lack of positive signals may be due to the fact that the pH was adjusted and/or that the assay lacked sensitivity as it was only possible to analyse diluted wine. To further evaluate the potential of receptomics for direct analysis of wine taste, it will be required to (i) identify and correct for the dip-inducing factor, (ii) analyse non-bitter wines after the addition of bitter compounds as positive controls and compare them to bitter wines, and (iii) repeat the tests with pH-insensitive reporters of receptor activation.

Introduction

Since the discovery of the human sensory receptor repertoire almost 20 years ago, it has been a holy grail of the food, flavour and fragrance industry to obtain receptor fingerprints as a proxy for traditional sensory evaluation to guide product development processes and assess product quality. The available technology so far has mostly relied on analyses that require multiple, single-use microtiter plates for the analysis of a single sample against all ~430 different olfactory and taste receptors, and these methods are not suitable for complex samples like wine. Thus, scans of the full receptor repertoire become prohibitively expensive for most applications when using the current approach.

Opportunities exist to overcome this problem. In all existing assays with live cells, the single cell (10 μm) is the elementary sensor and monitoring 10–100 cells is sufficient to evaluate any receptor activation event. Furthermore, cells may be activated repeatedly and will yield similar, reproducible signals (Roelse et al. 2018). This implies that there is plenty of scope for developing smaller assay systems, larger arrays and reusable chips. This will save greatly on costs and increase throughput, while generating much more data.

Pioneering this vision is a 'tongue-on-a-chip' platform developed at Wageningen Research that aims to functionally emulate the whole receptor diversity of the tongue on one cm^2 chips that are operated as flow cells (Roelse et al. 2013, 2018, 2019; Henquet et al. 2016; Wehrens et al. 2019). Typically, in this system the current size and input reduction factor is 100-fold compared to microtiter plate systems, and it allows sequential analysis of at least 10 samples at time intervals of typically 1–5 minutes (see www.receptomics.com for an animation). This biosynthetic 'tongue' has been able to differentiate different qualities of bitter vegetables, coffee varieties and roasts, and bitters in beer. Computational approaches have recently been developed that deal with non-specific host cell responses and sample colour by performing sample comparisons (Wehrens et al. 2019) and internal calibrations.

Receptor studies on wine have been limited so far to the study of purified compounds by Soares et al. (2013, 2016, 2018). They concluded that among a set of four selected polyphenolic compounds, malvidin-3-glucoside was the compound most likely to be involved in the bitter taste of (some) red wines because only the receptor TAS2R7 responded in the known concentration range of that compound in wines (Soares et al. 2013). This is somewhat surprising, given that malvidin-3-glucoside can be present in gram amounts in red grapes and is not known for contributing a bitter taste to wine (Brossaud et al. 2001). A subsequent study also identified receptors TAS2R5, 14, 30 and 39 using a broader set of polyphenol compounds that may be relevant for the bitter taste of red wine (Soares et al. 2018). Interestingly, using a different approach, a genetic association study identified a common genetic PAV variant of TAS2R38 as a determinant of reported differences in wine bitterness. The lack of overlap with the cell assays may be related in the genetic study to low sample size and low frequency of single nucleotide polymorphisms with relevance for the bitter taste. The association with TAS2R38 genotype PAV does suggest, however, that there may be bitter compounds in wine beyond the reported polyphenols (Carrai et al. 2017).

This paper reports on a small feasibility study evaluating the ability to analyse wine samples directly using the receptomics approach.

Results and discussion

A white and a red wine were chosen for the first analysis with the microfluidic taste receptor array. Gewürztraminer (Vin d'Alsace, Paul Mittnacht, 2016) was chosen as the white wine because it was expected that it might exhibit some bitter notes that could be picked up by the analysis. A South Australian Shiraz (Kavel Estate, Pastor's Promise, labelled by retailers in the Netherlands) was chosen as the red wine because it is a heavy-bodied wine rich in polyphenols with the potential to contain bitter compounds. Neither wine was evaluated by a tasting panel prior to experimentation. Experiments were set up to analyse bitter responses on a single array, with samples injected in a series with increasing concentrations (16×, 8×, 4×, 2×, 0× dilution). Receptor arrays on slides were used that contained 10 replica spots for 24 out of 25 known different bitter receptors and several genetic variants, plus mock-transfected and calcium insensitive (YC-) controls. As ethanol is incompatible with cell assays at concentrations above 0.3%, the alcohol was removed by a speedvac method (the sample was centrifuged for 45 minutes at low temperature and under vacuum, resulting in complete evaporation of the ethanol) until the volume had been reduced by about 35%. Distilled water was then added to the alcohol-reduced sample to restore the initial volume,

and the stepwise dilution series was prepared. Typically, four reference samples were injected prior to the experimental samples (300 µL each of buffer blank, 5 µM ATP, 2 mM pure bitter D-salicin, 1 µM fluorescein) to characterise the receptor array quality, determine host cell response to ATP and perform background fluorescence corrections by computational approaches. The first analysis (not shown) failed at the 4× and lower dilutions because the acidity (pH 4) of both wines strongly affected the signal of the fluorophore, leading to artefacts. The analysis was then repeated with wine that had been first titrated to pH 7 with 40–50 mM NaOH.

The raw taste receptors' responses to red wine measured via a calcium probe are shown in Figure 1. The Twitch-2B calcium probe is a genetically encoded protein consisting of a cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) connected by a calcium binding domain. Calcium binding induces a conformational change that brings CFP and YFP closer together, resulting in an increase in the transmission of energy from CFP to YFP. To observe transmission of energy, only CFP is excited, and the fluorescent emission light is separately monitored at wavelengths specific for CFP and YFP. In a typical experiment, the ratio of YFP/CFP is taken to report the calcium concentration in the cell cytoplasm. The ATP injection (injections 2 and 10) shows the normal pattern expected in the absence of sample autofluorescence: the calcium insensitive probe (YC-, green line in Figure 1) shows no response with either CFP or YFP, and the mock control (black line in Figure 1) shows a reduction in CFP fluorescence and a peak in YFP fluorescence. The ratio of YFP/CFP shows up as a peak for the ATP injections. However, with wine it is obvious that the YC-probe shows positive signals in both the CFP and YFP channels, which will superimpose any signal that is obtained by the calcium-sensitive probe. To eliminate this autofluorescence signal a computational normalisation method is applied (Figure 1, autofluorescence corrected panel). As a result, the fluorescein signal (injection 4) is now no longer visible and the peaks of the YC-probe have mostly disappeared, except those in the non-diluted wine sample where there is still a small signal visible after autofluorescence correction (injection 9) due to limitations of the current measurement set-up. For this reason, the undiluted data were not used further in the data analysis. Looking at the corrected data, both the mock and the TAS2R16 receptor shown in this example respond to the diluted wine samples with a reduction in corrected data in calcium-related fluorescence. These dips in the autofluorescence corrected data increase with the concentration of the wine in the samples (except for the undiluted sample where the mock shows a peak inside the broader dip, which is probably due to a failed correction of the autofluorescence). In general, the shape of the dips is different from the shape of the peaks for D-salicin, which would appear to indicate that the process causing the lowering of calcium levels in these cells may not be mediated by GPCR recep-

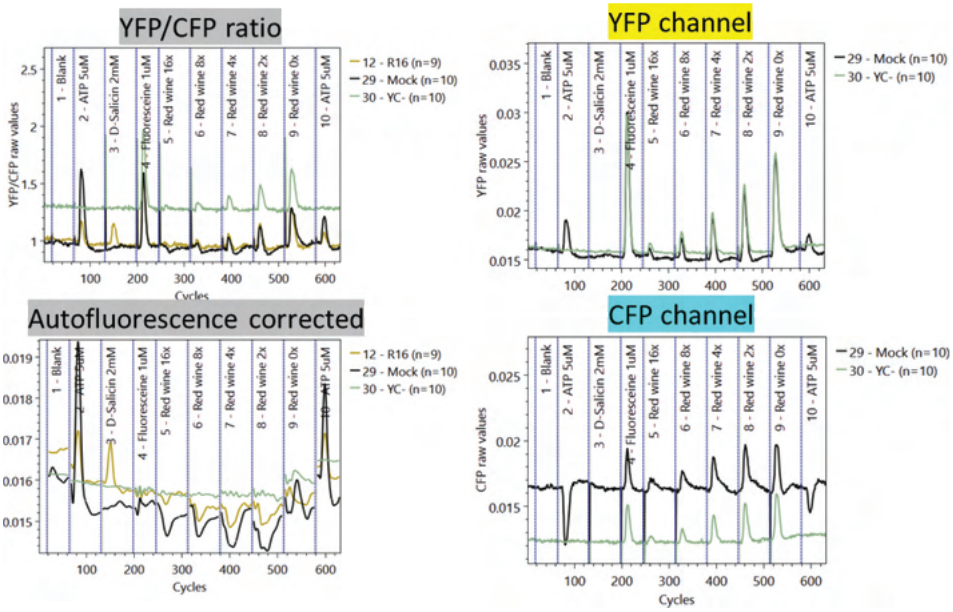


Figure 1. Correction of autofluorescence signals in samples of red wine. A dilution series of red wine was analysed as shown. The x-axis represents the time and each injection takes about five minutes. Shown are the responses of bitter receptor TAS2R16, the mock (receptor-free control) and YC- (a sensor control that is not responsive to calcium and indicates autofluorescence). The right two panels show the signals on the separate CFP and YFP channel wavelengths. It is clear from the YC- signal in the wine samples in the panels on the right that there is strong autofluorescence in the wine samples. As a result, the FRET (Fluorescence Resonance Energy Transfer) ratio signal of YFP/CFP (top left panel) shows a YC- signal as well, which is eliminated in the lower left panel by a computational normalisation. After the autofluorescence normalisation, the mock and TAS2R16 show negative peaks or dips representing a decrease in intracellular calcium levels.

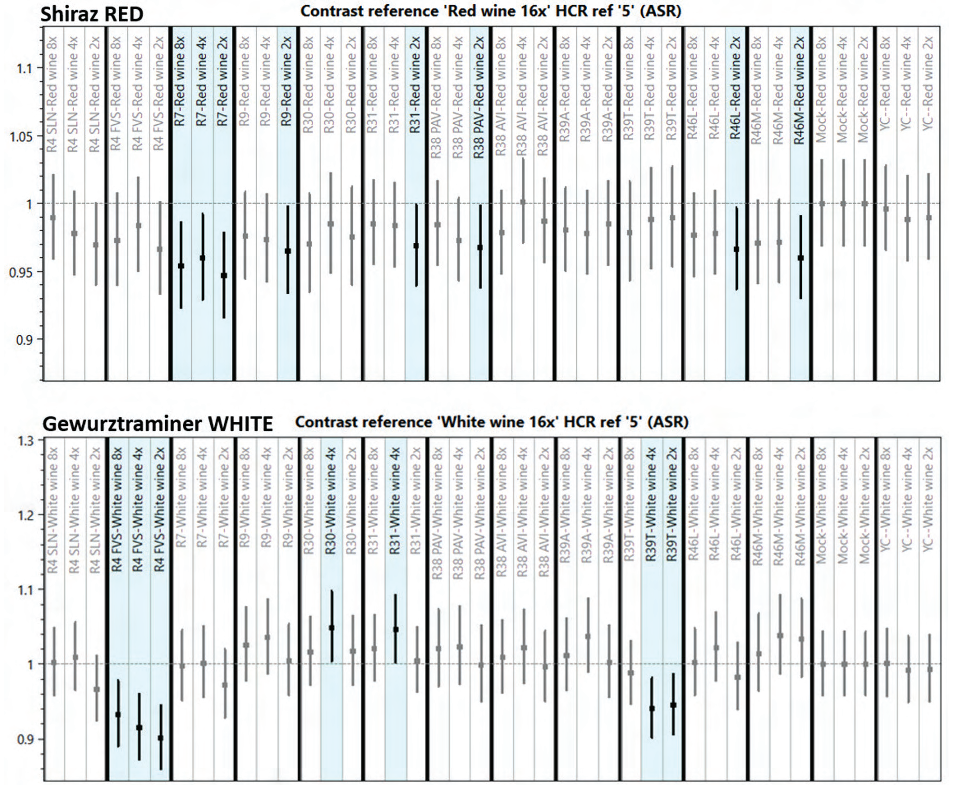


Figure 2. Contrasts of 2-, 4-, and 8-times diluted Shiraz and Gewürztraminer wines with 16x diluted wine. A value smaller than one indicates a calcium dip and a value larger than one indicates a calcium peak. Shown are signals from responsive TAS2R receptors that give a significant result. For TASR4, -38, -39 and -46, two genetic variants are shown giving distinct responses for all except TAS46L/M. TASR38-PAV was found in genetic association studies to be important for differences in bitterness of wines. TASR7 is sensitive to malvidin-3-glucoside at concentrations found in wine.

tors but by some other interaction. Direct comparison of the mock to the receptor-specific signals is complicated by the presence of these dips because the expression of a receptor generally affects the calcium responsiveness and concomitant signal in unpredictable ways, preventing a simple subtraction of the mock response. To solve this, a computational correction is generally applied, based on the signals obtained from the injection of ATP. ATP generates an increase in intracellular calcium leading to a response signal peak, and with quite a few other samples the correlation with ATP tends to be as good as the correlation between two dilutions of a sample if not too many receptors are activated by the sample (R^2 ranging 0.6–0.9). In the case of the wine samples, the correlation of signals from 16× diluted wine with ATP was very low (R^2~0.2, not shown) and disappeared completely for more concentrated wine samples, suggesting that ATP does not properly mimic the generic response of the cells to wine samples. A different approach was therefore employed, contrasting the wine samples with 2- to 8-fold dilution to the 16-fold dilution. In this case, the best correlation was ~0.62. This approach also worked well in the analysis of beer as an alternative to ATP-based normalisation, although the method is slightly less sensitive due to weak activation of bitter receptors in the most diluted sample.

Next, statistical analysis of the data was carried out with a script that automatically identifies the sample start and peak/dip height and takes the ratio of both values as described by Wehrens et al. (2019). Typically, this ratio is then compared to the blank or to another sample (of lower dilution) to obtain a relative value for the signal. On average, each receptor spot was represented around 10 times on the chip, which allowed a statistical analysis for the response of each receptor. Figure 2 shows the results obtained for the analysis applying the host cell correction based on a contrast of 16-times diluted wine to 2-, 4-, 8-times diluted wine. Unless the bitter compound is already saturating the bitter receptor at 16× dilution, this allows the visualisation of an increasing bitter response at lower dilutions. Surprisingly, in the tested Shiraz wine only significant reductions of the calcium concentrations were observed, not the expected increases. The previously described most relevant receptor TAS2R7 for red wine (Soares et al. 2013) is yielding the strongest reductions in calcium signal relative to a higher dilution. A significant negative (decreased calcium) signal from the TAS2R38-PAV variant is also seen; a receptor that was shown to be genetically correlated to the perceived bitterness of red wine (Carrai et al. 2017). Similar puzzling results were observed in Gewürztraminer where especially TAS2R39 gave a significant negative signal. TAS2R39 is known to react to certain polyphenols found in tea but not in wine. This suggests that wine might contain bitter compounds that have not yet been identified.

Conclusion

In conclusion, the results to date demonstrate that it is possible to obtain sample-specific signals from taste receptor assays using not only pure compounds in water, but also wine as a complex sample matrix. However, additional research and development are required before it can be concluded that the receptomics method is suitable for direct wine analysis and as a proxy for sensory analysis. Specifically, the following will be required:

(i) Isolation and identification of the factor which causes the unexpected reduction in the calcium-dependent signal (dip). This would also allow replacement of the 16× dilution as the control injection and separation of the specific response from the generic response.

(ii) Reconstitution experiments with additions of known bitter compounds to non-bitter wines and buffer solutions, preferably at concentrations typically found in wine, are necessary to show that bitter-specific signals that are visible in buffer samples can also be recorded in the wine matrix. Once this has been demonstrated, known bitter wines can be tested to evaluate the method with real samples and the receptor arrays can be cross-validated with data from traditional human sensory experiments.

(iii) Vidal et al. (2003, 2004), Lea and Arnold (1978) and Peleg et al. (1999) suggest that with an increasing degree of polymerization polyphenols appear to be perceived as less bitter but increase their astringency. It would be interesting to test receptor activation by monomers such as malvidin-3-glucoside or catechin and polymers of these molecules (both separate and mixed) to see their interaction at the receptor level. Possibly, polymerized polyphenols that are known to bind to proteins can directly bind to G protein docking sites or modify their confirmation through crosslinking or other non-specific surface modifications. Consequently, they may disable low-level spontaneous interaction of the gustducin G protein with the receptor causing the observed dips. Conceivably this effect would be the strongest with receptors that can accommodate the compounds best.

(iv) The neutralisation of the wine strongly alters its colour and possibly its bitterness or bitterness perception. As a solution, alternative pH-insensitive sensors of receptor activation should be evaluated.

References

Brossaud, F.; Cheynier, V.; Noble, A.C. (2001) Bitterness and astringency of grape and wine polyphenols. Aust. J. Grape Wine Res. 7: 33–39.

Carrai, M.; Campa, D.; Vodicka, P.; Flamini, R.; Martelli, I.; Slyskova, J.; Jiraskova, K.; Rejhova, A.; Vodenkova, S.; Canzian, F.; Bertelli, A.; Dalla Vedova, A.; Bavaresco, L.; Vodickova, L.; Barale, R. (2017) Association between taste receptor (TAS) genes and the perception of wine characteristics. Sci. Rep. 7: 9239.

Henquet, M.G.L.; Roelse, M.; de Vos, R.C.H.; Schipper, A.; Polder, G.; de Ruijter, N.C.A.; Hall, R.D.; Jongsma, M.A. (2016) Metabolomics meets functional assays: coupling LC-MS and microfluidic cell-based receptor-ligand analyses. Metabolomics 12: 115.

Lea, A.G.H.; Arnold, G.M. (1978) The phenolics of ciders: Bitterness and astringency. J. Sci. Food Agric. 29: 478–483.

Peleg, H.; Gacon, K.; Schlich, P.; Noble, A.C. (1999) Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. J. Sci. Food Agric. 79: 1123–1128.

Roelse, M.; de Ruijter, N.C.; Vrouwe, E.X.; Jongsma, M.A. (2013) A generic microfluidic biosensor of G protein-coupled receptor activation - monitoring cytoplasmic [Ca^{2+}] changes in human HEK293 cells. Biosens. Bioelectron. 47: 436–444.

Roelse, M.; Henquet, M.G.L.; Verhoeven, H.A.; De Ruijter, N.C.A.; Wehrens, R.; Van Lenthe, M.S.; Witkamp, R.F.; Hall, R.D.; Jongsma, M.A. (2018) Calcium imaging of GPCR activation using arrays of reverse transfected HEK293 cells in a microfluidic system. Sensors 18: 602.

Roelse, M.; Wehrens, R.; Henquet, M.G.L.; Witkamp, R.F.; Hall, R.D.; Jongsma, M.A. (2019) The effect of calcium buffering and calcium sensor type on the sensitivity of an array-based bitter receptor screening assay. Chem. Senses 44(7): 497–505.

Soares, S.; Brandão, E.; Mateus, N.; de Freitas, V. (2016) Sensorial properties of red wine polyphenols: Astringency and bitterness. Crit. Rev. Food Sci. Nutr. 57(5): 937–948.

Soares, S.; Kohl, S.; Thalmann, S.; Mateus, N.; Meyerhof, W. de Freitas, V. (2013) Different phenolic compounds activate distinct human bitter taste receptors. J. Agric. Food Chem. 61(7): 1525–1533.

Soares, S.; Silva, M.S.; García-Estevez, I.; Groβmann, P.; Brás, N.; Brandão, E.; Mateus, N.; de Freitas, V.; Behrens, M.; Meyerhof, W. (2018) Human Bitter Taste Receptors are Activated by Different Classes of Polyphenols. J. Agric. Food Chem. 66(33): 8814–8823.

Vidal, S.; Francis, L.; Guyot, S.; Marnet, N.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E.J. (2003) The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J. Sci. Food Agric. 83: 564–573.

Vidal, S.; Francis, L.; Williams, P.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. (2004) The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. Food Chem. 85(4): 519–525.

Wehrens, R.; Roelse, M.; Henquet, M.G.L.; van Lenthe, M.; Goedhart, P.W.; Jongsma, M.A. (2019) Statistical models discriminating between samples measured with microfluidic receptor cell arrays. PLoS One 14(4): e0214878.

AgTech in horticulture: opportunities from a practitioner's perspective

M. Fealy

Nuffield Scholar, Blue Sky Produce, Mareeba, Qld
matt@blueskyproduce.com

Abstract

Robots, automation and blockchain are promising to revolutionise farming forever, but why are farmers only reading about these technologies, rather than implementing them? The key objective of this presentation is to deliver a practical and realistic 'farmer first' overview of technology. Technology is bringing change at a pace that is difficult to keep up with. Drawing on visits to America, Europe, Asia, the Middle East and the Netherlands where I shared a coffee, a beer and a burrito with some of the most innovative farmers in the world, my research provides a practical breakdown of technology that can make a difference to farming practices today, particularly for horticulture and orchard production. My presentation canvases numerous risks to agriculture, such as the increasing unpopularity of temporary worker schemes, rising production costs, urbanisation and food safety and regulatory demands on traceability. I then explore currently available technology designed to address these challenges. AgTech has the potential to become a future pillar of rural economic development. As adoption of these technologies increases, so will the need for research and development, sales, service and support, resulting in the creation of new jobs for rural communities. As the world continues to rapidly change and farm productivity growth has all but stagnated, farmers must look outside the box for new innovations, from new industries, for solutions. It will take brave innovators across all sectors of production, industry and government to lead change and assist in the adoption of these technologies for the advancement of Australian agriculture.

This work has been published in a report by Matthew Fealy for Nuffield Australia Farming Scholars:

Fealy, M. (2017) Robotics, automation and emerging technologies for the future of Australian horticulture. Nuffield Australia Project No 1719: https://nuffieldinternational.org/live/Reports

Digital opportunities: from the vineyard to the winery door

E.J. Edwards[1], M.R. Thomas[1], S. Gensemer[2], P. Moghadam[3], T. Lowe[3], D. Wang[4], R. Lagerstrom[4], C. Hargrave[5], J. Ralston[5]

[1]CSIRO Agriculture and Food, Urrbrae, SA
[2]CSIRO Manufacturing, Urrbrae, SA
[3]CSIRO Data61, Pullenvale, Qld
[4]CSIRO Data61, Marsfield, NSW
[5]CSIRO Mineral Resources, Pullenvale, Qld
everard.edwards@csiro.au

Abstract

New and developing technologies, which provide sensors and the software systems for using and interpreting them, are becoming pervasive through our lives and society. This technological revolution has the potential to monitor all aspects of vineyard activity, assisting growers to make the management choices they need to achieve the outcomes they want. For example, a future vineyard may possess automated imaging that generates a three-dimensional model of the vine canopy, highlighting differences from the desired structure and how to use canopy management to improve fruit composition. Or, the imaging may generate maps with yield estimates and measurements of berry composition throughout the growing season. Further, that same imaging may also provide whole-of-vineyard data on vine nutrition or early warning of disease, allowing proactive management on a rapid time-scale. Sensors currently being trialled in vineyards include colour imaging (both still and video), hyperspectral imaging (the use of many spectral bands, typically in the near infra-red), LiDAR (3D laser scanning) and foliage penetrating (FOPEN) technologies. These can potentially be deployed proximally (from a vehicle) or remotely (from a drone or aircraft). The data from these sensors can be analysed using a wide range of traditional or novel techniques, such as machine learning. The outputs of different sensor technologies can even be combined and analysed as one dataset. For example, videos can be analysed as a moving image, split into individual frames or used to generate 3D imagery using photogrammetry or stereo imaging. Perhaps the biggest challenge in developing new digital tools, however, is to provide a demonstrably useful outcome for the grower at a commercially viable price.

Introduction

The technological revolution that we are currently living through, based on cheap and easily available computing and communications, has impacted the lives of much of the world's population and the operations of most of the world's businesses. As computing platforms have matured there has been a new focus on the development of sensors and the software systems for using them. From smartphones to cars to farm machinery, all increasingly contain a range of sensors that are monitored automatically, with intelligent software providing us with the information we want, when we want it.

These technological developments have greatly increased our ability to collect data from vineyards and wineries and to collate those data at the enterprise level. The resulting information can be used to improve the logistics of the business, from allocating fermenters to allocating marketing; to improve the management of inputs, such as labour, water, chemicals and fertiliser; and to enhance the industry's capacity to achieve desired fruit composition. Minimising the cost of production and maximising the value of the fruit, whether by more consistently meeting required specifications or by reaching higher specifications, will improve vineyard profitability and underpin the economic sustainability of the wine industry.

Sensor systems have the potential to monitor all aspects of vineyard activity. For example, a future vineyard may possess automated imaging that generates a three-dimensional model of the vine, highlighting the position of bunches and tracking their development through the season, or highlighting differences in the canopy between actual and desired structure. That same imaging may provide whole-of-vineyard data on fruit composition, vine nutrition or early warning of mildew infection, allowing proactive management on a rapid time-scale.

While the prospect of digital viticulture for improving vineyard productivity and efficiency has been explored for over a decade, the reality is that many of the requisite digital technologies have still not reached the level of maturity that is needed to enable broad industry-wide adoption. As a result, there is a clear gap in technology readiness as well as industry awareness to realise the benefits of digital viticulture in production vineyard operations.

The motivation, however, to pursue ongoing developments remains clear. Information on a whole-block scale will provide a step change in the accuracy of crop monitoring, but requires 'on-the-go' systems. Advances in computing power, whether local or in the cloud, computer vision and robotics provide the potential to deliver such capability. This combination of image processing, computer vision and machine learning techniques is becoming widely available and is already being used in management tools in some areas of agriculture (Kamilaris and Prenafeta-Boldú 2018).

Developing such systems requires a significant investment of effort, but more importantly, such development must be built on a solid knowledge base. The work described in this paper aims to develop solutions for key areas of viticulture, ranging from yield estimation to canopy structure assessment to non-destructive measurements of fruit composition and vine nutrient status.

Yield prediction and estimation

Improved yield estimation is a key target for many agricultural industries, including the wine industry. Wine companies desire accurate yield estimates for everything from logistical planning to crop management to marketing. Currently, yield estimation is typically based on hand counts on a small number of vines, or simply visual estimates by experienced staff, combined with historical information on yield for a particular block. On-the-go digital tools provide the opportunity to reduce the manual labour involved in this and also to vastly increase the proportion of a block that is used for yield estimation, thereby improving accuracy.

Early yield prediction

Grapevine yield comprises three factors: vines per block, bunch number per vine and bunch weight. Bunch weight is made up of berry number per bunch and average berry weight, giving a total of four primary factors, of which vine number will not normally vary. Bunch number is usually considered to be the largest single driver of season-to-season yield variation in Australian viticulture (Clingeleffer et al. 1997). Furthermore, where significant thinning of productive shoots, inflorescences or bunches does not occur, inflorescence number will

determine bunch number and can, therefore, be used to predict yield shortly after budburst, in combination with historical data on bunch weight. Even where thinning does occur, inflorescence count has value in ensuring the extent of thinning is matched to yield potential for a given season.

A range of sensors could potentially be used to enumerate inflorescences in the vineyard, but given the largely two-dimensional shape of the vine early in the season in most Australian vineyards and the low cost of RGB (colour) video sensors, we chose to use basic consumer action cameras (Go-Pro Hero 5/6/7, JB Hi-Fi, SA) mounted on a standard vineyard vehicle (Kubota RTV 500, Kubpower, SA) to record continuous videos of entire vine rows. We then developed an analysis pipeline using computer vision and machine learning techniques, which detects inflorescences in each frame and tracks them through the video to count them. The videos were recorded between E-L stages 12 and 14 (Coombe 1995) when inflorescences were clearly separated from the developing shoot tip, but occlusion by leaves was minimal. For each row, the number of inflorescences was counted by hand by two people operating separately. Where their estimates varied by more than 5%, a third count was also made. The detector was developed using a deep learning approach, including a convolutional neural network (CNN) trained using a set of hand-labelled images. Approximately 300 images, taken from videos recorded in three different vineyard blocks, were hand-labelled by drawing a rectangle over each visible inflorescence. This resulted in several thousand individually labelled inflorescences. The trained detector was then able to output each frame from each video with all detected inflorescences labelled. An example is given in Figure 1.

Following the detection of inflorescences in each frame, tracking through multiple frames was implemented using a second machine learning component, based on Siamese Net (Leal-Taixé et al. 2016). The tracking is required to prevent individual inflorescences being counted many times, as each normally occurs in many video frames.

Figure 1. An example of a hand-labelled video frame (top) and the same frame with the output from the inflorescence detector (bottom)

This also assists in eliminating false positives, as detected inflorescences that appear in only a very small number of frames cannot be tracked and thus are not counted. Typically, such detections are not of real inflorescences. Figure 2 provides a video screenshot with the tracking used to provide a count.

Yield prediction post fruit set

The same process can be used to detect grape bunches. Detectors were developed, as for the inflorescences, for pre-veraison (Figure 3), partial veraison (mixed green and red fruit) and post-veraison fruit (Figure 4). While there is value in estimating bunch number as the bunch develops, particularly where thinning of some sort has occurred after E-L 14 (time of inflorescence imaging), estimating the size of the bunch from the same images can be used to improve yield predictions. In fact, prototype digital tools have already been developed to do the latter step (Diago et al. 2014; Liu et al. 2018). However, these have mostly been developed using single images of exposed bunches and there are a number of difficulties in doing this for an on-the-go system. Firstly, the greater canopy development, compared with the time of inflorescence imaging, means that partial or significant occlusion of bunches is common. Secondly, the structure of the vine has much greater width at this time of the season, at least for training systems other than very highly managed VSP, resulting in bunches that are more spread out on the x plane (i.e. between the cordon and the mid-row/camera). As a result, the apparent size of a bunch in a video frame is determined not only by the actual bunch size, but also by the distance from the camera.

To assist with the first problem, the hand labelling of bunches in the video frames included a classification (full bunch, partial bunch, few berries), which could then be used in the output results to direct further steps in a yield prediction pipeline (Figures 3, 4). To assist with the second problem, multiple means for determining distance from video images are currently being assessed and will be reported at a later date.

Pre-harvest yield estimation

The problem of occlusion of fruit by the canopy could be avoided by using a technology that can 'see' through leaves. The use of radar to penetrate foliage and detect objects of interest (FOPEN) is widespread for both military surveillance and civilian mapping applications (Davis 2011). We have explored the potential use of a similar technology to quantify fruit volume in the vineyard using a vehicle mounted on-the-go sensor. Computational electromagnetic modelling using model fruit, leaf and cane properties was undertaken and indicated a frequency region of interest for practical application of radar (data not shown). This was followed by the development of a laboratory test rig (Figure 5 upper), where hardware could be trialled under known conditions. Testing using this rig indicated that just a few berries could be detected by the chosen sensor behind a small

Figure 2. Screenshot of video with detector and tracking algorithms producing an incrementing inflorescence count

Figure 3. An example of a hand-labelled video frame (top) and the same frame with the output from the pre-veraison detector (bottom)

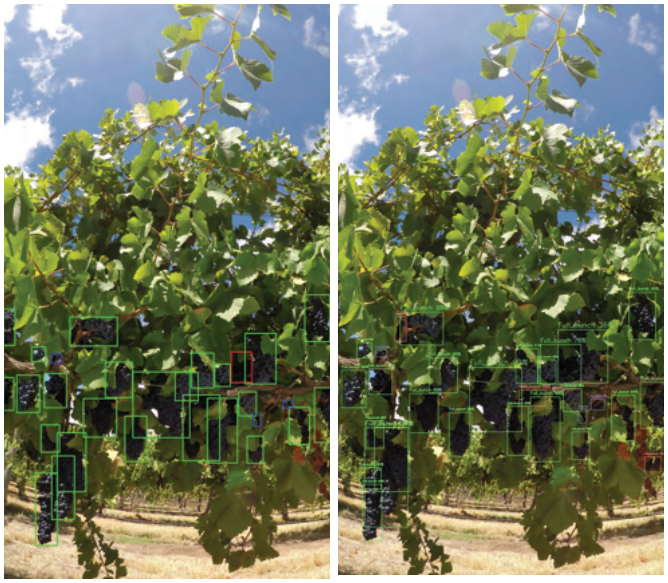


Figure 4. An example of a hand-labelled video frame (left) and the same frame with the output from the post-veraison detector (right)

amount of foliage at a distance of over 0.5 m (Figure 5 lower).

Initial field trials were undertaken with the sensor mounted on a utility terrain vehicle and driven down a typical row in a commercial vineyard. Canopy and fruit were removed in stages between runs and the results demonstrated significant changes in the size and intensity of radar returns. Figure 6 provides an example of single returns from an intact section of row following canopy removal and following fruit removal. The results clearly demonstrate the potential for this system to be used prior to harvest for yield estimation. It is expected that the system could be used any time after the berry water content has peaked following veraison.

Canopy size and structure

Canopy management plays a key role in viticulture, both to optimise the balance between fruit mass and photosynthetic capacity (Edwards et al. 2017) and to provide the appropriate microclimate to maximise fruit quality (e.g. Smart 1985). Management occurs through choice of trellis system, rootstock, pruning regime, irrigation management and within-season leaf removal. Irrespective of management type, we are lacking reliable, objective measures of canopy structure, often strongly relying on the personal experience of the vineyard manager; and where leaf removal has been done we are also committing to a significant labour cost.

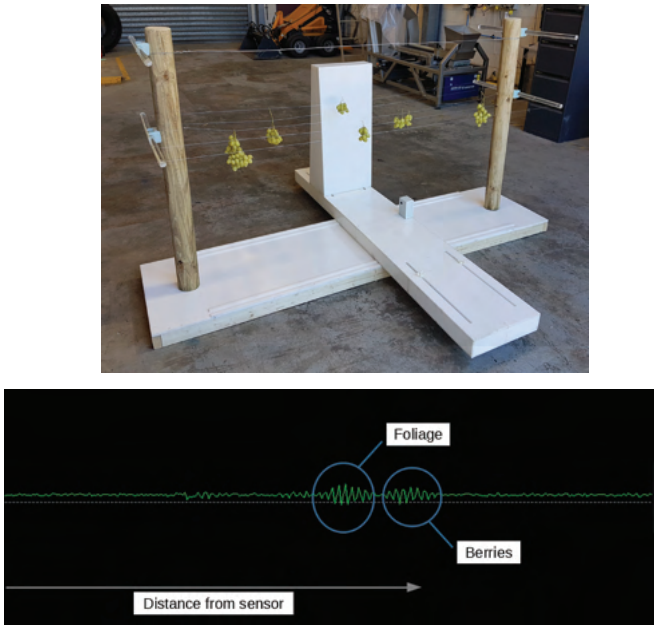


Figure 5. Laboratory-based radar test rig (upper) and sample signal return showing berry target obscured by leaves (lower)

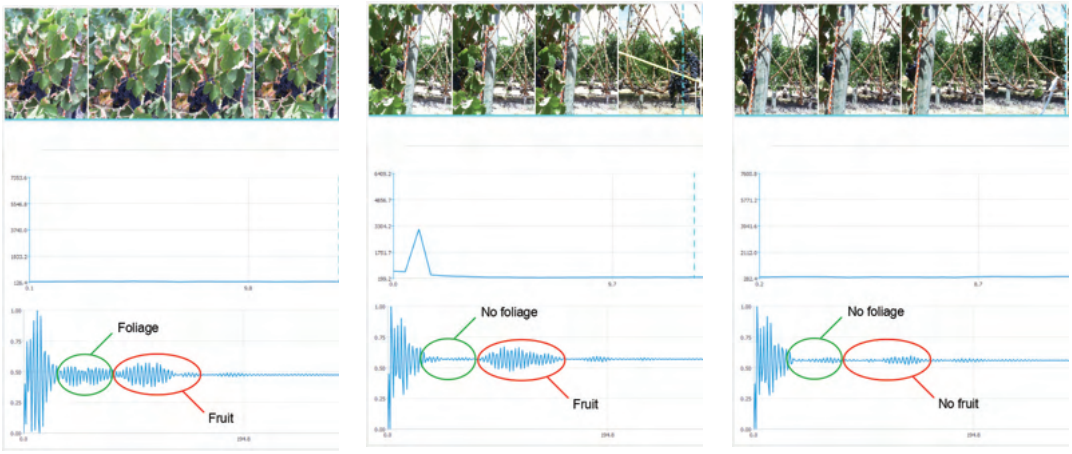


Figure 6. Demonstration of the use of radar to detect fruit embedded within a grapevine canopy. Single return signal from intact vine (left), vine with the leaves removed (centre) and a vine with both leaves and canopy removed (right)

Tools for assessing canopy size and structure are largely limited to research use, due to their cost and complexity. However, using modern technologies to put effective tools into the hands of the grower will allow canopy management to be undertaken in a far more objective manner and enable viticulturists to develop management techniques that are better able to optimise fruit microclimate. Such tools also offer the prospect of on-the-go optimisation of automated equipment, such as leaf plucking machines. One example of an existing tool is the VitiCanopy app (De Bei et al. 2016), which uses a smartphone camera to estimate leaf area index (LAI); however, this tool is static in nature and provides limited structural information.

Using drones

Drones have the advantage of quickly covering a large area of ground, but for a row crop the resulting flat orthomosaic image can make it difficult to differentiate between canopy and ground, particularly where a live cover crop is in place. However, where drone flights have a large amount of overlap between adjacent images, each part of the scene is viewed from many slightly different angles. This can be used to generate 3D data for the vineyard through photogrammetry (also known as 'structure from motion'). Figure 7 (top) provides an example image of a 3D point cloud of a section of vineyard generated from drone video imagery, where the separation of canopy and ground are clearly visible. We have used this to build a preliminary pipeline to map vineyard canopy cover using a standard consumer drone. This uses OpenDroneMap (https://www.opendronemap.org) to build a point cloud from drone data, a statistical analysis of the point cloud (Figure 7 bottom) to separate canopy from ground and then a number of steps to recognise the rows and output a percentage canopy cover per metre of row (Figure 8).

2D proximal imaging

Photogrammetry is computationally intensive and requires a high-performance computing facility to run in a reasonable timeframe. Furthermore, the use of drones—even small consumer drones—for commercial purposes is quite highly regulated. The VitiCanopy app (see above) uses a gap-fraction analysis originally developed for forestry (Macfarlane et al. 2007) which can be calculated using far less computing power. At present this is not on-the-go and requires significant labour to collect a dense dataset for a vineyard block. By mounting a consumer action-camera on a vineyard vehicle and viewing up through the canopy from below it is possible to use the same mathematics to determine a continuous leaf area index (LAI) down a vineyard row (Figure 9). This can then be averaged along a part row (e.g. per metre or per panel) to generate a map for the vineyard block (data not shown).

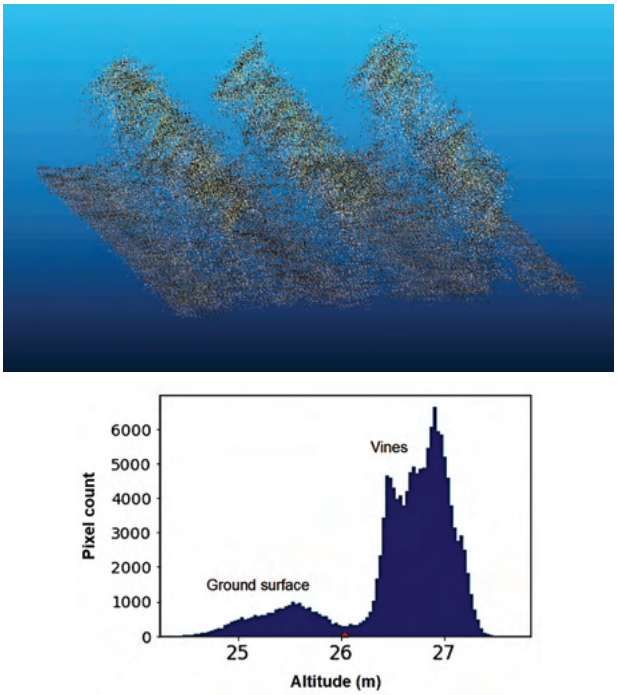


Figure 7. 3D point cloud for part of a vineyard (top) and a histogram of the pixels per height above sea level labelled for ground and canopy (bottom)

LiDAR

An active sensor (one not reliant on an external source of radiation) could examine the canopy from any angle, rather than from above (reflected sunlight) or below (transmitted sunlight), and could provide detailed structural information, such as fruiting zone porosity. This would offer the potential to develop more detailed and objective canopy management strategies, currently difficult to assess. One such sensor is LiDAR (light detection and ranging). In fact, trawling LiDAR systems have already been developed as a phenotyping tool for grapevines (Siebers et al. 2018) and for automating adjustment of variable rate sprayers (Llorens et al. 2011).

We have used a LiDAR that spins at a rate of 0.5 Hz (Figure 10 top), which creates a variation in observation angle due to the spin and the movement along the vine row. This allows greater penetration of the laser into the canopy and, therefore, improved observation of the canopy structure. Unlike existing methods, we also use a SLAM algorithm to globally register the LiDAR data into a self-consistent map, which is geolocated and aligned using GPS. This provides not simply a point cloud, but a ray cloud, where the direction of every laser pulse is recorded as well as the position of any return from both sides of a row. The system allows an entire vineyard block to be mapped in 3D in a single run (Figure 10 bottom).

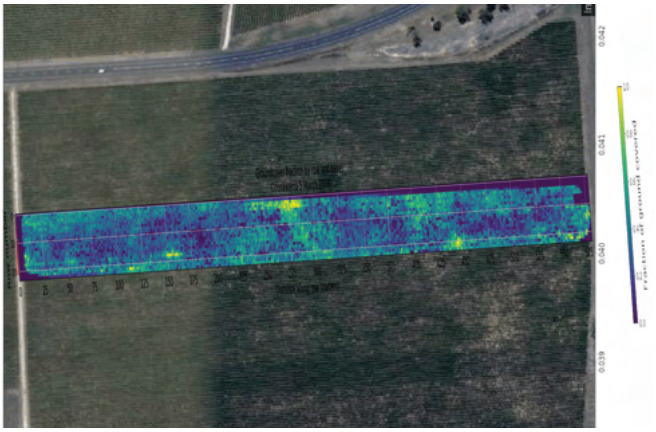


Figure 8. Map of canopy cover per metre of row overlaid over an aerial image of the vineyard

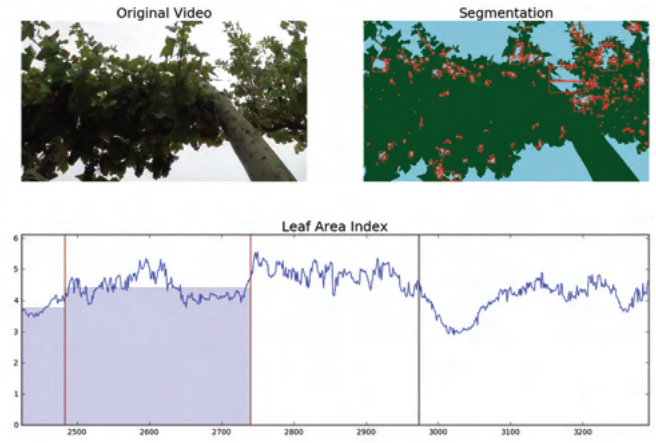


Figure 9. Original video still (top left), thresholded image with gaps marked (top right) and continuous leaf area index, average per panel (bottom)

Although the 3D imagery (point cloud) is potentially useful in its own right, by using the ray cloud more information is available to develop novel indices that can be used to reproducibly quantify various aspects of the vineyard. We have developed a canopy density index which is calculated on a per 12 cm cube (voxel) basis for the entire vineyard block (Figure 11). These values are still on a three-dimensional basis and can then be collapsed into two dimensions as required to give plan, side or end views of the vineyard (Figure 12). It is anticipated that such data will allow greater flexibility in canopy management as results can be more easily related to application. Furthermore, implementation of canopy management can be more easily mapped and controlled than previously.

Fruit and leaf composition

At present fruit maturation is most commonly tracked by taking a small number of field samples and measuring the total soluble solid content of the juice using a refractometer as an estimate of sugar content. Other components of fruit composition, during maturation or at harvest, are usually measured in the winery laboratory using some form of absorbance spectroscopy (e.g. ultraviolet-visible or Fourier transform infrared [FTIR]). Red-green-blue (RGB) imaging, such as described above, can provide some colour information, but is unable to be used to provide detailed compositional information, whether of the fruit or of the canopy. Absorbance spectroscopy is impractical in the field as an on-the-go solution, due to the need to have a light source and sensor on different sides of the tissue to be analysed. However, reflectance spectroscopy also provides information on chemical composition and can be used on-the-go in the field with a suitable sensor, such as a hyperspectral line scanner (Fernández-Novales et al. 2019). This produces an image, where each pixel of the image does not simply contain RGB values (three colours), but can contain hundreds of different wavebands, providing a spectrum that can be used to identify and quantify chemical components. This image is often referred to as a data cube.

Determining the concentration of a chemical component of a tissue, such as sugar in fruit or nutrients in leaves, from a hyperspectral data cube, requires identifying the pixels of interest and then using a calibration for that chemical component. The pixel identification can be done using traditional or machine learning forms of image analysis, but we can also use the spectral information itself. To test the potential of this for vineyard-derived data we imaged several hundred samples of non-lignified canes, inflorescences, leaves, shoot apices and tendrils using a laboratory-based hyperspectral camera (Headwall Micro e-VNIR, PAS, Gosford, NSW) and ran a variety of statistical tools, including machine learning techniques, to generate classifiers for these five types of green tissue. An estimate of the accuracy of these is provided in Table 1. Classifiers for green and red berries were also developed (not shown). The classifiers were subse-

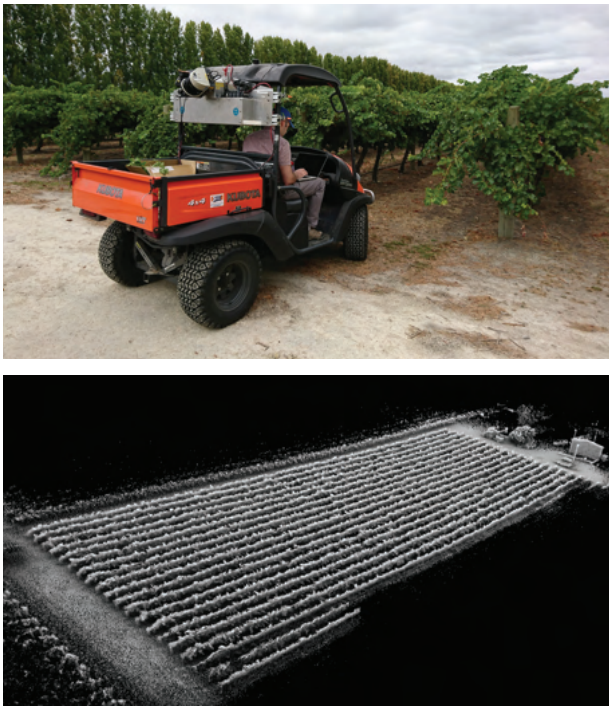


Figure 10. Photograph of the prototype spinning LiDAR system (AgScan3D+) in use (top) and 3D point cloud of a vineyard block in McLaren Vale, SA (bottom)

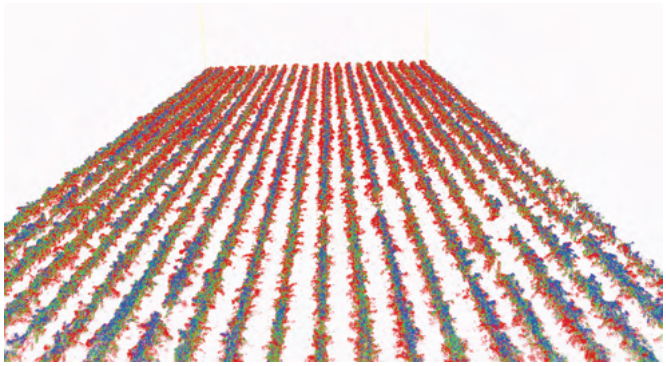


Figure 11. Colour-coded point cloud, with colour representing canopy density index for the McLaren Vale vineyard in Figure 10

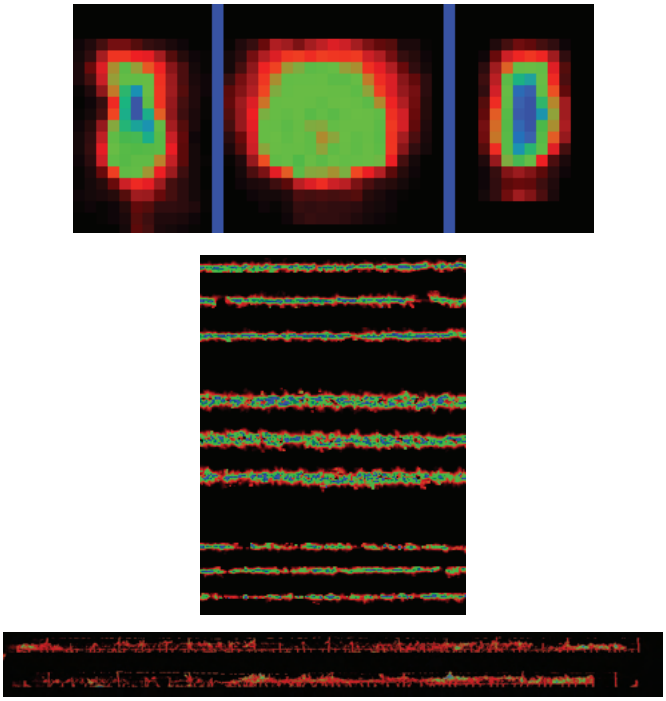


Figure 12. Two-dimensional views of canopy density derived from the three-dimensional data by averaging through the third dimension. Single rows from three vineyards to provide density variation as a cross-section (top), groups of three rows from three vineyards to provide density variation in a plan view (centre) and side view of two rows at leaf-fall to provide density variation with row height (bottom)

Table 1. Accuracy of tissue classification algorithm based on hyperspectral imaging. Rows provide the number of predictions per tissue type, where columns indicate the actual tissue type imaged

Predicted identity	Green cane	Inflorescence	Leaf	Shoot tip	Tendril
Green cane	268	0	0	0	2
Inflorescence	21	2203	0	17	8
Leaf	0	1	432	11	2
Shoot tip	0	0	0	92	0
Tendril	3	10	7	4	962

quently used in hyperspectral data analysis pipelines, whether laboratory or field data.

Prior to field deployment, a laboratory-based analytical tool was developed. This encompassed the hyperspectral-imaging and wet-laboratory analysis (using an OenoFoss, Foss Analytics, Mulgrave, Vic.) of over 3,000 individual berries from a wide range of genetic and environmental backgrounds. Berries used ranged in developmental state from pea-sized to overripe and the juice was analysed for ten different compositional parameters (Table 2). The highest correlation was achieved for juice soluble solids (°Brix) and Figure 13 provides a visual representation of this result.

Calibrations made using individual berry data were then successfully used to analyse whole bunches in the laboratory (data not shown but see Figure 14 for visual representation) and initial on-the-go imaging of small sections in the field (data not shown). The same equipment and approach were used to image and analyse leaf composition, with chlorophyll and a range of common nutrients (including N, P and K) targeted. The calibrations are still being developed, but initial results produced a coefficient of determination (r^2) of 0.80 for nitrogen and 0.95 for chlorophyll.

Table 2. Accuracy of juice composition calibrations for eVNIR hyperspectral data vs wet-laboratory analysis. Results are expressed as the coefficient of determination (r^2) and root mean squared error (RMSE) for each parameter and given for calibrations made using multiple genotypes (n > 3,000) and a single genotype (Sauvignon Blanc, n > 1,000).

Juice parameter	Sauvignon Blanc training	Multi-genotype training
pH	0.96 (0.23)	0.94 (0.33)
Total acid	0.93 (2.20)	0.90 (2.77)
Malic acid	0.94 (3.20)	0.92 (3.39)
Tartaric acid	0.60 (1.23)	0.38 (1.36)
Total soluble solids	0.99 (1.10)	0.98 (1.19)
Volatile acid	0.83 (0.05)	0.79 (0.67)
Yeast assimilable nitrogen	0.53 (89.6)	0.36 (96.3)
Alpha amino nitrogen	0.64 (47.7)	0.40 (61.9)
Gluconic acid	0.86 (0.42)	0.87 (0.45)
Ammonia	0.81 (55.5)	0.76 (60.5)

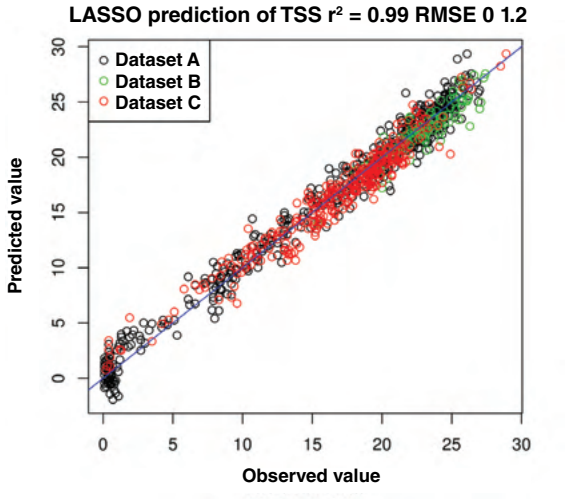


Figure 13. Predicted total soluble solids (TSS) using a calibration developed from hyperspectral imaging data plotted against observed TSS using a commercially available FTIR instrument, n > 3,000

Conclusions

Our work to develop digital tools for viticulture has encompassed a wide range of viticultural targets and a broad range of sensing technologies. Although these are at various states of development, all have demonstrated potential applicability to on-the-go vineyard use. Future work will include developing these tools further and engaging with partners to bring them to Australian viticulturists.

Acknowledgements

This research was supported by funding from the Department of Agriculture, Water and the Environment through their Rural R&D for Profit scheme and Wine Australia. Wine Australia invests in and manages research, development and extension on behalf of Australia's grapegrowers and winemakers and the Australian Government. The authors would also like to acknowledge Accolade Wines, Katnook Estate, Rymill Wine and their staff and Adam Loveys for their assistance and access to their vineyards. Finally, the authors gratefully acknowledge the assistance of Changming Sun and a number of CSIRO technical staff, particularly Don Mackenzie, Adam Smith, Najid Pereira-Ishak, Muhammad Rizwan Khokher, Qiyu Liao, Maoying Qiao and Alex Pitt.

References

Clingeleffer, P.R.; Sommer, K.J.; Krstic, M.P.; Small, G.; Welsh, M. (1997) Winegrape crop prediction and management. Aust. N.Z. Wine Ind. J. 12: 354–359.

Coombe, B.G. (1995) Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages. Aust. J. Grape Wine Res. 1: 104–110.

Davis, M. (2011) Foliage Penetration Radar: Detection and Characterization of Objects Under Trees. Stevenage: The Institution of Engineering and Technology.

De Bei, R.; Fuentes, S.; Gilliham, M.; Tyerman, S.; Edwards, E.; Bianchini, N.; Smith, J.; Collins, C. (2016) VitiCanopy: A Free Computer App to Estimate Canopy Vigor and Porosity for Grapevine. Sensors 16: 585.

Diago, M.P.; Sanz-Garcia, A.; Millan, B.; Blasco, J.; Tardaguila, J. (2014) Assessment of flower number per inflorescence in grapevine by image analysis under field conditions. J. Sci. Food Agric. 94: 1981–1987.

Edwards, E.J.; Smith, J.; Walker, A.; Barril, C.; Boettcher, A.; Foster, D. (2017) Targeted manipulation of vine balance: does vine balance directly affect fruit composition? Beames, K.S.; Robinson, E.M.C.; Dry, P.R.; Johnson, D.L. (eds) Proceedings of the 16th Australian Wine Industry Technical Conference, Adelaide, SA, 24-28 July 2016. Adelaide, SA: Australian Wine Industry Technical Conference Inc.: 96–100.

Fernández-Novales, J.; Tardáguila, J.; Gutiérrez, S.; Diago, M.P. (2019) On-The-Go VIS + SW − NIR Spectroscopy as a Reliable Monitoring Tool for Grape Composition within the Vineyard. Molecules 24: 2795.

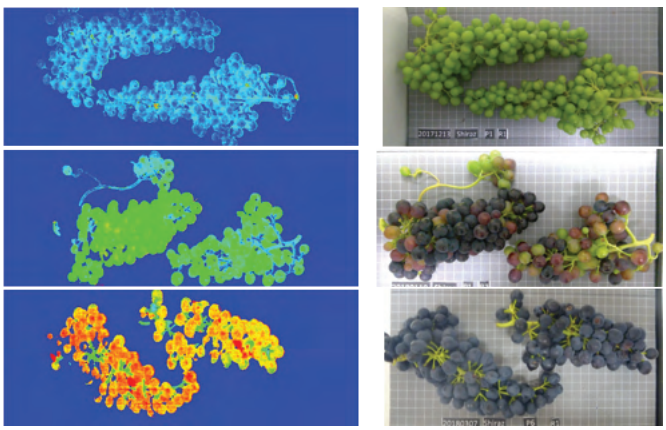


Figure 14. Whole bunch analysis of TSS using hyperspectral imaging. Left panels provide visual estimate of TSS, ranging from low (blue) to high (red); right panels provide colour photographs of the same bunches. Bunch development ranges from pre-veraison (top), to 80% veraison (middle) to maturity (bottom).

Kamilaris, A.; Prenafeta-Boldú, F.X. (2018) Deep learning in agriculture: a survey. Comput. Electron. Agric. 147: 70–90.

Leal-Taixé, L.; Canton-Ferrer, C.; Schindler, K. (2016) Learning by tracking: Siamese CNN for robust target association. 34th Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition Workshops. Las Vegas: IEEE: 33–40.

Liu, S.; Li, X.; Wu, H.; Xin, B.; Tang, J.; Petrie, P. R.; Whitty, M. (2018) A robust automated flower estimation system for grape vines. Biosyst. Eng. 172: 110–123.

Llorens, J.; Gil, E.; Llop, J.; Escolà, A. (2011) Ultrasonic and LIDAR Sensors for Electronic Canopy Characterization in Vineyards: Advances to Improve Pesticide Application Methods. Sensors 11: 2177–2194.

Macfarlane, C.; Hoffman, M.; Eamus, D.; Kerp, N. Higginson, S.; McMurtrie, R.; Adams, M. (2007) Estimation of leaf area index in eucalypt forest using digital photography. Agric. For. Meteorol. 143: 176–188.

Siebers, M.; Edwards, E.; Jimenez-Berni, J.; Thomas, M.; Salim, M.; Walker, R. (2018) Fast Phenomics in Vineyards: Development of GRover, the Grapevine Rover, and LiDAR for Assessing Grapevine Traits in the Field. Sensors 18: 2924.

Smart, R.E. (1985) Principles of grapevine manipulation with implications for yield and quality – a review. Am. J. Enol. Vitic. 36: 230–239.

Whole bunch fermentation of Shiraz and Pinot Noir: influence on 'green' characters and astringency

M.P. Day[1], D.L. Capone[2], S. Kassara[1], E.O. Bilogrevic[1], D. Espinase Nandorfy[1], F.T. Watson[1], I.L. Francis[1], K.A. Bindon[1]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]ARC Training Centre for Innovative Wine Production, The University of Adelaide, Urrbrae, SA
eleanor.bilogrevic@awri.com.au

Abstract

Although the berries of grape varieties such as Pinot Noir and Shiraz do not contain methoxypyrazines (the volatile compounds that impart 'green capsicum' and 'herbaceous' notes to wine), it has long been known that the rachis (stalks, stems) of some varieties can contain high levels of this compound. There is therefore potential that winemaking practices such as whole bunch fermentation might impart 'green' notes to Shiraz or Pinot Noir wines. The work presented here investigated the extent to which the addition of whole bunches during fermentation contributes to 'green capsicum' characters in wine. It was also of interest to determine how much additional tannin could be extracted from stem contact, which was expected to affect wine astringency. Pinot Noir and Shiraz grapes were fermented either completely crushed and destemmed, as a control, or with whole bunches added at 25%, 50%, 75% or 100%. For both varieties, the sensory scores for 'green capsicum' and the concentration of IBMP (3-isobutyl-2-methoxypyrazine, the compound most responsible for the 'green capsicum' aroma) were highly correlated with the proportion of whole bunches in the ferment. For Shiraz, the concentration of tannins and the perception of astringency were also highly correlated with the proportion of whole bunches. Differences in red/brown hue were also observed. This study clearly demonstrates how winemakers can fine-tune sensory outcomes by controlling the proportion of whole bunches used.

Introduction

In recent years, there has been a rise in popularity of the use of whole bunches in fermentation during winemaking (Godden 2018). This technique has most commonly been used in the production of Pinot Noir and Shiraz from cooler regions in Australia, with the practice also applied in Burgundy and Northern Rhône, and elsewhere internationally. Inclusion rates can range from anywhere between 10% and 100% whole bunches, with the aim of adding complexity to the wines by changing texture/mouth-feel attributes or aromas and flavours. Whole bunch fermentation is not typically associated with varieties such as Cabernet Sauvignon, as inclusion of whole bunches for this variety has been shown to result in excessive 'green' characters and astringency (Godden 2018). Previous studies have shown that most consumers do not respond positively to these characters in wine (Francis and Williamson 2015).

The compound 3-isobutyl-2-methoxypyrazine (IBMP) can be found in the berries of grape varieties such as Cabernet Sauvignon, Cabernet Franc, Merlot and Sauvignon Blanc (Harris et al. 2012). Descriptors for the aroma of this compound in wines include 'fresh green beans', 'green capsicum', 'grassy' or 'herbal'. These characters are quite commonly observed in these varieties and can be considered a signature of their styles. Pinot Noir and Shiraz, on the other hand, do not produce IBMP in the berry (Koch et al. 2010).

A recent study looking at material other than grapes (MOG) in fermentations, found that the inclusion of rachis (stems) in a cool climate Shiraz fermentation increased 'green' characters such as 'green capsicum' and 'green stalks' as well as astringency (Capone et al. 2018). The 'green' characters were a result of an increase in IBMP in the wine due to extraction from the rachis, with no IBMP detected in the ferments that were made from a hand-plucked fruit (berries only) treatment (Capone et al. 2018).

Following on from this initial study, an investigation into the sensory and compositional effects of different proportions of whole bunch fermentation in both Pinot Noir and Shiraz was conducted.

Winemaking

During the 2018 vintage, Pinot Noir and Shiraz grapes were harvested by hand from premium vineyards in the Adelaide Hills and small-lot replicated winemaking was completed by WIC Winemaking Services at the Waite Campus, Urrbrae, SA. The bunches were randomly separated into five treatments: no whole bunch inclusion (control, all destemmed and crushed fruit) and inclusion of crushed whole bunches at 25%, 50%, 75% and 100%. Whole bunches were placed at the bottom of a 50 kg fermenter together with a small amount of inoculated must, and destemmed and crushed fruit was then added in the required proportions. All replicates received hand-plunging twice a day for the duration of fermentation. After pressing, all ferments went through malolactic fermentation and received no oak treatment. The wines were bottled and stored for approximately nine months, after which time quantitative sensory descriptive analysis using a panel of highly trained and experienced assessors and comprehensive chemical analysis were completed.

Shiraz results

From the sensory descriptive analysis of the Shiraz wines, there was a significant increase in colour intensity of the wines with whole bunches included compared to the control treatment (Figure 1), with no significant difference between the different whole bunch treatments. This colour variance was also evident in the UV-visible spectral measures. The whole bunch treatments also exhibited lower 'red fruit' aroma than the control sample, but there was no significant difference for the 'dark fruit' aroma attribute. 'Stalky'/'capsicum' aroma and flavour were rated higher in the treatments with greater percentages of whole bunches, and there was a strong linear relationship between the 'stalky'/'capsicum' character and IBMP concentration, with the wines made with the highest proportion of whole bunches (Figure 2) highest in both measures.

There was also a positive relationship between the percentage of whole bunches and the perceived astringency in the treatments

(Figure 3). This was well correlated with the tannin concentration in the wines, which was between 596 mg/L and 1,360 mg/L. All other palate/mouth-feel attributes were not rated significantly differently across the wines.

In addition, there was a significant increase in a 'medicinal'/'Band-Aid' aroma for the 100% whole bunch treatment, which was related to the concentration of guaiacol (data not shown), although this tentative link requires further investigation.

Pinot Noir results

From the sensory descriptive analysis study of the Pinot Noir wines, there was a significant increase in the red colour intensity of the wines with 75% and 100% whole bunches included compared to the control treatment. In addition to the changes in red colour, there was a strong linear decrease in brown tint with inclusion of whole bunches, giving the higher whole bunch treatments a more vibrant red appearance (Figures 4 and 5). The higher percentage whole bunch treatments also had a significantly higher intensity of 'red fruit' and 'red confectionary' aromas than the 0 to 50% treatments. From the chemical analysis data, a number of fermentation-derived esters were related to this increase in 'red fruit'/'confectionary' aromas. There was no significant difference in 'dark fruit' or 'sweet spice' aroma among the treatments.

As was observed for the Shiraz wines, the 'capsicum' aroma attribute in the Pinot Noir wines was strongly related to the proportion of whole bunches (Figure 6) and again there was a correlation with the

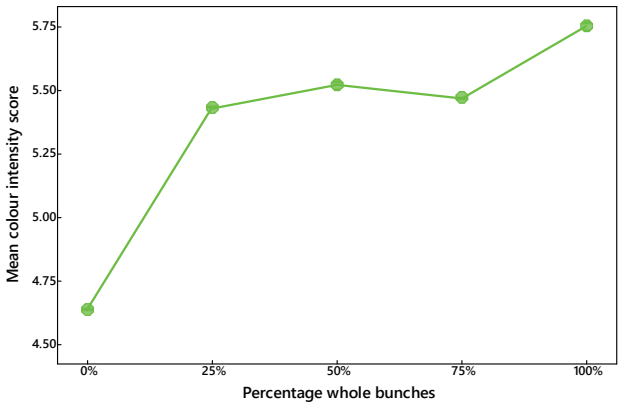


Figure 1. Relationship between mean colour intensity scores of the Shiraz wines and the percentage of whole bunches included in the fermentation

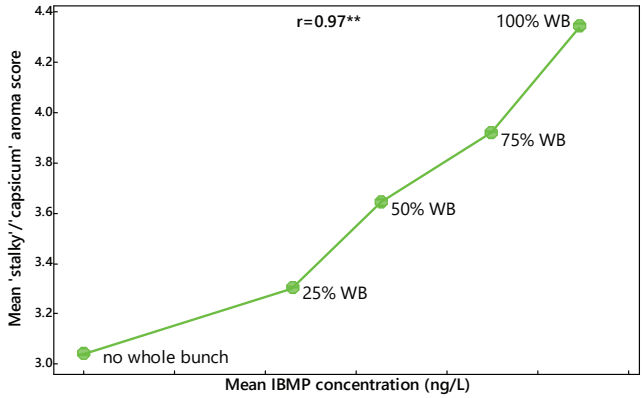


Figure 2. Relationship between mean 'stalky'/'capsicum' aroma score and the concentration of IBMP for each of the whole bunch (WB) treatments of Shiraz. (No whole bunch treatment: IBMP not detected, 100% whole bunch treatment IBMP concentration = 5.5 ng/L)

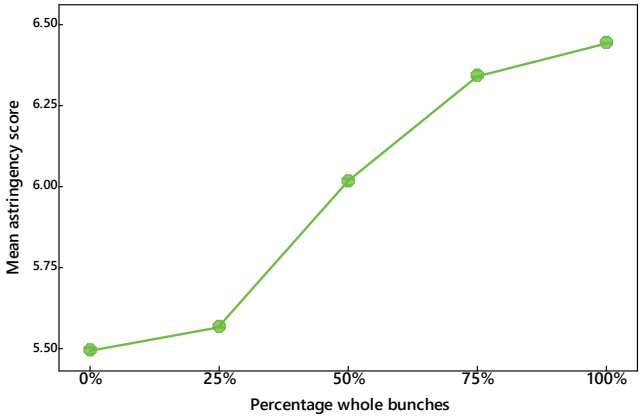


Figure 3. Relationship between mean astringency scores of the Shiraz wines and the percentage of whole bunches included in the fermentation

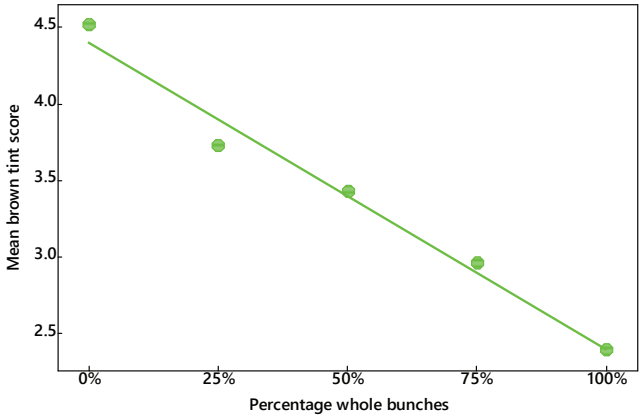


Figure 4. Relationship between mean brown tint scores of the Pinot Noir wines and the percentage of whole bunches included in the fermentation

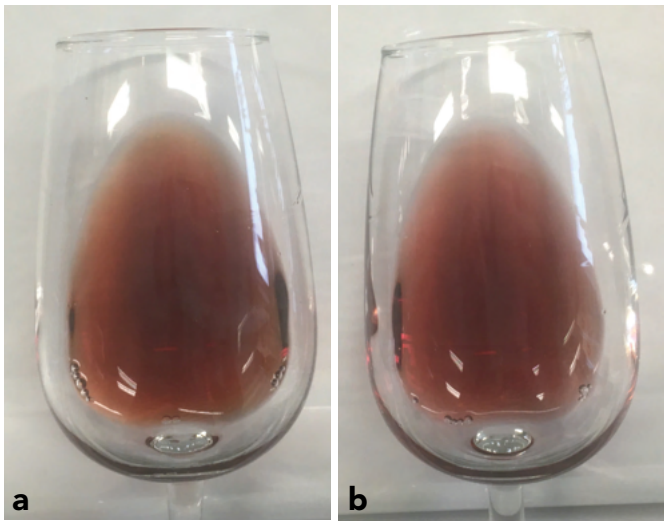


Figure 5. The colour difference between the Pinot Noir treatments: a) no whole bunches and b) 100% whole bunches

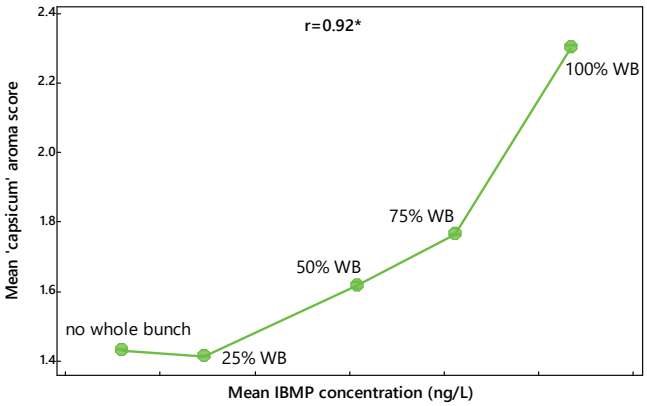


Figure 6. Relationship between mean 'capsicum' aroma scores and the concentration of IBMP for each of the whole bunch (WB) treatments of Pinot Noir. (No whole bunch treatment: IBMP not detected, 100% whole bunch treatment: IBMP concentration = 6.3 ng/L)

concentration of IBMP, with the 100% whole bunch treatment having a mean IBMP concentration of 6.3 ng/L. However, unlike the Shiraz, there were no significant textural/mouth-feel differences among the treatments. There were also significantly higher intensity scores for a 'cooked vegetable' aroma, which decreased as the proportion of whole bunches increased.

Conclusion

Whole bunch inclusion was found to result in different sensory effects for the two grape varieties studied. While both sample sets saw changes in appearance as well as increases in 'green' characters as the percentage of whole bunches increased, for the Pinot Noir there was an increase in 'red fruit' and 'confectionary' aromas, while the Shiraz had an increase in both tannin and astringency.

This study clearly showed that the inclusion of the rachis in Shiraz and Pinot Noir fermentations can result in detectable and sensorially significant increases in IBMP concentration, and winemakers would need to consider the trade-off between the generation of arguably undesirable 'green' characters against enhancement of wine colour, desirable flavour changes and increased tannin (Shiraz) when adding whole bunches to their ferments. It should be noted that this study was conducted in only one season with fruit from a single cool climate vineyard. Further studies investigating aspects such as the degree of lignification of the rachis and influence of inclusion of whole berries (Cowey 2018) would be beneficial.

Acknowledgements

This work is supported by Australia's grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster in Adelaide.

The contributions of the sensory panellists, WIC Winemaking Services, AWRI Commercial Services and the volunteer grape-pickers and whole-bunch sorters are gratefully acknowledged.

References

Capone, D.; Pearson, W.; Bindon, K.; Kassara, S.; Solomon, M.; Bey, L.; Francis, L.; Herderich, M.; Johnson, D. (2018) What makes a red wine green? Wine Vitic. J. 33(2): 32–35.

Cowey, G. (2018) Ask the AWRI: Carbonic maceration. Aust. N.Z. Grapegrower Winemaker 651: 70–71.

Francis, I.L.; Williamson, P.O. (2015) Application of consumer sensory science in wine research. Aust. J. Grape Wine Res. 21: 554–567.

Godden, P. (2018) Ask the AWRI: Understanding whole-bunch fermentation. Aust. N.Z. Grapegrower Winemaker 652: 63.

Harris, S.A.; Ryona, I.; Sacks, G.L. (2012) Behavior of 3-Isobutyl-2-hydroxypyrazine (IBHP), a key intermediate in 3-isobutyl-2-methoxypyrazine (IBMP) metabolism, in ripening wine grapes. J. Agric. Food Chem. 60(48): 11901–11908.

Koch, A.; Doyle, C.L.; Matthews, M.A.; Williams, L.E.; Ebeler, S.E. (2010) 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. Phytochemistry 71: 2190–2198.

Remediating 'reductive' characters in wine

M.Z. Bekker[1], D. Espinase Nandorfy[1], A.C. Kulcsar[1], A. Faucon[1], P.A. Smith[1,2]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]Current address: Wine Australia, Adelaide, SA
marlize.bekker@awri.com.au

Abstract

Winemakers use various remediation strategies to manage unwanted volatile sulfur compounds (VSCs) associated with 'reductive' aromas in wine. Remediation strategies such as diammonium phosphate (DAP) addition, copper fining, oxidative handling and racking, and fresh lees addition are commonly employed to remove unwanted VSCs. In this study, the effectiveness of five unique remediation strategies was evaluated over the course of 12 months. All the remediation techniques had varying levels of success in removing 'reductive' aromas. Remediating the wines early using macro-oxygenation appeared to be the most effective in producing wines with the lowest 'reduction'-related attributes while enhancing 'red fruit' attributes.

Introduction

Managing 'reductive' aromas in wines remains an important consideration for winemakers. Compounds such as hydrogen sulfide (H_2S), methanethiol (MeSH) and thioacetates, for example, have significant impacts on wine aroma and consumer preference. Various remediation strategies exist for the removal of these unwanted compounds, but each remediation strategy has its strengths and weaknesses. For example, copper fining is only effective in removing sulfhydryls (plus disulfides after they have been reduced back to their original sulfhydryl products) but it is not effective in remediating thioacetates or dialkyl sulfides. Copper fining may appear to be very effective immediately after treatment; however, if increased residual copper remains in wine post-bottling this may lead to the recurrence of 'reductive' aromas a few months or up to a year later (Bekker et al. 2018; Ugliano et al. 2011b; Viviers et al. 2013). Similarly, supplementation with diammonium phosphate (DAP) has been shown to cause increased H_2S concentrations in certain instances (Ugliano et al. 2009; Ugliano et al. 2011a; Waterhouse et al. 2016), even though DAP is commonly used to limit the risk of H_2S formation. Yeast strains have different abilities to metabolise DAP and certain strains are more prone to produce VSCs (Ugliano et al. 2009; Ugliano et al. 2011a; Waterhouse et al. 2016). In other instances, the remediation of VSCs is an additional benefit to already well-established winemaking strategies. For example, using oxygen effectively during winemaking is beneficial for yeast health and promotes fermentation efficiency (Day et al. 2015). Recent studies have demonstrated that an additional benefit of using aerative winemaking techniques, such as macro-oxygenation during active ferment, is that they produce wines with low 'reductive' characters and increased 'fruity' aromas (Bekker et al. 2016). Other strategies such as adding clean lees or using lees products to 'freshen up' wines may be effective through binding of some of the unwanted sulfur compounds. However, there are risks of introducing VSCs through lees autolysis or through the action of active enzymes that could cleave sulfur-containing amino acids.

With all these remediation strategies available to winemakers, each with its own set of risks and benefits, it becomes challenging to select the most beneficial option. With this in mind, this study was designed to evaluate the relative effectiveness of five commonly used 'reductive' aroma remediation strategies employed on the same wine with pronounced 'reductive' characters. The strategies tested were: supplementation with diammonium phosphate (DAP), copper fining, macro-oxygenation, a combination of copper fining and macro-oxygenation, and scalping VSCs with fresh wine lees (Figure 1).

Remediation strategies

Shiraz grapes were harvested and crushed, and six sets of triplicate wines were prepared using a standard winemaking procedure under particularly 'reductive' conditions to support increased production of VSCs. On the onset of 'reductive' aromas, each triplicate set of wines received individual remediation treatments (summarised in Figure 1):

- 'Control' wines received no remediation treatment
- 'DAP' wines received sequential DAP additions of 200 mg/L and 150 mg/L, totalling 350 mg/L
- 'Macro-Ox' and 'Macro-Ox + Copper' ferments were sparged with compressed air at a rate of 1 L/min for 120 minutes for five consecutive days (Day 3 to Day 7) using a drop-in t-piece sparger fitted with four 2 micron sinters
- 'Copper' and 'Macro-Ox + Copper' wines received an addition of 1.0 mg/L and 0.15 mg/L of $CuSO_4 \cdot 5H_2O$, respectively, once ferments reached approximately 1 Bé
- 'Macro-Ox + Copper' ferments received the same sparging as the 'Macro-Ox' treatment plus an addition of 0.15 mg/L of $CuSO_4 \cdot 5H_2O$ once ferments reached approximately 1 Bé
- 'Lees' wines were treated with 1.5 L of fresh clean lees after inoculation with malolactic bacteria.

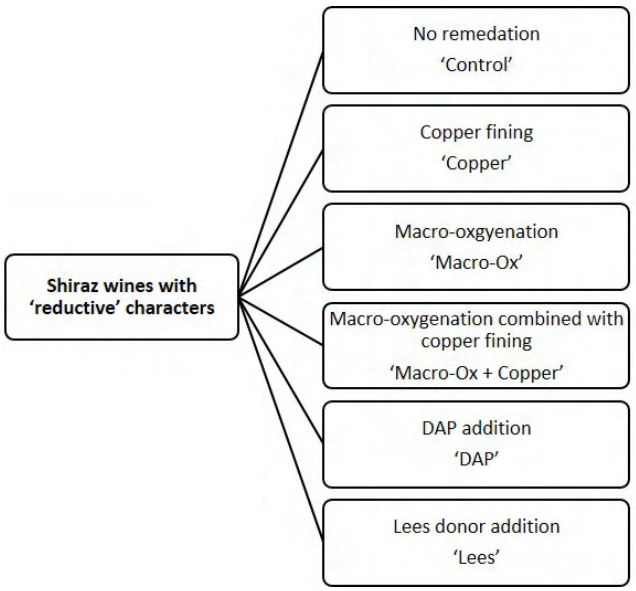


Figure 1. Summary of remediation strategies for 'reductive' characters in red wine employed in this study

Results and discussions

When evaluating the effects of the remediation treatments on individual VSCs associated with 'reductive' aromas that were measured in this study, it was clear that certain strategies were associated with decreased VSC concentrations, while others resulted in elevated VSC concentrations. The 'Macro-Ox' and 'Macro-Ox + Copper' treatments were successful in remediating H_2S concentrations in the wines for up to 12 months post-bottling (Figure 2). Significantly lower H_2S concentrations were measured in the 'Macro-Ox' treatments (at 'Bottling' timepoint P-value 0.015, at 'Month 12' timepoint P-value <0.001) and 'Macro-Ox + Copper' treatments (at 'Bottling' timepoint P-value 0.009; at 'Month 6' timepoint P-value <0.001) when compared to the control wines (Figure 2). In contrast, the 'Lees' treatment was associated with increased H_2S concentrations 12 months post-bottling (P-value <0.001) (Figure 2).

Similarly, 'Macro-Ox' and 'Macro-Ox + Copper' treatments were associated with significantly decreased MeSH concentrations when measured immediately post-bottling (P-values <0.001 and 0.002, respectively) (Figure 2). Copper fining also resulted in significantly decreased MeSH concentrations immediately after bottling (P-value 0.018) (Figure 2); however, this effect was short term, with no difference from the control wines observed when the wines were analysed 12 months post-bottling. Conversely, the 'DAP' and 'Lees' treatments resulted in significantly increased MeSH concentrations after bottling ('DAP' P-value 0.004, 'Lees' P-value <0.001) and the MeSH concentrations remained elevated in the 'Lees'-treated wines for 12 months post-bottling ('Lees' P-value 0.005) (Figure 2).

Interestingly, methylthioacetate (MeSAc) followed the same trends as MeSH in the remediated wines, with significantly decreased concentrations of MeSAc measured in the 'Macro-Ox' and 'Macro-Ox + Copper' treated wines (P-values 0.001 and <0.001, respectively), whereas 'DAP' and 'Lees' remediation treatments resulted in significantly increased MeSAc concentrations 12 months post-bottling (P-value <0.001 for both) (Figure 2).

Significant differences were found among the remediation treatments, mainly for attributes describing 'reductive' off-odours and

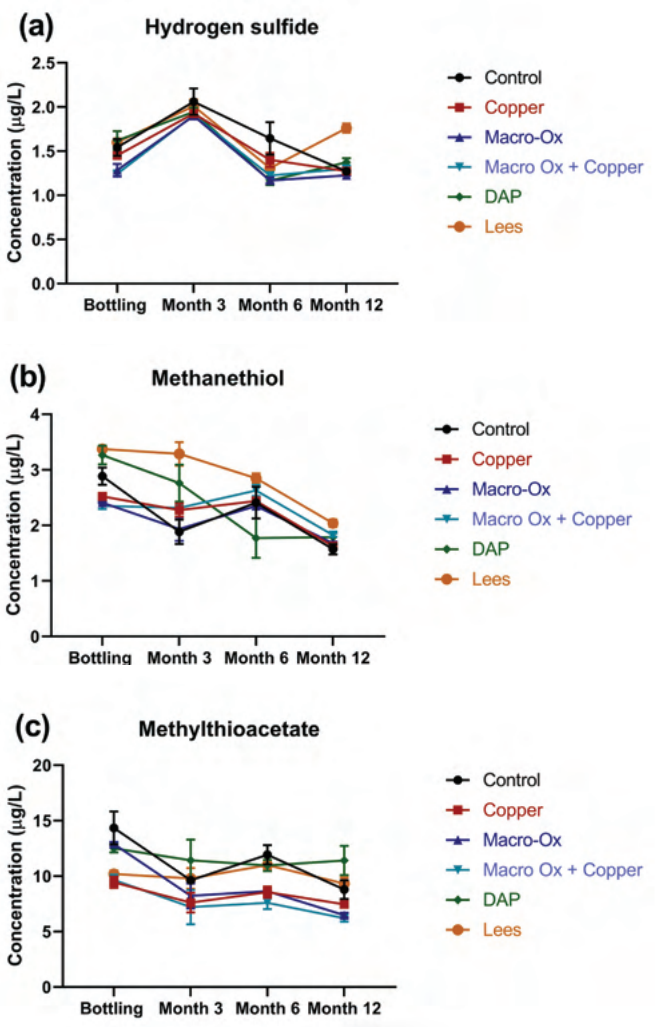


Figure 2. The effects over 12 months of treating 'reductive' wines using copper addition ('Copper'), macro-oxygenation ('Macro-Ox'), combined copper fining and macro-oxygenation ('Macro-Ox + Copper'), DAP addition ('DAP'), and lees treatment ('Lees') on the evolution of (a) hydrogen sulfide (H_2S), (b) methanethiol (MeSH), and (c) methylthioacate (MeSAc) measured in wines post-bottling

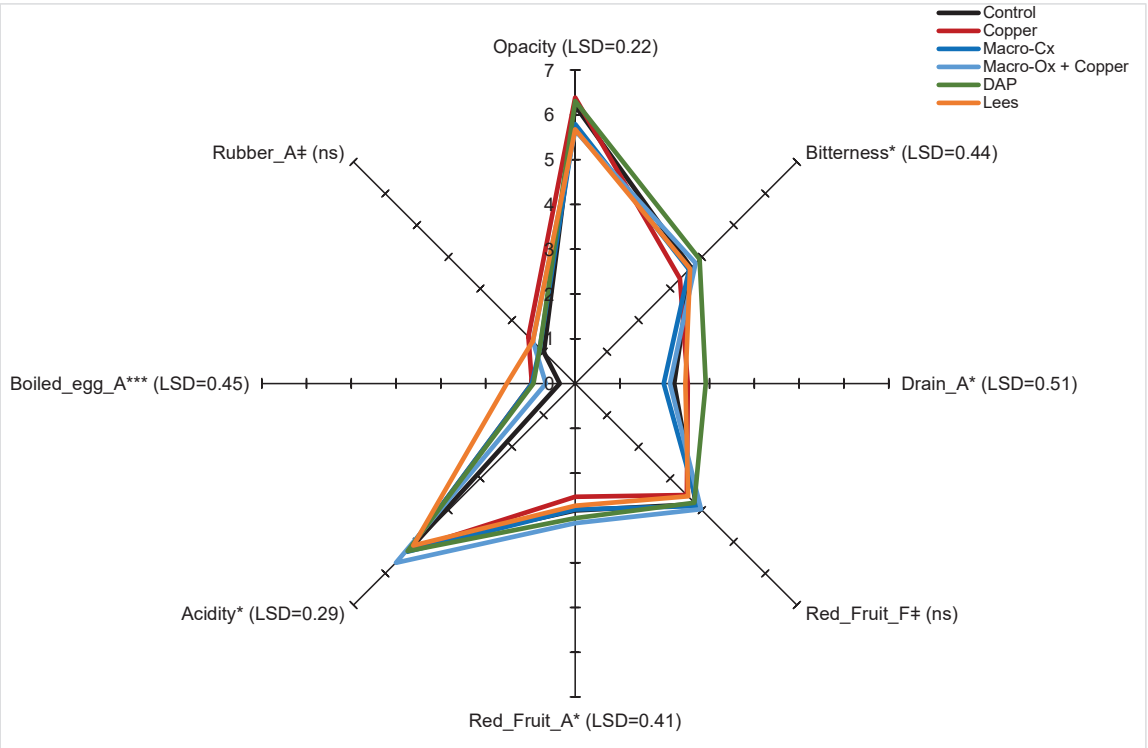


Figure 3. Mean sensory attribute intensity scores for significant attributes (*P < 0.05; **P < 0.01; ***P<0.001) and attributes approaching significance (‡ P < 0.16) for the 'reductive' aroma remediation treatments. Least significant difference (LSD) (P=0.05) values included for the significant attributes (P < 0.05).

'fruit' notes, which generally related well to the chemical results discussed above. The wines treated with 'Macro-Ox' and 'Macro-Ox + Copper' displayed lower 'boiled egg' and 'drain' aromas, and higher 'red fruit' aromas (Figure 3). The wines remediated with 'Copper', 'DAP', and 'Lees' treatments were characterised by 'drain', 'rubber' and 'boiled egg' aromas, and these characters were especially apparent in the 'Lees'-treated wines (Figure 3).

Conclusions

This work demonstrated that macro-oxygenation during fermentation was the most effective strategy over a period of 12 months for remediating 'reductive' characters in a Shiraz wine with pronounced 'reductive' characters. This strategy was associated with decreased VSC concentrations and their associated negative sensory attributes and increased 'fruity' notes. The macro-oxygenation and copper treatment did not produce wines with more preferred sensory profiles than macro-oxygenation treatment alone. The DAP, copper fining, and lees treatments were less successful in this study, with the sensory profiles of wines remediated with these treatments showing increased 'reductive' characters.

Acknowledgements

This work is supported by Australia's grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The Australian Wine Research Institute and the University of Adelaide are members of the Wine Innovation Cluster in Adelaide. This work is also supported by Metabolomics South Australia which is funded through Bioplatforms Australia Pty Ltd (BPA), a National Collaborative Research Infrastructure Strategy (NCRIS), and investment from the South Australian State Government and The Australian Wine Research Institute. The authors thank John Gledhill, Jules Gautier and Raquel Kallas from WIC Winemaking Services for winemaking; Mark Solomon, Stella Kassara and Song Qi for chemical analysis; and Mark Braybrook for technical support. The authors would also like to thank the sensory panellists involved in this study, as well as Leigh Francis, Wes Pearson and Eleanor Bilogrevic for their support with the sensory evaluation.

References

Bekker, M.Z.; Day, M.P.; Holt, H.; Wilkes, E.; Smith, P.A. (2016) Effect of oxygen exposure during fermentation on volatile sulfur compounds in Shiraz wine and a comparison of strategies for remediation of reductive character. Aust. J. Grape Wine Res. 22: 24–35.

Bekker, M.Z.; Wilkes, E.N.; Smith, P.A. (2018) Evaluation of putative precursors of key 'reductive' compounds in wines post-bottling. Food Chem. 245: 676–686.

Day, M.P.; Schmidt, S.A.; Smith, P.A.; Wilkes, E.N. (2015) Use and impact of oxygen during winemaking. Aust. J. Grape Wine Res. 21: 693–704.

Ugliano, M.; Fedrizzi, B.; Siebert, T.; Travis, B.; Magno, F.; Versini, G.; Henschke, P.A. (2009) Effect of nitrogen supplementation and *Saccharomyces* species on hydrogen sulfide and other volatile sulfur compounds in shiraz fermentation and wine. J. Agric. Food Chem. 57: 4948–4955.

Ugliano, M.; Kolouchova, R.; Henschke, P.A. (2011a) Occurrence of hydrogen sulfide in wine and in fermentation: influence of yeast strain and supplementation of yeast available nitrogen. J. Ind. Microbiol. Biotechnol. 38: 423–429.

Ugliano, M.; Kwiatkowski, M.; Vidal, S.; Capone, D.; Siebert, T.; Dieval, J.B.; Aagaard, O.; Waters, E.J. (2011b) Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. J. Agric. Food Chem. 59: 2564–2572.

Viviers, M.Z.; Smith, M.E.; Wilkes, E.; Smith, P. (2013) Effects of five metals on the evolution of hydrogen sulfide, methanethiol, and dimethyl sulfide during anaerobic storage of Chardonnay and Shiraz wines. J. Agric. Food Chem. 61: 12385–12396.

Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. (2016) Sulfur Metabolism. In: Understanding Wine Chemistry. Sussex, UK: John Wiley & Sons Ltd: 223–229.

The colorimetric measurement of free and bound Cu in white wine

A.C. Clark, N. Kontoudakis

National Wine and Grape Industry Centre, School of Wine and Food Sciences,
Charles Sturt University, Wagga Wagga, NSW
aclark@csu.edu.au

Abstract

Copper (Cu) is often added to wine to treat or prevent the accumulation of hydrogen sulfide and its associated odour. However, Cu in wine can affect oxidative and reductive ageing mechanisms and the rate at which they occur. Recently it has been shown that the Cu in wine exists predominantly in two forms whereby it is either bound to sulfide (bound) or not bound to sulfide (free). The measurement of free Cu can potentially provide a means of better understanding the activity of Cu in wine and how it affects reductive and oxidative aspects of wine. However, the current measures of Cu forms are labour-intensive and require expensive equipment not generally found in wineries. This paper describes the development of a routine colorimetric method to allow the determination of free Cu in white wine, which, combined with total Cu concentrations, also allows calculation of the bound Cu concentration. Further work is still required to understand exactly how and if the free Cu concentration of wine at bottling can be optimised to avoid the accumulation of 'reductive' aromas in wine.

Introduction

Copper (Cu) can find its way into wine from a variety of sources and as such, all wines will contain at least some of this metal. One source is the use of Cu-containing sprays in the vineyard (i.e. fungicides) that may remain on the grape and result in elevated Cu concentrations in the juice or must (Provenzano et al. 2010). Fortunately, the vast majority of this Cu is removed during primary fermentation, as it is readily precipitated and removed with yeast lees (Junghans and Straube 1991). However, increasing concentrations of Cu in juice can lead to elevated concentrations in the final white wine (Rousseva et al. 2016). The contact of juice or wine with brass equipment (e.g. fittings) in the winery also can be a source of Cu in the final wine (Boulton et al. 1999); however, with the advent of stainless steel in the winery this is now uncommon.

Often the major source of Cu in wine is its addition by winemakers to remove the off-aromas induced by hydrogen sulfide and other low molecular weight sulfur compounds such as methanethiol. Hydrogen sulfide can impart 'rotten egg'/'sewerage' aromas at low concentrations and has an aroma threshold in wine quoted as 1.1-1.6 µg/L, while methanethiol has an aroma of 'rotten cabbage'/'burnt rubber' and a higher aroma threshold of 1.8-3.1 µg/L (Siebert et al. 2010). The Cu added by winemakers readily reacts with hydrogen sulfide to form a copper sulfide complex that is non-volatile and hence the off-aroma associated with low molecular weight sulfur compounds is effectively eliminated (Clark et al. 2015). The strength of binding between Cu and the sulfur-containing compounds varies, as Cu will bind to hydrogen sulfide more efficiently than to thiol compounds, such as methanethiol (Franco-Luesma and Ferreira 2014). Copper is commonly added as aqueous solutions of copper(II) sulfate pentahydrate salt, but formulations of copper(II) citrate with or without bentonite are also used.

Copper sulfide: a complex complex

Once copper sulfide forms in wine, it is commonly assumed that the resulting product will readily precipitate and be removed by settling and/or filtration steps during wine production. However, recent work has shown that the copper sulfide forms nanoparticles in wine that do not readily settle (Kontoudakis et al. 2019a). Furthermore, the size of the copper sulfide particles is in the range of 0.10–0.25 µm, which means that even sterile filtration (0.20–0.45 µm) cannot ensure removal based on size exclusion mechanisms.

Additionally, during the reaction of copper(II) with hydrogen sulfide, it has been shown that a range of polysulfanes can be produced in certain circumstances (Bekker et al. 2018). The polysulfanes have multiple sulfur atoms bonded between the two thiol compounds (e.g. R-S-S-S-R, where R represents some additional chemical group). The polysulfanes have been identified in model wines where the copper sulfide was assigned as primarily a copper(I) sulfide species. In low oxygen conditions, and in the presence of sulfur dioxide and/or ascorbic acid, the polysulfanes have been linked with the release of hydrogen sulfide in model wine samples (Bekker et al. 2018). Therefore, although copper addition to wine can effectively remove sulfidic off-aromas, it may also set up the production of precursors to hydrogen sulfide that can release hydrogen sulfide in the future (i.e. during bottle ageing). Other wine components that can also act as a potential source of hydrogen sulfide in wine include residual protein, thiols and thioacetates (Kreitman et al. 2019). As yet, the importance of each potential hydrogen sulfide source in terms of yield of hydrogen sulfide is not known.

Why measure Cu forms?

Given the complexity of Cu reactions in wine, an approach to measure the different forms of Cu in wine was taken. Such a strategy has proved to be particularly effective for other wine additives, and none more so than in the use of sulfur dioxide in wine. For sulfur dioxide, free and bound forms are determined, and with pH measurement a further molecular fraction of sulfur dioxide can be calculated. The molecular sulfur dioxide is critical for microbial stability of wine, the free sulfur dioxide for oxidative stability and the total sulfur dioxide must be measured for regulatory reasons. It was envisaged that the measurement of different Cu forms may allow a more informed usage during wine production.

The electrochemical determination of Cu forms: free and bound Cu

The initial approach for Cu measurement in wine involved the electrochemical analysis of wine by a stripping potentiometry technique (Clark et al. 2016; Clark and Kontoudakis 2018). This technique simply classified Cu in wine as being electrochemically detectable or not. Once the method was optimised it was applied to 49 wines (Figure 1). From the results it was evident that the majority of Cu in the wines was in the non-detectable form, with minor amounts of

Cu in the detectable form. A survey of wine compounds was then conducted to determine which compounds could influence the electrochemical detection of Cu in wine (Table 1) (Kontoudakis et al. 2017). The compounds assessed included phenolic and thiol standards (e.g. (+)-catechin, methanethiol) as well as macromolecules extracted from wine, including white wine proteins, polysaccharides and red wine phenolic material. Upon addition of the wine components to a model wine system in combination with Cu it was apparent that only hydrogen sulfide could convert electrochemically detected Cu to the non-detected form.

In re-assessing Figure 1 in this context, it meant that the majority of Cu in wine is in a form whereby it is bound to sulfide. Alternatively, only minor amounts of 'non-sulfide-bound' Cu were present. To simplify the terminology, it was decided to refer to the different forms as 'bound Cu' and 'free Cu', respectively. It must be recognised that the 'free Cu' would still be largely bound to organic acids in the wine, and interact with phenolic compounds, but in terms of binding to sulfide it was considered as being 'free'. The results of Figure 1, with Cu mainly present in the sulfide-bound form, were consistent with previous studies showing that removal of the nanoparticle sulfide-bound Cu from wine is difficult. However, it was not possible to assess whether the bound form of Cu was generated before bottling, after bottling or a combination of both.

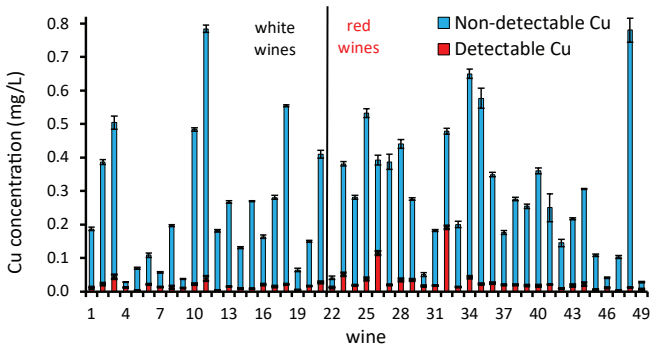


Figure 1. The electrochemically detectable Cu in wine. The electrochemical system consisted of medium exchange constant current stripping potentiometry using a thin mercury film on a screen-printed carbon electrode. The non-detectable Cu was later assigned as sulfide-bound Cu (bound Cu) and the detectable Cu as non-sulfide-bound Cu (free Cu).

Table 1. Components added separately to model wine in the presence of 0.40 mg/L of copper(II). The detection of copper was performed by the electrochemical stripping potentiometry technique.

Wine component	Concentration (mg/L)	Detectable Cu (mg/L)
Epicatechin	1,000	0.42 ± 0.01
Gallic acid	100	0.41 ± 0.01
Caffeic acid	100	0.43 ± 0.01
Quercetin-3O-glucoside	15	0.45 ± 0.01
Ellagic acid	10	0.41 ± 0.01
Red wine polyphenol (extract)	2,000	0.42 ± 0.01
Red wine tannin (extract)	1,000	0.38 ± 0.01
White wine protein (extract)	50	0.38 ± 0.01
White wine polysaccharide (extract)	200	0.40 ± 0.01
White wine polyphenol (extract)	200	0.39 ± 0.01
Hydrogen sulfide	0.22 (2:1 H_2S:Cu mole ratio)	0.01 ± 0.01
Methanethiol	0.30 (2:1 thiol:Cu mole ratio)	0.45 ± 0.01
Ethanethiol	0.40 (2:1 thiol:Cu mole ratio)	0.43 ± 0.01
Carbon disulfide	0.48 (2:1 thiol:Cu mole ratio)	0.39 ± 0.01
Dimethylsulfide	0.39 (2:1 thiol:Cu mole ratio)	0.38 ± 0.01
Cysteine	0.76 (2:1 thiol:Cu mole ratio)	0.39 ± 0.01
Glutathione	1.94 (2:1 thiol:Cu mole ratio)	0.45 ± 0.01
2-furanmethanethiol	0.72 (2:1 thiol:Cu mole ratio)	0.41 ± 0.01
4-mercapto-4-methyl-2-pentanone	0.83 (2:1 thiol:Cu mole ratio)	0.46 ± 0.01

Links between free Cu and 'reductive' characters in wine.

The wines from Figure 1 also underwent gas chromatography analysis to measure their free hydrogen sulfide concentration. This form of hydrogen sulfide is the component in wine not bound to metals and the component most easily perceived during sensory assessment, as opposed to the non-volatile metal-bound hydrogen sulfide. Figure 2 shows a plot for the free Cu concentrations versus the free hydrogen sulfide concentration in the wines (Kontoudakis et al. 2019b). The results show that the presence of free Cu prevents the accumulation of free hydrogen sulfide above its aroma threshold. Once the free Cu concentration becomes significantly low (i.e. less than 0.025 mg/L), in some wines the accumulation of free hydrogen sulfide then occurs. For those wines where the free hydrogen sulfide accumulated above the aroma threshold then a 'reductive' wine aroma would be likely. A similar relationship was observed between free Cu and free methanethiol (Figure 3) but with the accumulation of methanethiol occurring at slightly higher concentrations of free Cu than observed for hydrogen sulfide. This is consistent with Cu binding more weakly to methanethiol than hydrogen sulfide (Franco-Luesma and Ferreira 2014).

Although Figures 2 and 3 show potential for the use of free Cu to avoid 'reductive' odours in wine, other work has shown that in certain wines the free Cu concentration can decrease in low oxygen

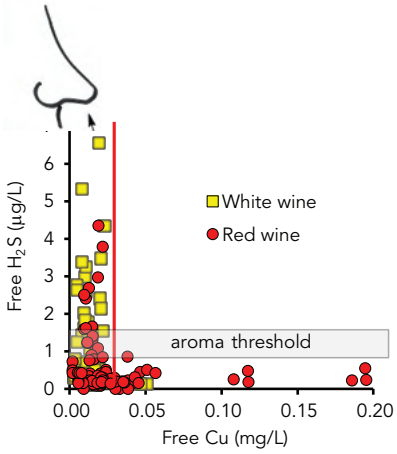


Figure 2. The concentration of free Cu versus free hydrogen sulfide in 49 white and red wines

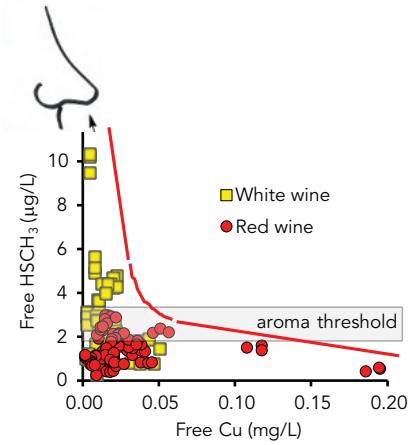


Figure 3. The concentration of free Cu versus free methanethiol in 49 white and red wines

conditions (e.g. bottle ageing) (Kontoudakis and Clark 2020). That is, under low oxygen conditions, the free Cu is able to sequester sulfide from some source in wine (e.g. polysulfanes) and thereby decrease. If it decreases to below 0.025 mg/L concentration then the accumulation of hydrogen sulfide and methanethiol can potentially eventuate. Consequently, the presence of free Cu at bottling may only provide temporary protection against hydrogen sulfide accumulation during the bottle ageing of certain wines. Further work is required to assess the variation in binding of free Cu in different wines at bottling, including the rate of binding and also whether the potential sulfide supply in a wine will be in excess of the free Cu concentration at bottling.

In any case, the determination of free Cu in wine may provide a means of minimising Cu additions to wine, especially if it can be identified whether a wine already has a significant amount of free Cu prior to any planned addition. The measurement of free Cu in a wine may also allow a means of indirectly tracking the production of hydrogen sulfide in a wine before it can be smelled, that is, by following decreases in free Cu. Although such post-bottling measurements would not allow any recuperative interventions by the winemaker, it may provide a knowledge base on the rates of hydrogen sulfide production in the same varieties of wine from year to year. This could provide better knowledge of targets for free Cu concentration at bottling for future vintages of the same wine. Other advantages of measuring the Cu forms in wine include the potential to perform a more targeted removal of Cu species during wine production should it be required. Research on mechanisms for the removal of Cu from wine based on its specific form is currently in progress at the National Wine and Grape Industry Centre.

Colorimeteric versus electrochemical measurement of free Cu

The electrochemical measurement of free Cu in wine is reproducible and accurate. However, the entire system requires integration of multiple pieces of hardware (e.g. peristaltic pumps, stand-alone liquid chromatography pump, potentiostat) that are not commercially available in a single package and do not have software for integrated control of all the separate hardware. This means that currently, manual operation of the electrochemical/chromatographic equipment is required. More crucially, the electrochemical system is relatively slow in the determination of free Cu, with a single wine determination and associated standards requiring about 3.5 hours. For example, the wines determined in Figure 1 required around 23 working days of analysis.

With this in mind, a colorimetric measure of free Cu was to be developed for white wine, which, in conjunction with total Cu measures, would allow calculation of the bound form of Cu. Previously a method for total Cu measurement in wine used bicinchoninic acid (BCA), ascorbic acid and silver(I) (Ag(I)) addition to wine, before a 30 minute incubation time, filtration and measurement of absorbance at 563 nm (see the supporting information). In this method, the Ag(I) was added to aid the release of Cu from binding to sulfide. For the determination of free Cu, the total Cu method was modified by avoiding Ag(I) addition to the sample so that the BCA ligand would only attach to the free Cu in wine rather than both the free and bound forms of Cu (Figure 4). A 40 mm cuvette was required for the method in order to have sufficient absorbance of the Cu-BCA complex to provide a limit of detection of 0.020 mg/L and quantify concentrations below the critical concentration of 0.025 mg/L free Cu (as per Figure 2). Unfortunately, steps to concentrate the wine and increase the concentration of free Cu to allow use of a 10 mm cuvette is not possible as concentrating would change the ratio between free and bound Cu in the wine.

Sixteen white wines were then measured by the electrochemical and colorimetric analyses and the results compared (Figure 5). The wines were either measured immediately upon opening of the bottle, or after addition of 0.080 mg/L Cu. The results showed excellent agreement between the different measures of free Cu in wine (Figure 5). A correlation graph was plotted (data not shown) and provided a gradient of 1.048 and a correlation coefficient of 0.8416. The limit of detection was 0.02 mg/L and the linearity 0.02–1.0 mg/L.

An example of changing forms of Cu in wine: high oxygen conditions

In order to illustrate the ability of the Cu forms to undergo transition in wine, the free and total Cu concentrations in a Chardonnay were measured as it was undergoing oxidation. A 3.0 L volume of the Chardonnay was saturated with air and left at room temperature, with stirring, for 12 days. As evident from Figure 6, initially the free Cu concentration in the wine was low (0.05 mg/L) compared to the total Cu concentration (0.46 mg/L). However, by 36 hours the free Cu concentration had increased to the total Cu concentration and after-

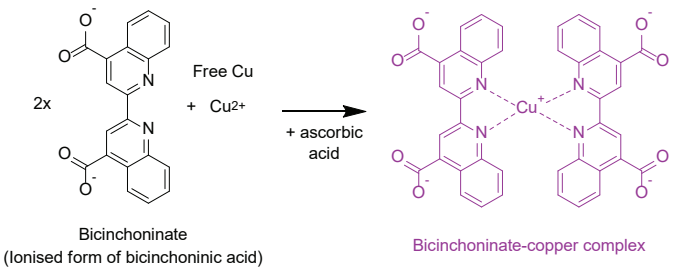


Figure 4. The reaction of bicinchoninic acid with free Cu in white wine

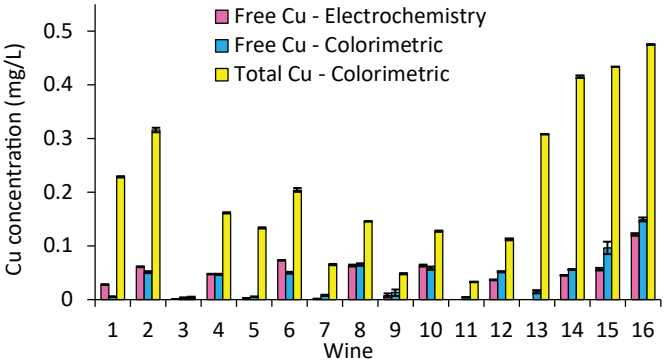


Figure 5. The total and free Cu concentrations in 16 white wines. The free Cu concentration was determined by both electrochemical and colorimetric methods.

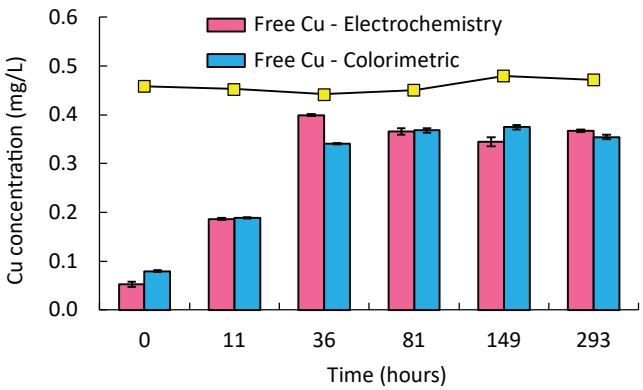


Figure 6. The change in free Cu during the oxidation of Chardonnay white wine. A 3.0 L volume of white wine was saturated with air and left to stir at room temperature with a 1.0 L ullage of air.

wards it remained just below the total Cu concentration. A similar increase was observed for another two white wines (data not shown), but the time required for the free Cu concentration in the wine to increase varied from wine to wine (84 hours and 150 hours, respectively). Again, good agreement was evident between the electrochemical and colorimetric measurement of free Cu.

Such a result is consistent with the production of *o*-quinone compounds from phenolic compounds during wine oxidation. Trace amounts of hydrogen sulfide can react with the *o*-quinone and in the process some sulfide-bound Cu will dissociate to hydrogen sulfide and free Cu. With progressive *o*-quinone production, increasing amounts of free Cu will be released from bound Cu until there is little bound Cu remaining. High concentrations of sulfur dioxide and/or ascorbic acid may slow this release of free Cu by competing with hydrogen sulfide for *o*-quinones. Another mechanism of hydrogen sulfide loss from the wine, and concomitant release of free Cu, may be due to volatile loss of hydrogen sulfide.

Conclusion

The Cu in wine can be classified in terms of its binding to sulfide, whereby it is either free or bound. The colorimetric analysis of free and total Cu in wine provides a simple, rapid and cheap means of determining free, bound and total Cu in wine using a spectrophotometer. Such a spectrophotometric technique enables ready access to this technique by winemakers, with the proviso that they can fit a 40 mm cuvette in their spectrophotometer.

The interaction of Cu forms with compounds responsible for 'reduced' aroma in wine can be summarised as follows:

1. Free Cu efficiently reacts with free hydrogen sulfide in wine and can immediately extinguish the off-aroma associated with free hydrogen sulfide.
2. The copper sulfide formed is not easily removed from wine.
3. In some instances, the reaction between free hydrogen sulfide and free copper can also produce precursors (e.g. polysulfanes) that may release hydrogen sulfide during the bottle ageing of wine.
4. The quantification of the hydrogen sulfide precursors (e.g. polysulfanes and others) in wine is not currently possible.
5. Release of hydrogen sulfide from precursors in wine will either lead to binding of free Cu or accumulation of free hydrogen sulfide in wine if free Cu is below a certain concentration. The latter will result in a 'reductive' aroma in wine.
6. The typical magnitude of free Cu that can be bound by hydrogen sulfide precursors during the bottle ageing of wine, and the rate at which this occurs, is not yet known.
7. High oxygen conditions can promote an increase in the proportion of free Cu but may also cause wine oxidation.

Point 6 above means that currently no certain recommendation can be provided for free Cu concentrations at bottling to avoid accumulation of 'reductive' aromas in wine. Future research will aim to provide answers as to whether recommendations for free Cu at bottling are possible. In the meantime, at the very least, the colorimetric measure of free and total Cu in wine will allow winemakers to understand how their current practices with Cu additions set up the different Cu forms at bottling along with total concentrations. If there are significant levels of free Cu at bottling, the technique will also allow the winemaker to gauge how quickly this free Cu is being bound by hydrogen sulfide released from precursors during wine ageing. Such insights will perhaps allow winemakers to refine their Cu usage protocol over time, and this may include targeting specific free Cu concentrations at bottling.

Supporting information

Protocols for the colorimetric measurement of Cu in wine:

1. https://cdn.csu.edu.au/__data/assets/pdf_file/0010/3053539/The-Determination-of-Total-Cu-in-White-Wine.pdf
2. Kontoudakis, N.; Smith, M.; Smith, P.A.; Wilkes, E.N.; Clark, A.C. (2020) The colorimetric determination of copper in wine: total copper. Aust. J. Grape Wine Res. 26: 121–129.
3. Contact aclark@csu.edu.au for a combined free and total Cu in wine methodology.

Acknowledgements

The method for total Cu determination in white wine was developed in collaboration with Dr Paul Smith, Dr Mark Smith and Dr Eric Wilkes (AWRI). The macromolecule binding agents (Table 1) were supplied by Dr Paul Smith (AWRI). This project (NWG1401) was funded by Wine Australia with funds from Australian grapegrowers and winemakers and matching funds from the Australian Government.

References

Bekker, M.Z.; Kreitman, G.Y.; Jeffery, D.W.; Danilewicz, J.C. (2018) Liberation of hydrogen sulfide from dicysteinyl polysulfanes in model wine. J. Agric. Food Chem. 66: 13483–13491.

Boulton, R.B.; Singleton, V.L.; Bisson, L.F.; Kunkee, R.E. (eds) (1999) Principles and practices of winemaking. New York: Springer: p. 339.

Clark, A.C.; Wilkes, E.N.; Scollary, G.R. (2015) Chemistry of copper in white wine: a review. Aust. J. Grape Wine Res. 21: 339–350.

Clark, A.C.; Kontoudakis, N.; Barril, C.; Schmidtke, L.M.; Scollary, G.R. (2016) Measurement of labile copper in wine by medium exchange stripping potentiometry utilising screen printed carbon electrodes. Talanta 154: 431–437.

Clark, A.C.; Kontoudakis, N. (2018) Increasing the efficiency and accuracy of labile Cu measurement in wine using screen-printed electrodes. Chemosensors 6: 35.

Franco-Luesma, E.; Ferreira, V. (2014) Quantitative analysis of free and bonded forms of volatile sulfur compounds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. J. Chromatogr. A 1359: 8–15.

Junghans, K.; Straube, G. (1991) Biosorption of copper by yeasts. Biol. Met. 4: 233–237.

Kontoudakis, N.; Smith, M.; Guo, A.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2017) The impact of wine components on fractionation of Cu and Fe in model wine systems: Macromolecules, phenolic and sulfur compounds. Food Res. Int. 98: 95–102.

Kontoudakis, N.; Mierczynska-Vasilev, A.; Guo, A.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2019a) Removal of sulfide-bound copper from white wine by membrane filtration. Aust. J. Grape Wine Res. 25: 53–61.

Kontoudakis, N.; Schmidtke, L.M.; Bekker, M.Z.; Smith, M.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2019b) Analytical strategies for the measurement of different forms of Cu and Fe in wine: Comparison between approaches in relation to wine composition. Food Chem. 274: 89–99.

Kontoudakis, N; Clark, A.C. (2020) Sulfide-binding to Cu(II) in wine: Impact on oxygen consumption rates. Food Chem. 305: doi.org/10.1016/j.foodchem.2020.126352

Kreitman, G.Y.; Elias, R.J.; Jeffery, D.W.; Sacks, G.L. (2019) Loss and formation of malodorous volatile sulfhydryl compounds during wine storage. Crit. Rev. Food Sci. Nutr. 59: 1728–1752.

Provenzano, M.R.; Bilali, H.E.; Simeone, V.; Baser, N.; Mondelli, D.; Cesari, G. (2010) Copper contents in grapes and wines from a Mediterranean organic vineyard. Food Chem. 122: 1338–1343.

Rousseva, M.; Kontoudakis, N.; Schmidtke, L.M.; Scollary, G.R.; Clark, A.C. (2016) Impact of wine production on the fractionation of copper and iron in Chardonnay wine: Implications for oxygen consumption. Food Chem. 203: 440–447.

Siebert, T.E.; Solomon, M.R.; Pollnitz, A.P.; Jeffery, D.W. (2010) Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. J. Agric. Food Chem. 58: 9454–9462.

wards it remained just below the total Cu concentration. A similar increase was observed for another two white wines (data not shown), but the time required for the free Cu concentration in the wine to increase varied from wine to wine (84 hours and 150 hours, respectively). Again, good agreement was evident between the electrochemical and colorimetric measurement of free Cu.

Such a result is consistent with the production of *o*-quinone compounds from phenolic compounds during wine oxidation. Trace amounts of hydrogen sulfide can react with the *o*-quinone and in the process some sulfide-bound Cu will dissociate to hydrogen sulfide and free Cu. With progressive *o*-quinone production, increasing amounts of free Cu will be released from bound Cu until there is little bound Cu remaining. High concentrations of sulfur dioxide and/or ascorbic acid may slow this release of free Cu by competing with hydrogen sulfide for *o*-quinones. Another mechanism of hydrogen sulfide loss from the wine, and concomitant release of free Cu, may be due to volatile loss of hydrogen sulfide.

Conclusion

The Cu in wine can be classified in terms of its binding to sulfide, whereby it is either free or bound. The colorimetric analysis of free and total Cu in wine provides a simple, rapid and cheap means of determining free, bound and total Cu in wine using a spectrophotometer. Such a spectrophotometric technique enables ready access to this technique by winemakers, with the proviso that they can fit a 40 mm cuvette in their spectrophotometer.

The interaction of Cu forms with compounds responsible for 'reduced' aroma in wine can be summarised as follows:

1. Free Cu efficiently reacts with free hydrogen sulfide in wine and can immediately extinguish the off-aroma associated with free hydrogen sulfide.
2. The copper sulfide formed is not easily removed from wine.
3. In some instances, the reaction between free hydrogen sulfide and free copper can also produce precursors (e.g. polysulfanes) that may release hydrogen sulfide during the bottle ageing of wine.
4. The quantification of the hydrogen sulfide precursors (e.g. polysulfanes and others) in wine is not currently possible.
5. Release of hydrogen sulfide from precursors in wine will either lead to binding of free Cu or accumulation of free hydrogen sulfide in wine if free Cu is below a certain concentration. The latter will result in a 'reductive' aroma in wine.
6. The typical magnitude of free Cu that can be bound by hydrogen sulfide precursors during the bottle ageing of wine, and the rate at which this occurs, is not yet known.
7. High oxygen conditions can promote an increase in the proportion of free Cu but may also cause wine oxidation.

Point 6 above means that currently no certain recommendation can be provided for free Cu concentrations at bottling to avoid accumulation of 'reductive' aromas in wine. Future research will aim to provide answers as to whether recommendations for free Cu at bottling are possible. In the meantime, at the very least, the colorimetric measure of free and total Cu in wine will allow winemakers to understand how their current practices with Cu additions set up the different Cu forms at bottling along with total concentrations. If there are significant levels of free Cu at bottling, the technique will also allow the winemaker to gauge how quickly this free Cu is being bound by hydrogen sulfide released from precursors during wine ageing. Such insights will perhaps allow winemakers to refine their Cu usage protocol over time, and this may include targeting specific free Cu concentrations at bottling.

Supporting information

Protocols for the colorimetric measurement of Cu in wine:

1. https://cdn.csu.edu.au/__data/assets/pdf_file/0010/3053539/The-Determination-of-Total-Cu-in-White-Wine.pdf
2. Kontoudakis, N.; Smith, M.; Smith, P.A.; Wilkes, E.N.; Clark, A.C. (2020) The colorimetric determination of copper in wine: total copper. Aust. J. Grape Wine Res. 26: 121–129.
3. Contact aclark@csu.edu.au for a combined free and total Cu in wine methodology.

Acknowledgements

The method for total Cu determination in white wine was developed in collaboration with Dr Paul Smith, Dr Mark Smith and Dr Eric Wilkes (AWRI). The macromolecule binding agents (Table 1) were supplied by Dr Paul Smith (AWRI). This project (NWG1401) was funded by Wine Australia with funds from Australian grapegrowers and winemakers and matching funds from the Australian Government.

References

Bekker, M.Z.; Kreitman, G.Y.; Jeffery, D.W.; Danilewicz, J.C. (2018) Liberation of hydrogen sulfide from dicysteinyl polysulfanes in model wine. J. Agric. Food Chem. 66: 13483–13491.

Boulton, R.B.; Singleton, V.L.; Bisson, L.F.; Kunkee, R.E. (eds) (1999) Principles and practices of winemaking. New York: Springer: p. 339.

Clark, A.C.; Wilkes, E.N.; Scollary, G.R. (2015) Chemistry of copper in white wine: a review. Aust. J. Grape Wine Res. 21: 339–350.

Clark, A.C.; Kontoudakis, N.; Barril, C.; Schmidtke, L.M.; Scollary, G.R. (2016) Measurement of labile copper in wine by medium exchange stripping potentiometry utilising screen printed carbon electrodes. Talanta 154: 431–437.

Clark, A.C.; Kontoudakis, N. (2018) Increasing the efficiency and accuracy of labile Cu measurement in wine using screen-printed electrodes. Chemosensors 6: 35.

Franco-Luesma, E.; Ferreira, V. (2014) Quantitative analysis of free and bonded forms of volatile sulfur compounds in wine. Basic methodologies and evidences showing the existence of reversible cation-complexed forms. J. Chromatogr. A 1359: 8–15.

Junghans, K.; Straube, G. (1991) Biosorption of copper by yeasts. Biol. Met. 4: 233–237.

Kontoudakis, N.; Smith, M.; Guo, A.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2017) The impact of wine components on fractionation of Cu and Fe in model wine systems: Macromolecules, phenolic and sulfur compounds. Food Res. Int. 98: 95–102.

Kontoudakis, N.; Mierczynska-Vasilev, A.; Guo, A.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2019a) Removal of sulfide-bound copper from white wine by membrane filtration. Aust. J. Grape Wine Res. 25: 53–61.

Kontoudakis, N.; Schmidtke, L.M.; Bekker, M.Z.; Smith, M.; Smith, P.A.; Scollary, G.R.; Wilkes, E.N.; Clark, A.C. (2019b) Analytical strategies for the measurement of different forms of Cu and Fe in wine: Comparison between approaches in relation to wine composition. Food Chem. 274: 89–99.

Kontoudakis, N; Clark, A.C. (2020) Sulfide-binding to Cu(II) in wine: Impact on oxygen consumption rates. Food Chem. 305: doi.org/10.1016/j.foodchem.2020.126352

Kreitman, G.Y.; Elias, R.J.; Jeffery, D.W.; Sacks, G.L. (2019) Loss and formation of malodorous volatile sulfhydryl compounds during wine storage. Crit. Rev. Food Sci. Nutr. 59: 1728–1752.

Provenzano, M.R.; Bilali, H.E.; Simeone, V.; Baser, N.; Mondelli, D.; Cesari, G. (2010) Copper contents in grapes and wines from a Mediterranean organic vineyard. Food Chem. 122: 1338–1343.

Rousseva, M.; Kontoudakis, N.; Schmidtke, L.M.; Scollary, G.R.; Clark, A.C. (2016) Impact of wine production on the fractionation of copper and iron in Chardonnay wine: Implications for oxygen consumption. Food Chem. 203: 440–447.

Siebert, T.E.; Solomon, M.R.; Pollnitz, A.P.; Jeffery, D.W. (2010) Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. J. Agric. Food Chem. 58: 9454–9462.

Yeast–bacteria compatibility in wine: it's complicated!

L. Bartle[1], K. Sumby[1,2], J. Sundstrom[1,2], J.G. Mitchell[3], V. Jiranek[1,2]

[1] Department of Wine and Food Science, The University of Adelaide, Urrbrae, SA
[2]Australian Research Council Training Centre for Innovative Wine Production, Urrbrae, SA
[3] Flinders University, College of Science and Engineering, Adelaide, SA
vladimir.jiranek@adelaide.edu.au

Abstract

Summers are getting hotter, vintages are becoming compressed and panic ensues. But not all hope is lost, because one way to start taking the pressure off is by having more efficient fermentation. Winemaking relies heavily on two types of microorganisms, yeast and lactic acid bacteria (LAB), to conduct alcoholic and malolactic fermentation (AF and MLF), respectively. Using a process called co-inoculation, yeast and LAB can simultaneously perform AF and MLF, thereby reducing overall fermentation time. However, this only works if yeast and LAB are compatible.

In this study, 72 commercial yeast-LAB pairs were tested for compatibility during co-inoculation in a chemically defined grape juice medium. From this, eight pairs were selected based on their ranking as the four most compatible and four least compatible. These pairs were further tested in sterile Shiraz juice, which revealed that compatibility of yeast and LAB was dependent on strain and a combination of metabolites that may affect MLF performance. Additionally, the ability of LAB to maintain a critical density was deemed an important factor for MLF completion.

As well as the results obtained from this study, other works have identified specific LAB modulation of yeast metabolism via induction of the [GAR$^+$] prion. This extends understanding of the complexity of yeast-LAB compatibility during fermentation.

Overall these studies show that compatibility is primarily dependent on yeast and LAB strain. Yeast [GAR$^+$] prion can only be induced by some LAB strains, so choosing a [GAR$^+$]-inducing LAB can lead to slower AF and successful MLF. Conversely, yeasts produce inhibitory compounds, such as succinic acid (ranging between 9 and 17 g/L), and therefore choosing low succinic acid-producing yeasts may help ensure co-inoculation success.

Introduction

The impact of a compressed vintage was evident again in the 2019 Australian harvest (Fulloon 2019; Halliday Wine Companion 2019). Many regions around Australia experienced fast ripening of both white and red fruit resulting in a need for efficient winery processes. One way that wineries may be able to manage a compressed vintage is by using co-inoculation fermentation strategies. Co-inoculation is the addition of yeast and bacteria to a juice within 48 hours of each other, allowing simultaneous AF and MLF (Bartle et al. 2019). Malolactic fermentation is performed with some white grape varieties but is most often implemented for red wines. Co-inoculation of yeast and LAB can provide numerous benefits, the most noticeable being reduced overall fermentation time. But co-inoculation only occurs efficiently if the yeast and bacteria are compatible.

Compatibility between yeast and LAB is confounded by numerous chemical and physical parameters. Yeast metabolites such as SO_2, ethanol and medium-chain fatty acids, as well as the ability of LAB to modulate yeast behaviour, all play a role in the complexity of yeast-bacteria compatibility (Capucho and San Romão 1994; Lonvaud-Funel 1995; Ramakrishnan et al. 2016). This paper presents key findings from a study of yeast-bacteria compatibility in a synthetic medium and subsequent Shiraz juice fermentations.

Compatibility in a synthetic juice

There are hundreds of yeasts and bacteria available for winemakers to purchase for their fermentations. It is understood that not all yeast and bacteria will work effectively together, so an experiment was designed to test compatibility in a synthetic juice. Synthetic juice provides a controllable and repeatable medium to test fermentation, and was the first step in observing compatibility between multiple yeast and bacteria strains. Fermentations were performed under controlled temperature at a laboratory scale of 100 mL. The AF and MLF performance of eight yeast with nine bacteria was assessed to generate a compatibility list for the 72 combinations. The eight yeast consisted of five *Saccharomyces cerevisiae* strains (SC1 to SC5), one *Saccharomyces uvarum* strain (SU) and two non-*Saccharomyces* strains (LT, TD). The nine bacteria tested included seven *Oenococcus oeni* strains (O1 to O7) and two *Lactobacillus plantarum* strains (Lp1 and Lp2).

Compatibility between yeast and bacteria was determined by bacteria completing MLF (reducing L-malic acid concentration to less than 0.1 g/L), and yeast AF progress remaining unaffected by co-inoculation (determined by AF comparison with yeast-only controls). It was expected that LT and TD would not complete AF because non-*Saccharomyces* yeast often do not complete AF alone, and need an inoculation with *S. cerevisiae* to complete AF (Ciani et al. 2016; Bartle et al. 2019). Despite this, gathering information about pure non-*Saccharomyces* compatibility with bacteria is useful information for the current trends of indigenous and organic winemaking.

Both *Lactobacillus plantarum* strains were incompatible with all yeast. They were unable to survive the fermentation conditions, which indicated that the synthetic medium used for these experiments may not be suitable for *Lb. plantarum* growth, or the yeast strains used were incompatible specifically with *Lb. plantarum*. Much more work is needed to elucidate a suitable growth medium for co-inoculation experiments using *Lb. plantarum* and a greater list of yeast-*Lb. plantarum* co-inoculations should be performed.

Two of the five *S. cerevisiae* strains used in this project, SC3 and SC5, were incompatible with all nine bacteria. The incompatibility seen for both of these strains could not be fully attributed to AF completion speed, and a more in-depth analysis of these strains is also required.

For the remaining 42 yeast-*O. oeni* co-inoculation combinations, 24 were compatible and 18 were incompatible. Non-*Saccharomyces* strains LT and TD were compatible with O1-3, O5 and O6. The SU strain was compatible with O1, O3-4 and O6-7. Strains SC1, SC2 and SC4 were compatible with the following bacteria: SC1 and O3, O6-7; SC2 and O1, O6; SC4 and O2, O4-5 and O7.

It was observed from this initial work that compatibility between yeast and bacteria is strain dependent, which led to a subsequent experiment in Shiraz juice.

The list of 72 yeast-bacteria pairs was reduced to eight pairs for further testing in Shiraz juice. More specifically, a 'top four' and 'bottom four' were chosen based on the following information:

Top four: four fastest completers of MLF: SC2 + O6, SC4 + O4, SC4 + O7 and SU + O7.

Bottom four: four pairs with highest residual malic acid concentration (excluding *Lb. plantarum* strains): SC1 + O1, SC1 + O4, SC2 + O5 and SC4 + O1.

Compatibility in Shiraz juice

Fermentations of sterile-filtered Shiraz grape juice were also conducted at laboratory scale, (150 mL) and kept at a constant temperature. A range of compounds and parameters were measured to determine factors that may influence yeast-bacteria compatibility. These included: pH; final SO_2 concentration; volatile compounds (GC-MS); organic compounds (HPLC); final ethanol concentration; yeast and bacterial viability (flow cytometry and spot plating methods); starting and final amino acid concentration (HPLC); and AF and MLF progression (enzymatic assays).

Of the eight yeast-bacteria pairs tested, four switched compatibility status: SC1 + O1, SC1 + O4 and SC2 + O5 switched from incompatible in synthetic medium to compatible in Shiraz juice; and SC4 + O4 switched from being compatible in synthetic medium to incompatible in Shiraz juice. The switch in compatibility status cannot be explained by the data analyses performed, but is most likely a consequence of using a more complex growth environment, Shiraz juice, compared to the synthetic medium that may not include other required nutrients (that are currently unknown) for growth, AF and MLF processes.

Concentrations of volatile compounds, ethanol and SO_2 were correlated with yeast strain, while pH was correlated with successful co-inoculation. These results were not surprising due to the well-documented ability of yeast to drive volatile compound production (Antonelli et al. 1999; Lopandic et al. 2007), ethanol and SO_2 release (Osborne and Edwards 2006; Wells and Osborne 2011). Different yeast strains produce different concentrations of important sensory compounds in wine and also produce varying concentrations of ethanol and SO_2 during AF.

The correlation of pH with successful co-inoculation was also unsurprising since MLF is used to de-acidify wines. The production of the less acidic lactic acid during MLF contributes greatly to the increase in pH.

The experiment revealed that succinic acid concentration inversely correlated with compatibility (Table 1). In fermentations where MLF was successful, the succinic acid concentration was significantly lower than the yeast-only control ($p < 0.005$). This could be explained by two potential mechanisms: the bacteria taking up succinic acid from the environment and/or the bacteria modulating the succinic acid production of the yeast. If bacteria were taking succinic acid from the medium, it would be expected that bacteria with a higher uptake would conduct slower MLF. This assumption is based on succinic acid acting as a competitive inhibitor of the malolactic enzyme (Reguant et al. 2005). Succinic acid competes with malic acid for the active site of the malolactic enzyme in *Oenococcus oeni*, thereby slowing or inhibiting MLF. However, the results in this study did not agree with this assumption since succinic acid uptake did not positively correlate with MLF speed (Table 1). This may be due to a matrix effect involving citric acid and ethanol, which are two other known competitive inhibitors for the malolactic enzyme (Reguant et al. 2005).

Alternatively, the ability of bacteria to modulate yeast's production of certain molecules is not unreasonable since bacterial modulation of yeast behaviour has been reported previously (Garcia et al. 2016; Ramakrishnan et al. 2016; Gonzalez et al. 2018).

In the two fermentations where MLF was not completed by bacteria, there was significantly more succinic acid produced compared to the yeast-only control ($p < 0.005$). From this observation it is speculated that yeast may be producing more succinic acid in response to co-inoculation with bacteria, although no significant changes in succinic acid have been reported previously (Abrahamse and Bartowsky 2012). For the other co-inoculations where MLF was successful, it is unknown whether yeast were producing more succinic acid since only the succinic acid concentration at the end point of fermentations were measured. A more in-depth study of succinic acid production over time comparing yeast alone and in co-inoculation with bacteria should be performed to identify if succinic acid production by yeast is a response to bacterial competition.

Bacterial modulation of yeast behaviour

Lactic acid bacteria may have the ability to modulate yeast behaviour. One example of this is *O. oeni's* ability to induce the yeast [*GAR*$^+$] prion in wine-like conditions (Garcia et al. 2016; Ramakrishnan et al. 2016; Gonzalez et al. 2018). Prions are misfolded proteins that cause otherwise healthy proteins to misfold and perform a particular function. The [*GAR*$^+$] prion causes a shift in the preferential glucose metabolism of *S. cerevisiae* that leads to utilisation of alternative carbon sources (Jarosz et al. 2014; Walker et al. 2016). It has been reported that under wine-like conditions the [*GAR*$^+$] prion occurs in 50-60% of the yeast population (Ramakrishnan et al. 2016) and therefore does not affect the completion rate of AF. It has also been reported that [*GAR*$^+$] prion phenotypes are not present in every *S. cerevisiae* strain (Gonzalez et al. 2018).

The [*GAR*$^+$] prion has been proposed to be induced by several mechanisms involving *O. oeni*. Lactic acid (Garcia et al. 2016) and acetic acid (Ramakrishnan et al. 2016) have both been identified as molecules that induce [*GAR*$^+$] prion in *S. cerevisiae*, as well as physical proximity of *O. oeni* to the yeast (Ramakrishnan et al. 2016). The ability of *O. oeni* to induce the [*GAR*$^+$] prion is strain specific, with commercial strains reportedly having a higher likelihood of prion induction (Ramakrishnan et al. 2016).

Just as is seen with [*GAR*$^+$] prion induction, wine bacteria may be able to modulate the production of other metabolites, including succinic acid. However more research is needed to identify mechanisms involved in bacterial modulation of yeast metabolism during co-inoculation.

Table 1. Succinic acid concentration (g/L) measured at the end of fermentations. Different letters indicate significant differences $p < 0.005$

Yeast	Bacteria	Succinic acid (g/L)	MLF completed?	MLF speed
SC1	None	9.7 ± 0.2 B,D	n/a	n/a
	O1	7.6 ± 0.1 E	Yes	Slow
	O4	7.7 ± 0.0 E	Yes	Slow
SC2	None	9.6 ± 0.2 A,D	n/a	n/a
	O5	6.8 ± 0.3 C	Yes	Fast
	O6	7.1 ± 0.1 C,E	Yes	Slow
SC4	None	9.0 ± 0.0 A	n/a	n/a
	O1	9.9 ± 0.3 B,D	No	n/a
	O4	10.3 ± 0.1 B	No	n/a
	O7	7.4 ± 0.0 C,E	Yes	Fast
SU	None	17.1 ± 0.3 F	n/a	n/a
	O7	15.3 ± 0.2 G	Yes	Slow

What does all of this mean?

Predicting yeast-bacteria compatibility for co-inoculation is not straightforward. Compatibility between yeast and bacteria is confounded heavily by strain, juice and vintage. This study provides a foundation for future research into identification of key factors that may be used to indicate the likelihood of yeast-bacteria compatibility in different juice types. A comprehensive survey of different yeast-bacteria pairs in a range of juice types is needed to pinpoint the main drivers of compatibility. Once this work has been completed, scaling up to volumes more comparable to commercial winemaking and investigating the influence of large-scale winemaking conditions (e.g. unregulated temperatures, mechanical processes) on compatibility outcomes should be performed.

Acknowledgements

This work was supported by Australia's grapegrowers and winemakers through their investment body, Wine Australia, with matching funds from the Australian Government. Louise Bartle is supported by scholarships from the University of Adelaide and Wine Australia (AGW Ph 1510). Vladimir Jiranek and Joanna Sundstrom are supported by Wine Australia project funding (UA 1803-2.1). Joanna Sundstrom, Krista Sumby and Vladimir Jiranek are supported by 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 and industry partners. The University of Adelaide is a member of the Wine Innovation Cluster in Adelaide.

References

Abrahamse, C.E.; Bartowsky, E.J. (2012) Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. World J. Microbiol. Biotechnol. 28: 255–265.

Antonelli, A.; Castellari, L.; Zambonelli, C.; Carnacini, A. (1999) Yeast influence on volatile composition of wines. J. Agric. Food Chem. 47: 1139–1144.

Bartle, L.; Sumby, K.; Sundstrom, J.; Jiranek, V. (2019) The microbial challenge of winemaking: yeast-bacteria compatibility. FEMS Yeast Res. 19: foz040.

Capucho, I.; San Romão, M.V. (1994) Effect of ethanol and fatty acids on malolactic activity of *Leuconostoc oenos*. Appl. Microbiol. Biotechnol. 42: 391–395.

Ciani, M.; Morales, P.; Comitini, F.; Tronchoni, J.; Canonico, L.; Curiel, J.A.; Oro, L.; Rodrigues, A.J.; Gonzalez, R. (2016) Non-conventional yeast species for lowering ethanol content of wines. Front. Microbiol. 7: 642.

Fulloon, S. (2019) Australia's wine industry under threat after record summer heatwaves. SBS News. 4 March: https://www.sbs.com.au/news/australia-s-wine-industry-under-threat-after-record-summer-heatwaves

Garcia, D.M.; Dietrich, D.; Clardy, J; Jarosz, D.F. (2016) A common bacterial metabolite elicits prion-based bypass of glucose repression. Elife. 5: e17978.

Gonzalez, R.; Tronchoni, J.; Mencher, A.; Curiel, J.A.; Rodrigues, A.J.; López-Berges, L.; Juez, C.; Patil, K.R.; Jouhten, P.; Gallego, N.; Omarini, A. (2018) Low phenotypic penetrance and technological impact of yeast [*GAR*$^{+}$] prion-like elements on winemaking. Front. Microbiol. 9: 3311.

Halliday Wine Companion (2019) A first look into the Australian wine vintage 2019. 24 April: https://www.winecompanion.com.au/articles/news/a-first-look-into-australian-wine-vintage-2019

Jarosz, D.F.; Brown, J.C.; Walker, G.A.; Datta, M.S.; Ung, W.L.; Lancaster, A.K.; Rotem, A.; Chang, A.; Newby, G.A.; Weitz, D.A.; Bisson, L.F. (2014) Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism. Cell 158: 1083–1093.

Lonvaud-Funel, A. (1995) Microbiology of the malolactic fermentation: molecular aspects. FEMS Microbiol. Lett. 126: 209–214.

Lopandic, K.; Gangl, H.; Wallner, E.; Tscheik, G.; Leitner, G.; Querol, A.; Borth, N.; Breitenbach, M.; Prillinger, H.; Tiefenbrunner, W. (2007) Genetically different wine yeasts isolated from Austrian vine-growing regions influence wine aroma differently and contain putative hybrids between *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. FEMS Yeast Res. 7: 953–965.

Osborne, J.P; Edwards, C.G. (2006) Inhibition of malolactic fermentation by *Saccharomyces* during alcoholic fermentation under low-and high-nitrogen conditions: a study in synthetic media. Aust. J. Grape Wine Res. 12: 69–78.

Ramakrishnan, V.; Walker, G.A.; Fan, Q.; Ogawa, M.; Luo, Y.; Luong, P.; Joseph, C.M.; Bisson, L.F. (2016) Inter-kingdom modification of metabolic behavior: [*GAR*+] prion induction in *Saccharomyces cerevisiae* mediated by wine ecosystem bacteria. Front. Ecol. Environ. 4: 137.

Reguant, C.; Carreté, R.; Ferrer, N.; Bordons, A. (2005) Molecular analysis of *Oenococcus oeni* population dynamics and the effect of aeration and temperature during alcoholic fermentation on malolactic fermentation. Int. J. Food Sci. Technol. 40: 451–459.

Walker, G.A.; Hjelmeland, A.; Bokulich, N.A.; Mills, D.A.; Ebeler S.E.; Bisson L.F. (2016) Impact of the [*GAR*+] prion on fermentation and bacterial community composition with *Saccharomyces cerevisiae* UCD932. Am. J. Enol. Vitic. 67: 296–307.

Wells, A.; Osborne, J.P. (2011) Production of SO_2 binding compounds and SO_2 by *Saccharomyces* during alcoholic fermentation and the impact on malolactic fermentation. S. Afr. J. Enol. Vitic. 32: 267–279.

Developing next-generation grapevine rootstocks with long-term resistance to phylloxera and root knot nematode

H.M. Smith, J.D. Dunlevy, N.B. Morales, A.A. Smith, P.R. Clingeleffer

CSIRO Agriculture and Food, Urrbrae, SA
harley.smith@csiro.au

Abstract

Rootstocks are a key management tool for increasing vineyard performance by safeguarding winegrape varieties from soil-borne pests. However, the current set of rootstocks that provide resistance to phylloxera and root knot nematode are derived from a limited number of breeding lines. As a result, these rootstocks likely inherited similar resistance mechanism(s) to phylloxera and root knot nematode. This is a major concern, as a breakdown in phylloxera and/or root knot nematode resistance would severely limit rootstock options for replanting. The CSIRO Rootstock Breeding Team is using next-generation technologies combined with rapid phenotyping methods to develop elite rootstocks with new pedigrees for long-term resistance to phylloxera and root knot nematode.

Introduction

Rootstocks are used throughout most grapevine production areas of the world, as a means to improve vineyard performance (Walker and Clingeleffer 2009; Whiting 2012). Rootstocks were initially bred to limit the impact of phylloxera on grapevine production (Dunlevy et al. 2019; Riaz et al. 2019). Additional traits were also selected to safeguard vines against other soil-borne pests including root knot nematode, as well as abiotic stresses. Most of the commercial rootstocks were developed over 100 years ago in Europe. As a result, these rootstocks lack key traits for optimal performance under Australian conditions (Walker and Clingeleffer 2009; Whiting 2012). Moreover, the narrow genetic base of resistance in commercial rootstocks combined with changes in climate and pest pressures indicate that these rootstocks lack durable resistance to phylloxera and root knot nematode (Dunlevy et al. 2019; Riaz et al. 2019). Whiting (2012) indicated that the development of new rootstocks for Australian conditions is required in order to provide the grapevine industry with a better selection of rootstocks for sustainable vineyard management and profitable winegrape production. To overcome the limitations of commercial rootstocks, the CSIRO Rootstock Breeding Team is using new breeding approaches to efficiently develop and evaluate new rootstocks for Australian conditions with durable resistance to phylloxera and root knot nematode (Dunlevy et al. 2019).

Biosecurity threats to Australian viticulture

The majority of winegrape varieties used in production are derived from *Vitis vinifera*. Current studies estimate that 75% of vines in South Australia are maintained on own roots (*Vitis vinifera*) (Logan 2018). In the Murray Darling and New South Wales, only 28% and 34% of the planted vines were established on rootstocks, respectively. Due to the lack of rootstock adoption in many winegrape production regions, grape phylloxera is the number one biosecurity risk to Australian viticulture.

Vitis vinifera winegrape varieties used in production are native to Eurasia and have no resistance to grape phylloxera, which is native to North America (This et al. 2006; Riaz et al. 2019). As a result, this insect pest effectively feeds and reproduces on young and lignified roots. Feeding sites established on lignified roots are a major problem, as these galls often crack, allowing pathogenic fungi to enter into the plant, which ultimately results in vine death. In contrast to *Vitis vinifera*, North American *Vitis* species that co-evolved with grape phylloxera display resistance or tolerance to this insect pest. In tolerant rootstocks, grape phylloxera feeding occurs only on young roots but not on lignified roots. Therefore, vine death does not occur in tolerant rootstocks, as galls produced on young roots do not provide an entry point for fungal pathogens.

In Australia, phylloxera is managed in part by quarantine or phylloxera management zones (Powell 2008). Phylloxera Infested Zones (PIZs) function to confine phylloxera by managing the movement of equipment and grape material in order to minimise the transfer of grape phylloxera to production regions devoid of this insect pest. To date, PIZs are located in Victoria and New South Wales, while South Australia, Tasmania and Western Australia are known as phylloxera-free zones. While PIZs provide a level of protection for the phylloxera-free zones, the restricted movement of this insect pest is not guaranteed. For example, since the first detection of grape phylloxera approximately 13 years ago, the Maroondah PIZ boundary has expanded nine times due to new detections of this insect. Since March 2019, three new detections have occurred in this PIZ, with two detections found outside the PIZ. The potential spread of phylloxera is a reminder that phylloxera-resistant rootstocks are the best management tool to protect vineyards from this insect pest.

In the mid-1800s, the accidental introduction of grape phylloxera from North America to Europe devastated the wine and grape production industries (Forneck and Huber 2009; Powell 2012). While *Vitis vinifera* varieties have no resistance to grape phylloxera, it was recognised that North American *Vitis* species could be used as rootstocks to protect the Eurasian winegrape varieties from this insect pest. As a result, rootstock breeding efforts, which involved the hybridisation of North American *Vitis* species, were initiated and the first set of phylloxera-resistant and tolerant rootstocks were released in the late 1800s in Europe (Ollat et al. 2016). Many of these rootstocks are used today throughout the world to maintain production in the presence of grape phylloxera. However, the genetic diversity of these rootstocks is narrow, as this material was developed from a limited number of *Vitis berlandieri*, *Vitis riparia* and *Vitis rupestris* varieties (Riaz et al. 2019). In California and Europe, studies indicate that phylloxera strains are adapting to feeding and reproducing on tolerant rootstocks with a *Vitis riparia* pedigree (including 101-14), as infestation levels have increased on the roots of these rootstocks (Kocsis et al. 1999, 2002; Lund et al. 2017; Riaz et al. 2019). Evaluation of roots from 101-14 grafted vines infested with grape phylloxera indicates that high levels of feeding are reducing vine health in some California vineyards (Cooper 2012; Stamp 2011). However, additional studies are required to further evaluate the impact of high grape phylloxera infestation on vine health.

Root knot nematodes are sedentary endoparasitic nematodes that feed and reproduce on the roots of susceptible *Vitis* species and winegrape varieties (Walker and Stirling 2008). Root knot nematodes are a major risk to Australian viticulture, particularly in sandy soil

Mediterranean environments, as new aggressive root knot nematodes have emerged in the past eight years (Dunlevy et al. 2019; Smith et al. 2016). Moreover, as the severity of heatwaves is expected to increase due to climate change, there is concern that the root knot nematode-resistant mechanism(s) displayed in a subset of rootstocks may be compromised when soil temperatures exceed 30°C (Ferris et al. 2013). The Ramsey rootstock provides sufficient protection against root knot nematode, which contributed to the popularity of this rootstock in the 1980s and early 1990s (Walker et al. 1994; Walker and Clingeleffer 2009; Smith et al. 2016). However, the use of Ramsey in hot climates reduces red wine quality (Walker and Clingeleffer 2009). It has been speculated that the increase in vigour induced by hot climate conditions contributes to the decrease in red wine quality. Currently, 1103 Paulsen is the most popular rootstock used in Australian grapevine production. Compared to Ramsey, 1103 Paulsen displays moderate to high resistance to root knot nematode with lower levels of vigour. The root knot nematode resistance and vigour traits, as well as other favourable characters, are associated with the increase in popularity of 1103 Paulsen, particularly in hot climates (Walker and Clingeleffer 2009). However, the durability of 1103 Paulsen's resistance to root knot nematode has been compromised in many production regions due to the emergence of new aggressive populations found in McLaren Vale, Barossa, Riverland and Sunraysia (Dunlevy et al. 2019). Due to widespread usage of 1103 Paulsen, the potential spread and emergence of aggressive root knot nematode populations could further impact grapevine production.

While rootstocks are a major sustainable management tool for increasing vineyard performance, the lack of genetic diversity in commercial rootstocks may not provide a long-term solution for dealing with phylloxera and root knot nematode. Therefore, there is an imperative need to develop durable phylloxera- and root knot nematode-resistant rootstocks for Australian conditions bred from unique and diverse North American *Vitis* species.

Next-generation mapping for phylloxera and root knot nematode resistance

Breeding new rootstocks with resistance to phylloxera and root knot nematode is costly and time-consuming. Moreover, phylloxera and root knot nematode resistance traits found in North American *Vitis* species are limited. Therefore, to safeguard these traits for long-term pest resistance it is recommended that two or more resistance traits for each pest be combined into a single rootstock (Dunlevy et al. 2019). For durability, it is essential that the resistance traits function in a non-redundant manner. The most effective approach for combining traits is DNA marker-assisted selection.

DNA markers used for selecting individuals with resistance traits are identified via genetic mapping. Next-generation sequencing is a rapid and cost-effective approach for DNA marker discovery and genetic mapping studies. Figure 1 illustrates the methodology used to genetically map the root knot nematode (*MELOIDOGYNE JAVANICA RESISTANCE 1*; *MJR1*) and phylloxera (*RESISTANCE TO DAKTULOSPHAIRA VITIFOLIAE 2*; *RDV2*) resistance traits from *Vitis cinerea* C2-50. A filial (F_1) population consisting of ~100 individuals was established by crossing *Vitis cinerea* C2-50 with the *Vitis vinifera* cultivar Riesling. C2-50 provides complete resistance to the aggressive root knot nematode, *Meloidogyne javanica* 'pt 1103P' and G1 grape phylloxera, while Riesling is highly susceptible to these pests (Smith et al. 2018a, b). *Meloidogyne javanica* 'pt 1103P' and G1 grape phylloxera-resistant and susceptible phenotypes for C2-50, Riesling and F_1 individuals were determined, and heritability studies showed that C2-50 harbours a single resistance trait for each soil pest (Figure 1). The next-generation DNA marker discovery approach called genotyping-by-sequencing was used to identify a set of segregating markers called single nucleotide polymorphisms (SNPs; Figure 1). After next-generation sequencing, the reads were aligned to the sequenced grape reference genome to identify a set of SNPs. SNPs were processed and parsed to identify two sets of high-quality segregating molecular markers, which were used to create genetic maps for C2-50 and Riesling (Figure 1). Results of genetic mapping showed that the *MJR1* and *RDV2* traits localised to chromosome 18 and 14, respectively, in C2-50. Moreover, numerous SNPs tightly linked to *MJR1* and *RDV2* were identified that flank and cosegregate with these traits (Smith et al. 2018a, b).

A subset of SNPs tightly linked to *MJR1* and *RDV2* have been developed for marker-assisted selection. These SNPs will be used to indirectly select root knot nematode and phylloxera resistance traits in progeny derived from targeted breeding crosses. As a result, hundreds to thousands of progeny derived from these breeding crosses can be rapidly screened with these molecular markers to identify individuals containing *MJR1* and *RDV2*. This molecular marker-based screening is key not only for stacking traits but it also overcomes the use of laborious glasshouse screening assays for identifying resistant individuals.

Rapid phenotyping of F_1 populations for the genetic mapping of root knot nematode resistance traits

Another major source of root knot nematode resistance is found in *Vitis champinii*, a North American grapevine species (Walker et al. 1994). The mode of resistance in *Vitis champinii* is non-redundant with *Vitis cinerea* (Cousins 2007). Therefore, durable resistance against root knot nematode can be achieved by combining the *MJR1* from *Vitis cinerea* C2-50 with *MJR2* from *Vitis champinii*. Mapping populations for *Vitis champinii* × Riesling have been established by the CSIRO Rootstock Breeding Team. Preliminary heritability studies indicate that *Vitis champinii* contains a single root knot nematode resistance trait called *MJR2*. Additional North American *Vitis* species resistant to root knot nematode have been identified in the CSIRO grapevine germplasm collection (Smith et al. 2016). The major challenge for mapping and identifying molecular markers linked to

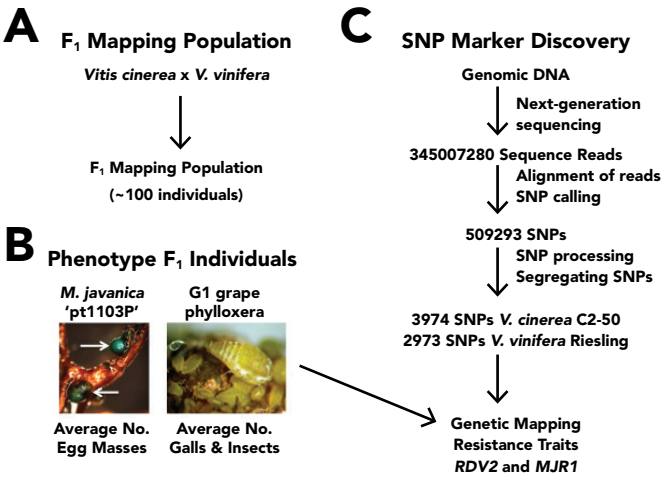


Figure 1. Summary of next-generation genetic mapping of phylloxera and root knot nematode resistance traits in *Vitis cinerea* C2-50. (A) A F_1 mapping population was created by crossing the phylloxera and root knot nematode resistance *Vitis cinerea* C2-50 with *Vitis vinifera* Riesling, which is highly susceptible to these soil pests. A F_1 mapping population consisting of ~100 individuals was established. (B) After propagation, F_1 individuals were screened in triplicate with G1 grape phylloxera and *Meloidogyne javanica* 'pt 1103P'. After screening, susceptible and resistant phenotypes were determined for each F_1 individual. (C) Genomic DNA was isolated from the F_1 individuals, *Vitis cinerea* C2-50 and *Vitis vinifera* Riesling and next-generation sequencing was performed. Sequence reads from each genotype were aligned to the grapevine reference genome and 509,293 molecular markers called single nucleotide polymorphisms (SNPs) were identified. Next, high quality SNPs were identified by processing and segregating SNPs for *Vitis cinerea* C2-50 and *Vitis vinifera* Riesling. The segregating SNPs were used to generate genetic maps for each parent. Finally, the phenotype data from the F_1 individuals was used to identify the genetic map position of *MJR1* and *RDV2* resistance traits in *Vitis cinerea* C2-50.

MJR2 and other root knot nematode resistance traits is to overcome the inefficiencies of the glasshouse-based screening assay used to phenotype F_1 individuals.

While the efficacy of the root knot nematode glasshouse screening assay has improved (Smith et al. 2016), it is very laborious and typically takes two years to determine the resistant and susceptible phenotypes for each F_1 individual (Figure 2). Furthermore, root knot nematode screening is restricted in that it can only be performed from October to April, as nematode activity is extremely low during the winter months in the glasshouse. Lastly, during the screening assay, the egg masses scored are used to identify F_1 individuals that are susceptible (Figure 2). Individuals with no egg masses are classified as resistant. It would be extremely beneficial to have a resistance phenotype to score in order to confirm the presence of a resistance trait in an F_1 individual.

The development of an *in vitro* root knot nematode screening assay was used to identify the biological basis of resistance in C2-50 (Smith et al. 2018b), as well as other North American *Vitis* species. A sterile population of *Meloidogyne javanica* 'pt 1103' was established and maintained on *Cucumis sativus* roots. In this procedure, infectious nematodes were harvested and used to infect the roots of sterile *Vitis* material maintained under aseptic conditions (Figure 3). In the absence of *Meloidogyne javanica* 'pt 1103', control roots were devoid of galls and localised areas of cell necrosis (Figure 3A, D). However, after inoculation with *Meloidogyne javanica* 'pt 1103', regions of cell necrosis in the root meristem or cortex was observed in the roots of resistant North American *Vitis* species (Figure 3B, C). In contrast, gall and egg masses developed on the roots of *Vitis vinifera* after inoculation with *Meloidogyne javanica* 'pt 1103' (Figure 3E, F).

The root knot nematode *in vitro* screening assay is a valuable tool used to increase the efficiency for phenotype determination (Figure 3). First, the ability to phenotype for resistance and susceptibility using the *in vitro* system increases the accuracy of phenotyping. Second, after inoculation with *Meloidogyne javanica* 'pt 1103', the resistant and susceptible phenotypes of the roots are determined within 14 days. In contrast to the glasshouse screening assay, the *in vitro* screen can be performed throughout the year, as the propagated grapevines and *Meloidogyne javanica* 'pt 1103' are maintained at constant temperature and light conditions. While establishing sterile grapevines for *in vitro* screening is time-consuming (Smith et al. 2018b), an aseptic population of F_1 plants can be easily propagated by simply sterilising seeds prior to germination. Compared to the glasshouse screening assay, the phenotype of F_1 plants can be determined in less than four months using the *in vitro* root knot nematode screening assay. As a result, the *in vitro* screening system serves as a rapid phenotyping system that substantially increases the efficiency of the genetic mapping of root knot nematode resistance traits (Figure 3). It would be extremely beneficial to establish a similar system for determining phylloxera resistance and susceptibility in F_1 populations.

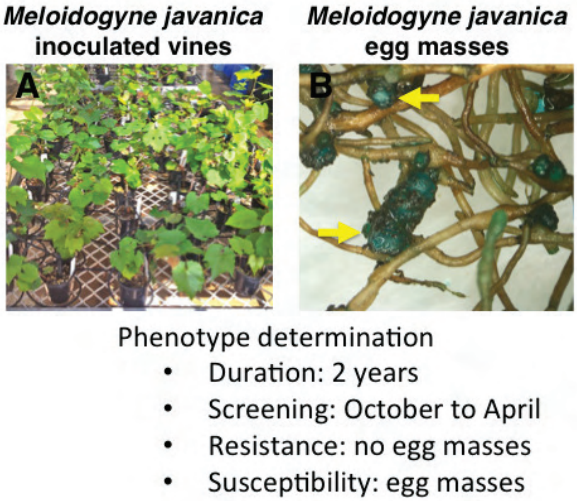


Figure 2. Phenotype determination for root knot nematode resistance using the glasshouse screening assay. The duration for determining the phenotype for each F_1 individual in triplicate is at least two years. This is primarily due to the fact that screening can only be performed from the end of October to the beginning of April, as nematode activity significantly declines during the winter months. (A) After inoculation, sufficient egg mass development occurs at 60 days and (B) the average number of egg masses are scored for each F_1 individual. F_1 vines containing egg masses on roots are classified as susceptible; whereas vines with no egg masses are classified as resistant.

First-generation rootstocks with durable resistance to phylloxera and root knot nematode

Börner is a rootstock derived from a cross between *Vitis riparia* and *Vitis cinerea* Arnold. To date, it is the only commercial rootstock that has complete resistance to phylloxera. Experimental studies showed that phylloxera resistance is mediated by a single resistance trait called *RESISTANCE TO DAKTULOSPHAIRA VITIFOLIAE 1* (*RDV1*), which is derived from *V. cinerea* Arnold (Zhang et al. 2009). DNA markers flanking *RDV1* were identified and used for marker-assisted selection of new grapevine varieties with phylloxera resistance (Hausmann et al. 2012). It is interesting to note that *RDV1* and *RDV2* are derived from two different *V. cinerea* accessions. The fact that *RDV1* and *RDV2* map to chromosome 13 and 14, respectively, indicates that the mode of resistance may be non-redundant. Therefore, combing *RDV1* with *RDV2* is a feasible approach to develop rootstocks with long-term resistance to phylloxera.

A breeding scheme has been developed to breed the first set of next-generation rootstocks via marker-assisted breeding (Dunlevy et al. 2019). The first step in the breeding scheme is to cross *Vitis cinerea* C2-50 × Börner. Seedlings produced from this cross will be screened with the *RDV1*- and *RDV2*-linked DNA markers to identify F_1 individuals containing these two phylloxera-resistance traits. Subsequently, DNA markers linked to *MJR1* will be used to identify seedlings containing this root knot nematode resistance trait. Selected individuals harbouring all three resistance traits will then be crossed to *Vitis champinii* to introduce *MJR2*, and seedling progeny

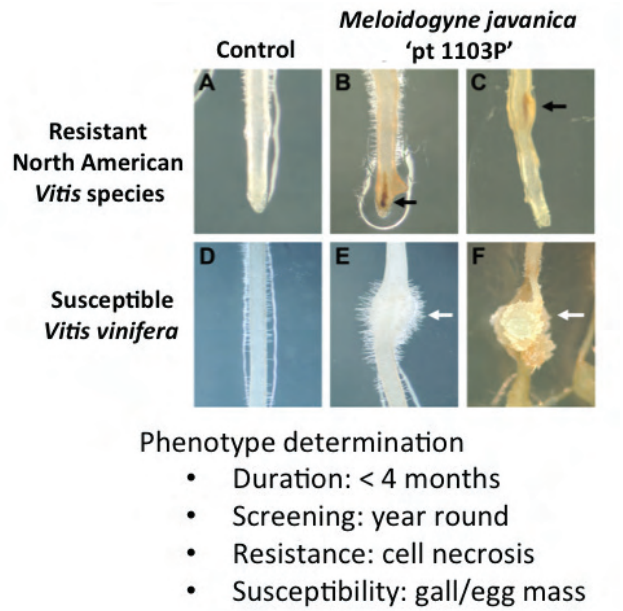


Figure 3. The *in vitro* root knot nematode screening assay is a rapid system to determine resistance and susceptibility phenotypes. (A and D) Control roots were devoid of cell necrosis as well as gall and egg mass development. (B and C) *Meloidogyne javanica* 'pt 1103P' induced cell necrosis in the roots of resistant North American *Vitis* species. Cell necrosis occurred in the root meristem and cortex cells of the roots. (E and F) After the addition of *Meloidogyne javanica* 'pt 1103P' to *Vitis vinifera* roots, gall and egg mass development occurred. The duration for determining the phenotype for each F_1 individual using the *in vitro* screening assay is <four months. This system is highly efficient, as screening can be performed year round in environmentally controlled incubators. Second, it takes approximately 14 days to score the roots of each vine. Furthermore, using this system, both resistance and susceptibility phenotypes can be scored by the presence of cell necrosis and gall/egg mass development, respectively.

from this cross will be screened with DNA markers linked to the two phylloxera and two root knot nematode resistance traits. Selected individuals containing *RDV1*, *RDV2*, *MJR1* and *MJR2* will undergo glasshouse phylloxera and root knot nematode screening assays to confirm resistance to these soil pests (Dunlevy et al. 2019).

The second set of next-generation rootstocks will be developed from additional North American species and hybrid material to further increase the genetic diversity of rootstocks. Marker-assisted selection will be used to combine new phylloxera and root knot nematode traits to maintain durability. In addition, DNA markers linked to salinity tolerance traits, which function to exclude sodium and chloride ions from the leaves and berries, will be used in this breeding scheme for increasing vineyard performance in production areas with saline soils.

Evaluation of marker-assisted selected rootstocks

Elite next-generation rootstocks will be selected in nursery, viticulture and wine trials. The first selection step will occur in nursery trials where rootstock material with high root formation and graft compatibility will be identified for further evaluation. Next, viticulture trials will be performed, and rootstock material will be evaluated and selected for vigour potential, potassium uptake, reproductive performance and berry composition and quality. Due to the correlation between high vigour, increased potassium uptake and reduced wine quality in hot climates (Kodur 2011; Walker and Clingeleffer 2016), rootstock material with low to medium vigour will primarily be selected. For cool climate conditions, high vigour rootstocks will also be selected, as long as there is little or no effect on wine quality. As water availability is predicted to be a limiting factor in vineyard production, rootstocks will be evaluated under reduced irrigation conditions to identify rootstocks that display good performance under drought conditions. Long-term pest resistance will also be evaluated by performing trials in phylloxera-infested vineyards, as well as vineyards suffering from root knot nematode. Lastly, winemaking trials will be performed on rootstocks adapted to Australian conditions to determine their impact on berry and wine composition and quality.

Conclusion

In conclusion, the development of next-generation rootstocks with long-term resistance to phylloxera and root knot nematode will be achieved by marker-assisted breeding. This approach will allow the stacking of multiple genetic resistance traits to effectively safeguard vines from phylloxera and root knot nematode. Next-generation genetic mapping combined with rapid phenotyping systems will increase the efficiency of identifying DNA markers linked to resistance traits for marker-assisted breeding. During the evaluation process, durable resistant rootstocks that perform the best under Australian conditions will be selected for release.

Acknowledgements

We thank Dr Catherine Clarke from Agriculture Victoria for providing the phylloxera image displayed in Figure 1. We acknowledge CSIRO Agriculture and Food and Wine Australia (CSA1701-1.3) for funding. CSIRO Agriculture and Food is a member of the Wine Innovation Cluster in Adelaide.

References

Cooper, M.L. (2012) Phylloxera Populations on Resistant Rootstocks. University of California Agriculture and National Resources: 20 June: http://cenapa.ucdavis.edu/newsletters/Vineyard_Views_Newsletter_-_Events43564.pdf

Cousins, P.; Johnston, D.; Switras-Meyer, S.; Boyden, L.; Vidmar, J.; Meyer, C. (2007) USDA ARS research in grape rootstock breeding and genetics. Acta Hortic. 733: 51–58.

Dunlevy, J.D.; Clingeleffer, P.R.; Smith, H.M. (2019) Breeding next-generation rootstocks with durable pest resistance using marker-assisted selection. Wine Vitic. J. 34: 40–45.

Ferris, H.; Zheng, L.; Walker, M.A. (2013) Soil temperature effects on the interaction of grape rootstocks and plant-parasitic nematodes. J. Nematol. 45: 49–57.

Forneck, A.; Huber, L. (2009) (A)sexual reproduction – a review of life cycles of grape phylloxera, *Daktulosphaira vitifoliae*. Entomol. Exp. Appl. 131: 1–10.

Hausmann, L.; Eibach, R.; Zyprian, E.; Topfer, R. (2012) Genetics of phylloxera root resistance in cultivar 'Börner'. Acta Hortic. 904: 47–52.

Kocsis, L.; Granett, J.; Walker, M.A.; Lin, H.; Omer A.D. (1999) Grape phylloxera populations adapted to *Vitis berlandieri* × *V. riparia* rootstocks. Am. J. Enol. Vitic. 50: 101–106.

Kocsis, L.; Granett, J.; Walker, M.A. (2002) Performance of Hungarian phylloxera strains on *Vitis riparia* rootstocks. J. Appl. Entomol. 126: 567–571.

Kodur, S. (2011) Effects of juice pH and potassium on juice and wine quality, and regulation of potassium in grapevines through rootstocks (*Vitis*): a short review. Vitis 50: 1–6.

Logan, S. (2018) Rootstock resistance – why isn't Australia doing better in its uptake of rootstocks for phylloxera control? Wine Vitic. J. 33: 40–43.

Lund, K.T.; Riaz, S.; Walker, M.A. (2017) Population structure, diversity and reproductive mode of the grape phylloxera (*Daktulosphaira vitifoliae*) across its native range. PLoS One 12: e0170678.

Ollat, N.; Bordenave, L.; Tandonnet, J.P.; Boursiquot, J.M.; Marguerit, E. (2016) Grapevine rootstocks: origins and perspectives. Acta Hortic. 1136: 11–22.

Powell, K.S. (2008) Grape phylloxera: an overview. In: Root Feeders: An Ecosystem Perspective. Johnson, S.N.; Murray, P.J. (eds) Oxfordshire: CAB International: 96–114.

Powell, K.S. (2012) A holistic approach to future management of grapevine phylloxera. In: Arthropod management in vineyards: pests, approaches and future directions. Bostanian, G.; Vincent, C.; Isaacs, R. (eds) London: Springer Science and Business: 219–252.

Riaz, S.; Pap, D.; Uretsky, J.; Laucou, V.; Boursiquot, J.M.; Kocsis, L.; Walker, M.A. (2019) Genetic diversity and parentage analysis of grape rootstocks. Theor. Appl. Genet. 132: 1847–1860.

Smith, B.P.; Morales, N.B.; Thomas, M. R.; Smith, H.M.; Clingeleffer, P.R. (2016) Grapevine rootstocks resistant to the root-knot nematode *Meloidogyne javanica*. Aust. J. Grape Wine Res. 23: 125–131.

Smith, H.M.; Clarke, C.W.; Smith, B.P.; Carmody, B.M.; Thomas, M.R.; Clingeleffer, P.R.; Powell, K.S. (2018a) Genetic identification of SNP markers linked to a new grape phylloxera resistant locus in *Vitis cinerea* for marker-assisted selection. BMC Plant Biol. 10: 360.

Smith, H.M.; Smith, B.P.; Morales, N.B.; Moskwa S.; Clingeleffer, P.R.; Thomas, M.R. (2018b) SNP markers tightly linked to root knot nematode resistance in grapevine (*Vitis cinerea*) identified by a genotyping-by-sequencing approach followed by Sequenom MassARRAY validation. PLoS One 13: e0193121.

Stamp, J.A. (2011) Vineyard Development: Principles, Problems and Perspectives. Wine Bus. Mon. December: 52–59: https://smallvines.com/wp-content/uploads/2014/08/Wine-Business-Monthly-Vineyard-Development.pdf

This, P.; Lacombe, T.; Thomas, M.R. (2006) Historical origins and genetic diversity of wine grapes. Trends Genet. 22: 511–519.

Walker, M.A.; Ferris, H.; Eyre, M. (1994) Resistance in *Vitis* and *Muscadinia* species to *Meloidogyne incognita*. Plant Dis. 78: 1055–1058.

Walker, G.E.; Stirling, G.R. (2008) Plant-parasitic nematodes in Australian viticulture: key pests, current management practices and opportunities for future improvements. Australas. Plant Pathol. 37: 268–278.

Walker, R.; Clingeleffer, P. (2009) Rootstock attributes and selection for Australian conditions. Aust. Vitic. 13: 70–76.

Whiting, J. (2012) Rootstock breeding and associated R&D in the viticulture and wine industry. Australian Government Grape and Wine Research Development Corporation: Rev. August: http://www.mvwi.com.au/items/517/Rootstock_Review_-_John_Whiting_FINAL_(email).pdf

Zhang, J.K.; Hausmann, L.; Eibach, R.; Welter, L.J.; Topfer, R.; Zyprian E.M. (2009) A framework map from grapevine V3125 (*Vitis vinifera* 'Schiava grossa' × 'Riesling') × rootstock cultivar 'Börner' (*Vitis riparia* × *Vitis cinerea*) to localize genetic determinants of phylloxera root resistance. Theor. Appl. Genet. 119: 1039–1051.

Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar Chardonnay

M.J. Roach[1], D.L. Johnson[1], J. Bohlmann[2,3], H.J.J. van Vuuren[2,3], S.J.M. Jones[4], I.S. Pretorius[5], S.A. Schmidt[1], A.R. Borneman[1,6]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]Michael Smith Laboratories, The University of British Columbia, Vancouver, British Columbia, Canada
[3]Wine Research Centre, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, British Columbia, Canada
[4]Michael Smith Genome Sciences Centre, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada
[5]Chancellery, Macquarie University, Sydney, NSW
[6]Department of Genetics and Evolution, The University of Adelaide, Adelaide, SA
michael.roach@awri.com.au

This work is published in PLoS Genetics: doi.org/10.1371/journal.pgen.1007807

Abstract

Chardonnay is the most widely grown white winegrape variety worldwide and Australian Chardonnay's success on the world stage has been largely underpinned by a historic international program of clonal selection. But what exactly is a 'clone'? Genetic mutations accumulate within a plant during successive propagations. This often causes phenotypic differences that alter yield, quality or sensory characteristics. Favourable phenotypes can be captured and amplified by using a single plant to establish a lineage for new plantings. Clonal plantings can improve vineyard performance or provide unique flavour and aroma profiles. Unfortunately, the genetics behind clonal differences is poorly understood. A clone sequencing program was undertaken to understand the scale and scope of genetic variation amongst clones of Chardonnay.

The clone sequencing program was initiated with the production of a high-quality 'diploid' assembly for Chardonnay. In addition, fifteen popular Chardonnay clones were sequenced, and 1,620 genetic markers identified that distinguish them, one of which is a marker for a known 'Muscat' mutation. Marker validation was undertaken through sequencing of plants from the same clonal lines but sourced from independent locations. Clones were able to be reliably identified using these markers and regional differences were also identified within clonal populations. Finally, it was shown that the Chardonnay genome contains extensive evidence of parental inbreeding, such that its parents, Pinot Noir and Gouais Blanc, may even represent first-degree relatives. This previously unreported finding sheds new light on the heritage of Chardonnay and Gouais Blanc.

Introduction

Chardonnay is used in some of the world's most iconic wines. It originated centuries ago in France as a cross between two ancient cultivars—Pinot Noir and Gouais Blanc (Bowers et al. 1999; Hunt et al. 2010). Both Pinot Noir and Gouais Blanc are parents to many important commercial cultivars grown today. However, Gouais Blanc itself is rarely cultivated as it's generally considered to produce low-quality wine (Hunt et al. 2010). Chardonnay quickly spread throughout the world, becoming the most widely grown white grape variety. It is especially important in the Australian wine industry as the most widely grown white grape variety and third most widely grown winegrape in Australia behind Shiraz (Syrah) and Cabernet Sauvignon (Wine Australia 2019).

Chardonnay's expansion in Australia in the 1980s coincided with the maturation of clonal selection programs in France, the USA and Australia, and with subsequent regional trials of these clones (Olmo 1980; Bernard 1995; Cirami and Ewart 1995). Clone I10V1 performed extremely well and was widely adopted, dominating Australian plantings of Chardonnay. Australian Chardonnay has since become very diverse in style, driven by regional variations, winemaking practices and the wide range of clones that are now available.

It is generally understood that random genetic mutations can occur during the growth of a grapevine, and that these mutations are passed on to future plants when propagated. The accumulation of mutations during successive propagations creates genetic drift and can cause phenotypic changes. Capturing this phenotypic variation forms the basis of clonal selection. The different clones for Chardonnay exhibit a range of characteristics including altered yields, quality and bunch morphology, as well as changes to the sensory profile of the resulting wines (Bettiga 2003; Reynolds et al. 2004; Fidelibus et al. 2006; Vouillamoz and Grando 2006; Anderson et al. 2008; Duchêne et al. 2009; Anderson and Aryal 2013). However, the genetics underpinning these phenotypic differences largely remains a mystery. Furthermore, there can be uncertainties surrounding the authenticity of clonal material, the origins and relatedness of many clones are still unknown, and ampelography is usually insufficient for distinguishing between clones. An analysis of the genetic mutations underpinning the different clones was therefore required. Identified clonal mutations could be used in a genetic test for confirming the identity of a clone and could give insights into what drives phenotypic differences. However, performing this type of analysis requires a reference genome assembly.

The first genome assemblies for grapevine were two assemblies for Pinot Noir, released in 2007 (Jaillon et al. 2007; Velasco et al. 2007). These genomes were produced from first-generation Sanger sequencing (very high cost, small volume of data) and second-generation (or 'next-gen') sequencing (low cost, large volume of data, short-read lengths). Genome assemblies at the time were notoriously difficult, and both of these genomes (like most at that time) are highly fragmented. The advent of third-generation sequencing has resulted in the rapid release of many high-quality genome assemblies (Chin et al. 2016; Fu et al. 2017; Khost et al. 2017; Yoshida et al. 2017; Jain et al. 2018). Third-generation sequencing reads are often 50−100× longer than typical second-generation sequencing, and the current longest reported individual read is over 2 million bases long (Payne et al. 2018). Longer reads make the assembly problem much simpler, resulting in much higher quality assemblies for far less effort.

The main aim of the study was to examine the diversity that is present in the clones of Chardonnay. This genetic diversity would then be used to explore a clonal authenticity or identification test. A reference genome assembly for Chardonnay was produced using the latest third-generation sequencing technology. Single-nucleotide polymorphism (SNP) and insertion/deletion (InDel) genetic mutations were identified that distinguish the Chardonnay clones,

and these were used in a proof-of-concept clonal identification test. The new Chardonnay assembly was also used to explore the heritage of Chardonnay and its parents—Pinot Noir and Gouais Blanc.

Materials and methods

Detailed materials and methods are available in Roach et al. (2018).

Reference genome for Chardonnay, significant improvements over Pinot Noir genome

To produce the Chardonnay reference genome assembly, the clone I10V1 was selected to use due to its prominence in the Australian wine industry. This clone was sequenced using PacBio RS-II SMRT (third-generation long-read) sequencing (Eid et al. 2009). Chardonnay was then assembled using a 'diploid' assembler (Chin et al. 2016), meaning that for organisms with two copies of each chromosome (such as grapevine) it will produce a primary assembly consisting of one copy of all the regions in the genome, and a secondary assembly consisting of the other copy.

The primary assembly for Chardonnay consists of only 854 contigs (fewer is better), whereas the more contiguous of the two Pinot Noir assemblies (PN40024) consists of 14,634 contigs. A Cabernet Sauvignon assembly, released two years prior to Chardonnay, had similarly excellent contiguity (Chin et al. 2016). The genome sizes for Chardonnay and Pinot Noir (PN40024) were similar at 490 Mb and 486 Mb respectively. Both Chardonnay and Cabernet Sauvignon represent order-of-magnitude improvements over the original Pinot Noir assemblies, and both capture the native heterozygosity of these cultivars.

Chardonnay genome provides insights into Chardonnay's heritage

As an early evaluation of the Chardonnay assembly, the 'third-gen' sequencing reads were aligned to the genome and the coverage was examined. Any regions where chromosomal copies were not separated would stand out as the read-depth would be approximately double that of the rest of the genome. This generally occurs when the region is homozygous (i.e. both chromosomal copies have identical sequence). Heterozygous SNPs were identified to also assess for homozygosity. The read-depth and the heterozygous SNP density was juxtaposed for Chromosome 2 in Figure 1. There is a large run of homozygosity along Chromosome 2 indicated by a doubling of the read-depth and a large drop in heterozygous SNP density. There were many of these throughout the genome. These runs of homozygosity can be caused by gene conversions early in Chardonnay's past, or as a result of inbreeding.

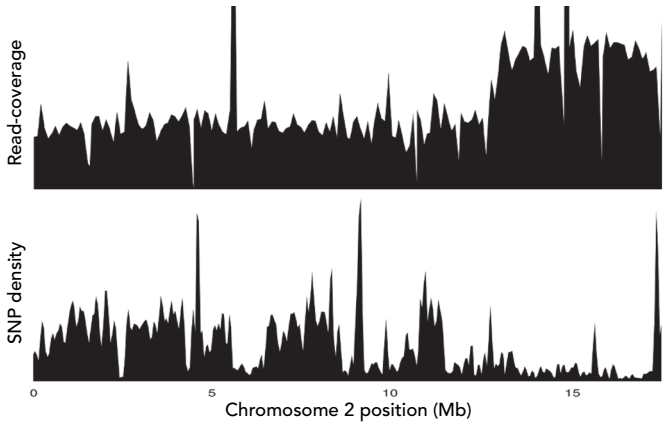


Figure 1. Run of homozygosity along Chromosome 2 of Chardonnay. The juxtaposed plots show the read-depth histogram for PacBio long-reads mapped to the primary contigs + haplotigs assembly (top), and the heterozygous SNP density (bottom). A run of homozygosity from approximately 12 Mb to almost the end of Chromosome 2 is indicated by an approximate doubling of the read-depth in the top track and large drop in the heterozygous SNP density in the bottom track.

The possibility of inbreeding was investigated further by assigning and examining inheritance over the entire Chardonnay genome. The analysis was limited to only consider the closely aligning sequence pairs between the chromosome copies. These were compared to sequencing data for Pinot Noir. Where the Pinot Noir sequence matched one copy but not the other, it could be determined that the matching copy was inherited from Pinot Noir and the mismatched copy was inherited from Gouais Blanc.

Using this strategy, it was possible to assign parentage over most of the Chardonnay genome. However, there were large tracks throughout the genome where both Chardonnay chromosome copies matched Pinot Noir, and as such it was impossible to determine which copy was inherited from Pinot Noir and which had come from Gouais Blanc (Figure 2a). It should be noted that the parentage appears to switch back and forth between the chromosomes. While some of these may be true biological events, most are simply artefacts of genome assembly that do not adversely affect downstream analyses.

It was evident that both Pinot Noir and Gouais Blanc shared large portions of DNA. DNA sequence data for Gouais Blanc was therefore required to resolve these unknown regions. Gouais Blanc was sequenced and the analysis repeated using the data from both parents. Within these regions, Gouais Blanc indeed matches only one of the two chromosome copies in Chardonnay. This allowed the parentage to be assigned over these regions (Figure 2b).

This SNP-based approach had its strengths as well as some limitations. There were gaps in the analysis due to restricting it to only the closely aligning chromosomal sequence pairs. There was also the possibility of biases resulting from evaluation of a subset of the genome, or from read-mapping and variant calling errors. An orthogonal approach was developed to assign parentage. Simply put, the approach involved extracting short sequences (known as 'kmers') from both chromosomal copies and searching the sequencing data of the parents for the presence or absence of these kmers. Regions that were missing lots of kmers from only one of the parents were determined to be inherited from the other parent. The SNP- and kmer-based parentage assignments were compared for the primary contigs and it was found that there was excellent consistency between the two approaches (Figure 2c).

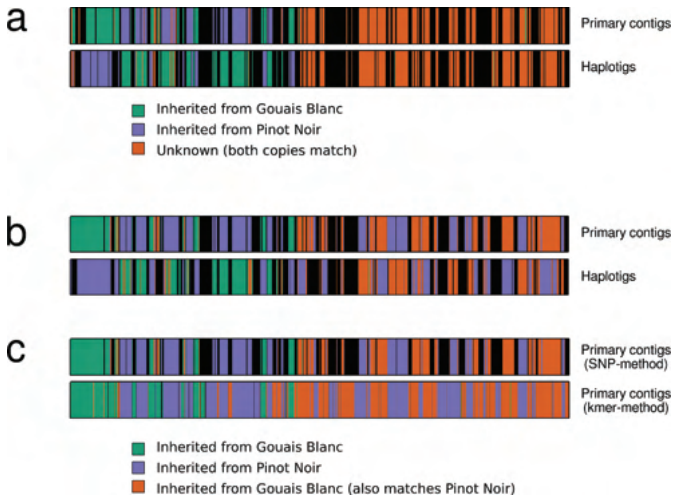


Figure 2. Inheritance mapping of Chardonnay Chromosome 10. Gaps in the analysis are coloured black. Inheritance was initially calculated using a SNP-based method using only sequencing data for Pinot Noir (a). There were large regions that could not be assigned due to both chromosomal copies matching the Pinot Noir sequencing data. Inheritance was later calculated using the same SNP-based method with sequencing data for both Pinot Noir and Gouais Blanc (b). The regions where both copies of Chardonnay matched the Pinot Noir sequencing data were able to be resolved with the inclusion of the Gouais Blanc sequencing. An orthogonal approach using kmers was developed to assign parentage and was compared to the SNP-based method (c). The SNP- and kmer-based methods exhibited excellent consistency of parentage assignments over the Chardonnay genome.

A genetic backcross between Pinot Noir and an ancestor of Gouais Blanc most likely had occurred. This would have resulted in a large portion of Gouais Blanc's genome originating from Pinot Noir. Previous studies that have examined the relationships of grapevine varieties identified numerous parent and child relationships, and possible sibling relations for varieties where parentage was unknown (Bowers et al. 1999; Lacombe et al. 2013). These studies relied on a small number of short simple repeat (SSR) sequences to identify immediate relations; however, the ability to accurately identify more distant relations is limited. Nevertheless, an examination of the SSR markers over these two studies shows that Pinot Noir and Gouais Blanc share at least one marker at 60% of the genomic locations that were tested, which supports this theory. Further work is needed to determine what the exact relationship is between Chardonnay's parents.

Genetic variation between clones of Chardonnay

Second-generation sequencing was performed on 15 clones of Chardonnay. The details of these clones are available in Roach et al. (2018). Mutations that are unique to a clone, or a group of related clones, are useful genetic 'markers' for identifying those clones. This is useful, for instance, when identifying the clone of an unknown Chardonnay plant sample. A marker discovery software pipeline was developed to identify genetic mutations that were different in at least one clone. The pipeline involves aligning the sequencing data to the Chardonnay reference genome, identifying potential marker SNP or InDel mutations, and finally kmer-based filtering to remove false positives.

In total, 1,620 marker mutations were identified among the clones of Chardonnay. These markers are tabled in S1 Dataset in Roach et al. (2018). The markers were used to generate a phylogeny of the Chardonnay clones, shown in Figure 3, together with the number of marker mutations for each clone. It should be noted that as some clones share certain markers, the total number of markers next to each clone identifier will be greater than 1,620. Most clonal marker mutations are unique to only one clone. There were several exceptions to this; clones CR red and Waite Star are both bud-sports of I10V1, demonstrated by them sharing all 90 of I10V1's mutations. As well as the I10V1 mutations, CR red and Waite Star also contain 14 and 24 extra mutations respectively. Clones 124 and 118—both commonly used for sparkling styles—also share a significant portion of their mutations and appear to be genetically similar.

Marker mutations can cause changes to a clone's phenotype. A mutation in a gene that changes the resulting protein sequence can alter the function of that protein. Changes to highly conserved amino acids or changes of amino acids to ones that are highly dissimilar are especially likely to affect the function of proteins. To predict which mutations might result in a phenotype change, marker mutations were filtered to only examine mutations that reside within genes. Of these, the mutations predicted to change the resulting translated protein sequence were scored for their likelihood of affecting protein function. Among the high-scoring mutations was a well-characterised 'Muscat' mutation (Emanuelli et al. 2010) detected in clone 809 (which was the only Muscat clone included in this study). This mutation arises as one of several possible non-synonymous mutations in the 1-deoxy-d-xylulose-5-phosphate synthase 1 (DXS1) gene that is associated with the production of higher levels of monoterpenoids; the resulting wine has a stronger floral 'Muscat' aroma. The marker mutations predicted to affect protein function are available in S1 Dataset in Roach et al. (2018). Further work is required to evaluate the phenotypic impacts of these mutations.

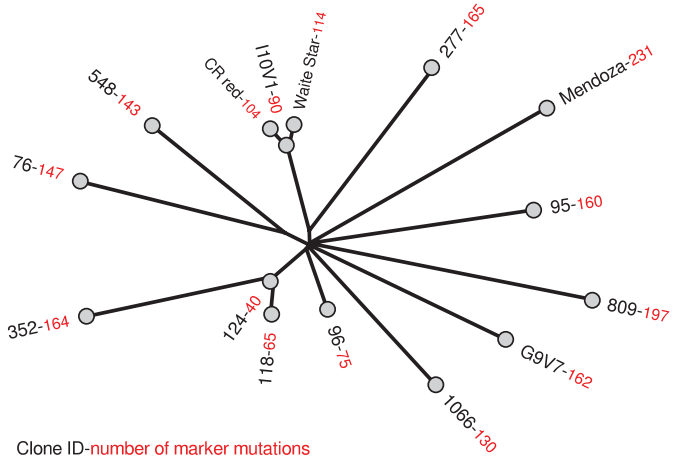


Figure 3. Phylogeny of Chardonnay clones according to clonal marker mutations. The clone identifier is indicated in black and the number of markers for that clone is indicated in red.

Marker screening pipeline

A software pipeline was developed for quickly and accurately screening clonal marker mutations directly from sequencing data. Simply put, a kmer database was generated from the sequencing data. Next, kmers previously generated for each of the clonal mutations were screened against the kmer database. This method proved to be extremely quick to perform. The kmer database was built typically in 20 to 30 minutes for high-coverage datasets, and a further 7 mins was required to screen for the marker kmers. The drawback to this method is that it still requires that whole genome shotgun sequencing be performed on the sample, which can be costly.

It was necessary to determine if these marker mutations could prove useful in an authenticity test. The markers needed to be reliably detected from different sequencing platforms. It was also necessary to ensure that clones sourced from different locations contained enough of the same clonal marker mutations to identify the clone. Finally, a minimum threshold for sequencing coverage was determined for reliably detecting marker mutations from sequencing data. The results of these experiments appear in S1 Dataset and Figure 5 in Roach et al. (2018).

Six of the clones were sequenced from the same Australian-sourced plants that were used for marker discovery; however, a different sequencing platform was used. These were then screened for the marker mutations. Between 30% and 72% of markers were identified and almost all missing markers were due to poor coverage at the genome locations of those markers.

Three clones that were sourced from a separate location (in North America) to the marker discovery material were sequenced at high coverage. Between 55% and 83% of the marker mutations were identified. Furthermore, between 14% and 44% of the markers were determined to be missing. This shows that despite both plants reportedly being the same clone, there were differences in the mutations present. One of these high-coverage sets was subsampled in order to assess what a minimum threshold might be for reliably identifying marker mutations. Even at a very low coverage of approximately 12-fold, there were still 35% of the total marker mutations identified.

Finally, low-coverage sequencing (between 9.8-fold and 24.8-fold) was performed on eight of the clones (also sourced from North America) and screened for markers. Between 8% and 42% of the markers in these samples were identified, and in all cases the markers that were identified were consistent with the identity of the clone that was screened. This demonstrates one possible affordable option for clonal identification.

There were differences in mutations within clonal populations that appeared to be dependent on the source location. It may be possible to leverage these differences in a clonal identification test to not only

identify the clone, but also the most likely source location. They also represent an opportunity to uncover how Chardonnay plants have been propagated and distributed around the world. Future work is needed to source and sequence clones from multiple locations to enable the identification and characterisation of subpopulations within clones. When combined with sample metadata, this will provide a thorough picture of the state of the clones of Chardonnay worldwide.

Acknowledgements

The authors thank Nick Dry (Yalumba Nursery) and Dr Michael McCarthy (South Australian Research and Development Institute) for provision of plant material; Assoc. Prof. Andrew Lonie and Assoc. Prof. Torsten Seemann (University of Melbourne), Dr Cihan Altinay and Derek Benson (University of Queensland), Stephen Crawley (QCIF), QRIScloud, and Melbourne Bioinformatics for assistance with computing resources; Dr Jason Chin, Dr Gregory Concepcion, Emily Hatas and Dr Sarah Kingan (Pacific Biosciences) for discussion and early access to FALCON Unzip; Dr Sean Myles, who acted as an advisor to Genome BC; Samantha Turner (University of British Columbia) for outstanding administrative duties; and Andrew Gilbert (Bioplatforms Australia) for supporting this work.

The AWRI, a member of the Wine Innovation Cluster in Adelaide, is supported by Australia's grapegrowers and winemakers through their investment body Wine Australia with matching funds from the Australian Government. This work was also supported by Bioplatforms Australia (BPA) through the Australian Government National Collaborative Research Infrastructure Strategy (NCRIS) scheme.

References

Anderson, K.; Aryal, N.R. (2013) Database of Regional, National and Global Winegrape Bearing Areas by Variety, 2000 and 2010. Wine Economics Research Centre, University of Adelaide (revised July 2014).

Anderson, M.M.; Smith, R.J.; Williams, M.A.; Wolpert, J.A. (2008) Viticultural evaluation of French and California Chardonnay clones grown for production of sparkling wine. Am. J. Enol. Vitic. 59: 73–77.

Bernard, R. (1995) Aspects of clonal selection in Burgundy. In: Wolpert, J.; Walker, M.A.; Roberts, D. (eds) Proceedings of the International Symposium on Clonal Selection: Portland, Oregon.

Bettiga, L. (2003) Comparison of seven chardonnay clonal selections in the Salinas Valley. Am. J. Enol. Vitic. 54: 203–206.

Bowers, J.; Boursiquot, J.-M.; This, P.; Chu, K.; Johansson, H.; Meredith, C. (1999) Historical Genetics: The Parentage of Chardonnay, Gamay, and Other Wine Grapes of Northeastern France. Science 285: 1562–1565.

Chin, C.-S.; Peluso, P.; Sedlazeck, F.J.; Nattestad, M.; Concepcion, G.T.; Clum, A.; Dunn, C.; O'Malley, R.; Figueroa-Balderas, R.; Morales-Cruz, A.; Cramer, G.R.; Delledonne, M.; Luo, C.; Ecker, J.R.; Cantu, D.; Rank, D.R.; Schatz, M.C. (2016) Phased diploid genome assembly with single-molecule real-time sequencing. Nat. Methods 13: 1050–1054.

Cirami, R.; Ewart, A.J.W. (1995) Clonal selection, evaluation and multiplication in Australia. In: Wolpert, J.; Walker, M.A.; Roberts, D. (eds) Proceedings of the International Symposium on Clonal Selection: Portland, Oregon.

Duchêne, E.; Legras, J.L.; Karst, F.; Merdinoglu, D.; Claudel, P.; Jaegli, N.; Pelsy, F. (2009) Variation of linalool and geraniol content within two pairs of aromatic and non-aromatic grapevine clones. Aust. J. Grape Wine Res. 15: 120–130.

Eid, J.; Fehr, A.; Gray, J.; Luong, K.; Lyle, J.; Otto, G.; Peluso, P.; Rank, D.; Baybayan, P.; Bettman, B.; Bibillo, A.; Bjornson, K.; Chaudhuri, B.; Christians, F.; Cicero, R.; Clark, S.; Dalal, R.; Dewinter, A.; Dixon, J.; Foquet, M.; Gaertner, A.; Hardenbol, P.; Heiner, C.; Hester, K.; Holden, D.; Kearns, G.; Kong, X.; Kuse, R.; Lacroix, Y.; Lin, S.; Lundquist, P.; Ma, C.; Marks, P.; Maxham, M.; Murphy, D.; Park, I.; Pham, T.; Phillips, M.; Roy, J.; Sebra, R.; Shen, G.; Sorenson, J.; Tomaney, A.; Travers, K.; Trulson, M.; Vieceli, J.; Wegener, J.; Wu, D.; Yang, A.; Zaccarin, D.; Zhao, P.; Zhong, F; Korlach, J.; Turner, S. (2009) Real-time DNA sequencing from single polymerase molecules. Science 323: 133–138.

Emanuelli, F.; Battilana, J.; Costantini, L.; Le Cunff, L.; Boursiquot, J.M.; This, P.; Grando, M.S. (2010) A candidate gene association study on muscat flavor in grapevine (*Vitis vinifera* L.). BMC Plant Biol. 10: 241.

Fidelibus, M.W.; Christensen, L.P.; Katayama, D.G.; Verdenal, P.-T. (2006) Yield Components and Fruit Composition of Six Chardonnay Grapevine Clones in the Central San Joaquin Valley, California. Am. J. Enol. Vitic. 57: 503–507.

Fu, X.; Li, J.; Tian, Y.; Quan, W.; Zhang, S.; Liu, Q.; Liang, F.; Zhu, X.; Zhang, L.; Wang, D.; Hu, J. (2017) Long-read sequence assembly of the firefly Pyrocoelia pectoralis genome. GigaScience 6: 1–7.

Hunt, H.V.; Lawes, M.C.; Bower, M.A.; Haeger, J.W.; Howe, C.J. (2010) A banned variety was the mother of several major wine grapes. Biol. Lett. 6: 367–369.

Jaillon, O.; Aury, J.M.; Noel, B.; Policriti, A.; Clepet, C.; Casagrande, A.; Choisne, N.; Aubourg, S.; Vitulo, N.; Jubin, C.; Vezzi, A.; Legeai, F.; Hugueney, P.; Dasilva, C.; Horner, D.; Mica, E.; Jublot, D.; Poulain, J.; Bruyere, C.; Billault, A.; Segurens, B.; Gouyvenoux, M.; Ugarte, E.; Cattonaro, F.; Anthouard, V.; Vico, V.; Del Fabbro, C.; Alaux, M.; Di Gaspero, G.; Dumas, V.; Felice, N.; Paillard, S.; Juman, I.; Moroldo, M.; Scalabrin, S.; Canaguier, A.; Le Clainche, I.; Malacrida, G.; Durand, E.; Pesole, G.; Laucou, V.; Chatelet, P.; Merdinoglu, D.; Delledonne, M.; Pezzotti, M.; Lecharny, A.; Scarpelli, C.; Artiguenave, F.; Pe, M.E.; Valle, G.; Morgante, M.; Caboche, M.; Adam-Blondon, A.F.; Weissenbach, J.; Quetier, F.; Wincker, P. (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449: 463–467.

Jain, M.; Koren, S.; Miga, K.H.; Quick, J.; Rand, A.C.; Sasani, T.A.; Tyson, J.R.; Beggs, A.D.; Dilthey, A.T.; Fiddes, I.T.; Malla, S.; Marriott, H.; Nieto, T.; O'Grady, J.; Olsen, H.E.; Pedersen, B.S.; Rhie, A.; Richardson, H.; Quinlan, A.R.; Snutch, T.P.; Tee, L.; Paten, B.; Phillippy, A.M.; Simpson, J.T.; Loman, N.J.; Loose, M. (2018) Nanopore sequencing and assembly of a human genome with ultra-long reads. Nature Biotechnol. 36: 338–345

Khost, D.E.; Eickbush, D.G.; Larracuente, A.M. (2017) Single-molecule sequencing resolves the detailed structure of complex satellite DNA loci in Drosophila melanogaster. Genome Res. 27: 709–721.

Lacombe, T.; Boursiquot, J.-M.; Laucou, V.; Di Vecchi-Staraz, M.; Péros, J.-P.; This, P. (2013) Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.). Theor. Appl. Genet. 126: 401–414.

Olmo, H.P. (1980) Selecting and breeding new grape varieties. Calif. Agric. 34: 23–24.

Payne, A.; Holme, N.; Rakyan, V.; Loose, M. (2018) Whale watching with BulkVis: A graphical viewer for Oxford Nanopore bulk fast5 files. bioRxiv 312256.

Reynolds, A.; Cliff, M.; Wardle, D.; King, M. (2004) Evaluation of winegrapes in British Columbia: 'Chardonnay' and 'Pinot noir' clones. Horttechnology 14: 594–602.

Roach, M.J.; Johnson, D.L.; Bohlmann, J.; Van Vuuren, H.J.J.; Jones, S.J.M.; Pretorius, I.S.; Schmidt, S.A.; Borneman, A.R. (2018) Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar Chardonnay. PLoS Genet. 14: doi.org/10.1371/journal.pgen.1007807

Velasco, R.; Zharkikh, A.; Troggio, M.; Cartwright, D.A.; Cestaro, A.; Pruss, D.; Pindo, M.; Fitzgerald, L.M.; Vezzulli, S.; Reid, J.; Malacarne, G.; Iliev, D.; Coppola, G.; Wardell, B.; Micheletti, D.; Macalma, T.; Facci, M.; Mitchell, J.T.; Perazzolli, M.; Eldredge, G.; Gatto, P.; Oyzerski, R.; Moretto, M.; Gutin, N.; Stefanini, M.; Chen, Y.; Segala, C.; Davenport, C.; Dematte, L.; Mraz, A.; Battilana, J.; Stormo, K.; Costa, F.; Tao, Q.; Si-Ammour, A.; Harkins, T.; Lackey, A.; Perbost, C.; Taillon, B.; Stella, A.; Solovyev, V.; Fawcett, J.A.; Sterck, L.; Vandepoele, K.; Grando, S.M.; Toppo, S.; Moser, C.; Lanchbury, J.; Bogden, R.; Skolnick, M.; Sgaramella, V.; Bhatnagar, S.K.; Fontana, P.; Gutin, A.; Van De Peer, Y.; Salamini, F.; Viola, R. (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS One 2: e1326.

Vouillamoz, J.F.; Grando, M.S. (2006) Genealogy of wine grape cultivars: 'Pinot' is related to 'Syrah'. Heredity 97: 102–110.

Wine Australia (2019) Australian Wine Sector 2018 at a glance: https://www.wineaustralia.com/market-insights/australian-wine-sector-at-a-glance

Yoshida, Y.; Koutsovoulos, G.; Laetsch, D.R.; Stevens, L.; Kumar, S.; Horikawa, D.D.; Ishino, K.; Komine, S.; Kunieda, T.; Tomita, M.; Blaxter, M.; Arakawa, K. (2017) Comparative genomics of the tardigrades *Hypsibius dujardini* and *Ramazzottius varieornatus*. PLoS Biol. 15: e2002266.

Inspirations from the past and opportunities for the future

S. Nordestgaard, E.N. Wilkes

The Australian Wine Research Institute, Urrbrae, SA
simon.nordestgaard@awri.com.au

Abstract

This paper discusses examples of technologies that have and have not been adopted in wineries, their history and opportunities for the future. Cross-flow filtration and flotation are examples of technologies that have now been successfully adopted by many wineries and have led to efficiency and/or quality improvements. Some of the biggest remaining opportunities for large wineries relate to automation. Many winemaking practices are still quite manual; for example, dips for volume measurement, sample collection and laboratory analysis of ferments, use of hoses, and sparging to adjust gas levels. The costs of some of the more automated approaches discussed in this article may be higher in the short term but they may also provide a path to continued improvements in quality and cost reduction in the longer term.

Introduction

This paper discusses some key technologies that have been adopted in wineries in recent years. It draws on data from the AWRI Vineyard and Winery Practices Survey (Nordestgaard 2019) and research on the history of winery equipment and practices (Nordestgaard 2020). Some areas of wine production where technology adoption has been low and some new opportunities are also outlined.

Cross-flow filtration – the most important practice change in wineries

The survey results for wine filtration technologies used in Australia in 2016 are presented in Figure 1. Cross-flow filtration has now been widely adopted by the Australian wine sector, particularly by larger wineries, with 95% of wineries crushing 10,000 tonnes of grapes or more a year using this technology. In the survey, cross-flow filtration was nominated more than any other newer winery practice as having had a positive impact in the last five years. One prominent winemaker described it as: 'the single biggest advance that we have made in quality improvement in the last 25 years'. Wine producers also mentioned health and safety benefits of replacing diatomaceous earth, reduced numbers of filtration stages and/or refiltrations and lower product dilution and wine losses than with pressure leaf filtration using diatomaceous earth. Automation is another major benefit of this technology—systems can run for long periods unsupervised, including overnight.

However, cross-flow filtration is not new for the wine industry and it was not always so popular. Systems were available as early as the 1980s and numerous studies were performed. For example, in 1985 in France, the Institut Technique de La Vigne et du Vin held a seminar on cross-flow filtration featuring multiple manufacturers and researchers and published a 250-page set of proceedings (ITV 1985). There was also interest in Australia from multiple companies and Bryce Rankine reports that the first system was used in 1986 (Gibson 1986; Rankine 1996).

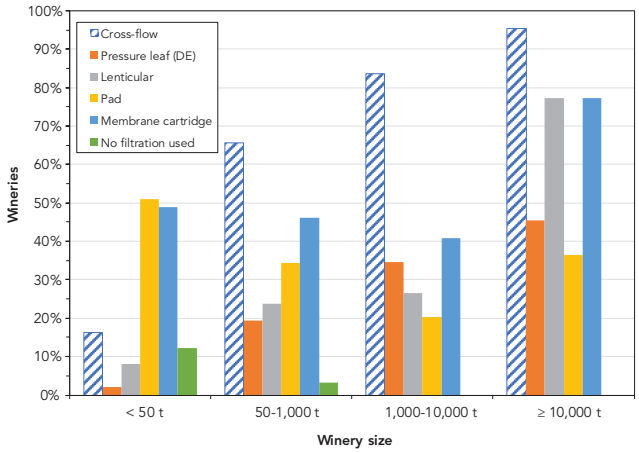


Figure 1. Wine filtration techniques used by Australian wineries in 2016

Uptake of cross-flow filtration in the 1980s was limited. Adoption did not really accelerate in Australia until the mid-2000s when a couple of big wine companies installed systems and put large quantities of wine through them. This likely illustrated the benefits of the technology and gradually gave others the confidence to adopt it. Prior to that, industry opinions of cross-flow filtration were typically negative. There were concerns about possible stripping of colloidal compounds and of wine warming and oxidation. The technology was also considered to be too expensive given that flow rates were much lower than with pressure leaf diatomaceous earth filtration. (This is still a criticism from some wineries and pressure leaf diatomaceous earth filtration is still used to some extent, Figure 1.)

Technical improvements in membranes and system design have addressed the initial quality concerns with cross-flow filtration. However, there remains ongoing industry interest in more robust cross-flow filtration membranes capable of higher flow rates as well as the most suitable membranes and systems for filtering lees. Adoption of cross-flow filtration in this application is currently much lower than it is for wine.

The adoption path of cross-flow microfiltration should serve as inspiration for other advanced technologies that industry sentiments can change. This technology has gone from being dismissed in the 1980s to being one that wineries have nominated as the best change that they have made.

One interesting aspect of the early days of cross-flow filtration in the wine industry was that there was also interest in ultrafiltration, not just the microfiltration that has now been so successful. Ultrafiltration uses membranes with smaller pores and can remove haze-forming proteins from white wine, negating the need for bentonite (Wucherpfennig 1978; Miller et al. 1985). However, it also strips out other desirable macromolecules and there were sometimes issues with incomplete protein removal by the membrane types/porosities used at the time (Hsu et al. 1987). Ultrafiltration has received relatively little attention in this application since and may be worth revisiting using new membranes in a multi-stage format to retain desirable macromolecules. Ultrafiltration has the potential to be integrated with microfiltration into a single clarification and protein stabilisation system. While it would take some development, this style of technology is desirable since it could be automated and

would be at lower risk from future regulatory changes than most alternatives since it would not use additives or processing aids.

Flotation – the second most important practice change in wineries (and a history across multiple industries)

In the AWRI Vineyard and Winery Practices Survey, flotation was the next most important practice change nominated by wineries. The 2016 adoption levels of flotation, either as a single-stage juice clarification process or as a secondary stage technique following centrifugation, are shown in Figure 2. Single-stage flotation is now used by around half of wineries that crush more than 1,000 tonnes of grapes per year.

Flotation has many benefits. It is faster than settling, requires less cooling, and less juice is generally lost in float lees than settled lees. Flotation systems are also cheaper than centrifuges. The uptake of single-stage flotation is still relatively new for the Australian wine industry, having happened predominantly in the last decade. However, flotation has been used in other industries for much longer, including for more than a century in the minerals industry.

While flotation has resulted in important efficiency improvements in wineries, it had an even bigger impact on minerals processing. Fuerstenau (2007) reports that 'no metallurgical process developed in the 20th century compares with that of froth flotation and the profound effect it had on the minerals industry'. Earlier, Milliken (1962) expressed similar sentiments saying, 'Without the development of froth flotation there would be no mining industry as we know it today. This is because virtually the entire world supply of copper, lead, zinc, and silver is first collected in the froth of the flotation process'. Prior to its use in wine production, flotation also made major contributions to wastewater clarification and potable water clarification (Wang et al. 2005; Edzwald and Haarhoff 2011), and it is from these applications, rather than from mining, that single-stage flotation technology likely crossed into the wine industry and evolved to its current state.

While flotation processes currently use gas bubbles, early flotation applications relied on oil, with the desirable hydrophobic mineral constituents being attracted to the oil. The Bessel brothers used oil for flotation of graphite particles but reported in their 1877 patent that the bubbles produced by boiling made the process more efficient (Fuerstenau 2007; Edzwald and Haarhoff 2011). They followed up with a patent that relied on acid reaction with carbonates to produce gas bubbles, but their work was abandoned and forgotten for many years, following the discovery of higher-grade graphite reserves.

Australia played a key role in the development of minerals froth flotation technology in the early 20th century (Fuerstenau 2007). One early Australian process was the Potter-Delprat process (Figure 3a) used at Broken Hill (Truscott 1923; BHP 2015). As with one of the Bessel patents, it relied on the generation of carbon dioxide gas from the reaction of acid with carbonates. The feed material naturally contained carbonates and therefore only the acid needed to be added (Truscott 1923).

Another method that was used to generate bubbles in some early flotation equipment was application of a vacuum, such as in the Elmore vacuum process (Figure 3b). Bubble generation/dispersion by mechanical aeration also came to be used. The early Minerals Separation cells (Figure 3c) relied on agitation for frothing, while later equipment such as the Ruth cell (Figure 3d) specifically introduced air below the surface of the liquid and then mechanically dispersed it. While less sophisticated, this last design is conceptually not dissimilar from many modern minerals flotation cells that rely on air introduction (via natural aspiration or using compressed air) followed by mechanical dispersion of this air using an agitator (e.g. Figure 4). In minerals flotation, an array of different chemicals can be used to suit

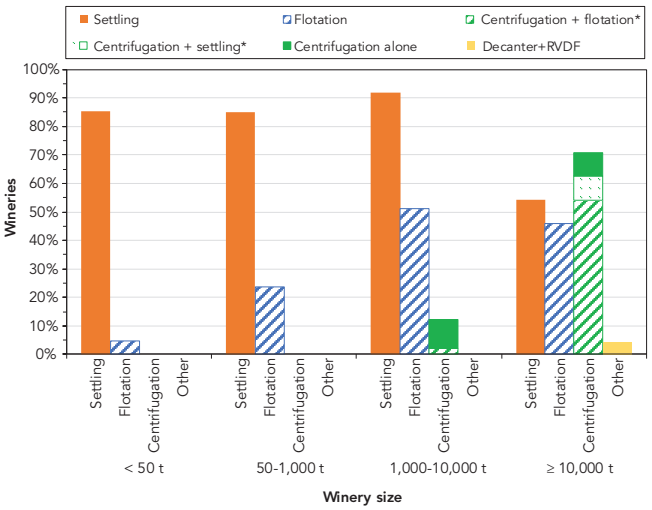


Figure 2. Juice clarification techniques used by Australian wineries in 2016

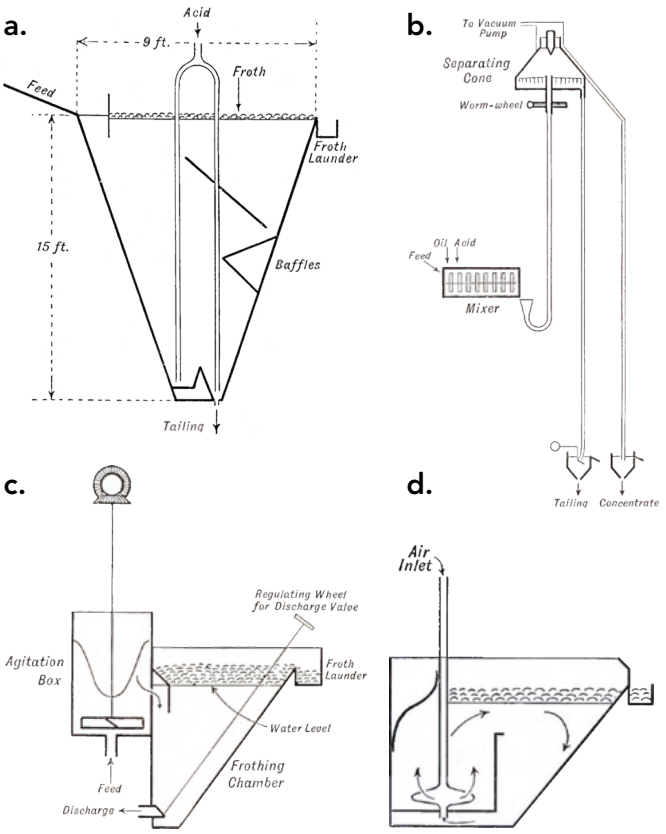


Figure 3. Some early mineral flotations equipment: (a) Potter-Delprat acid-carbonate flotation process, (b) Elmore oil-vacuum flotation process, (c) Minerals Separation cell with agitation box, (d) Ruth sub-aeration mechanical dispersion cell (adapted from Truscott 1923)

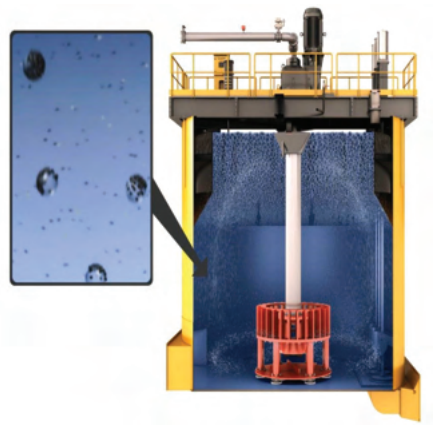


Figure 4. A modern mechanical dispersion flotation cell (Outotec, supplied)

the specific separation application – frothers, collectors, activators, depressants, modifiers and flocculants (Fuerstenau 2007). The use of chemicals is much more restrictive in juice clarification since the end product is for human consumption. Also, unlike juice clarification, in minerals processing the valuable material is generally in the froth/floats rather than in the phase below them.

Flotation for wastewater and water clarification has generally relied on dissolved gas bubble generation, in contrast to the mechanical dispersion techniques used in minerals processing. In this technique gas (usually air) is dissolved under pressure and that pressure is then released, producing bubbles that are usually smaller and more uniform than those achieved with mechanical dispersion processes (Pedersen 1921; Shammas and Bennett 2010; Edzwald and Haarhoff 2011). The small bubbles provide more surface area for collisions with solids and the lack of an agitator means that they are less likely to be sheared. Wastewater and water solids typically have low densities compared with many minerals, so large bubbles are not required to lift them (Edzwald and Haarhoff 2011).

The first use of flotation in water processing was in the 1920s for clarifying wastewater from the Scandinavian paper industry. The original Sveen-Pedersen process (Figure 5) used dissolved air flotation. It is referred to as the Sveen-Pedersen process because Pedersen designed the equipment, but it was only successful once Sveen's 'glue' was dosed to enhance flocculation (Pedersen and Sveen 1930; Klinger 1958). This dosing principle is amazingly similar to current wine industry flotation practices since the 'glue' was mainly protein, like the gelatine which is still used today in juice clarification (although gelatine is gradually being substituted with other non-animal and non-allergenic additives like pea and potato proteins and fungally derived chitosan). Flotation was later adopted for other industrial wastewater treatment and finally for potable water clarification. There were various advances along the way including dissolving air in a small part of a recycle stream instead of in the entire feed to save power, different configurations of flotation basin (e.g. Figure 6) and dissolved air flotation-filtration (DAFF) whereby depth filtration is integrated at the bottom of the flotation basin.

Single-stage flotation in the wine industry has been experimented with since the 1970s (e.g. Boulton and Green 1977). The first widespread application of flotation, however, appears to have been in Australia as a secondary stage after centrifugation and this technique is still widely practised today (Figure 2). When centrifuges started to be used for juice clarification it was found that air was being dissolved under pressure and, when released, the air bubbles floated fine particles in the product tank (Heinz Eibner, pers. comm.). Systems were later refined to use nitrogen instead of air and to specifically take advantage of this phenomenon (Chan 1984). By using a flotation step, much higher flow rates through the centrifuge could be used and/or a secondary settling stage prior to fermentation avoided.

Modern-day winery single-stage flotation originated in Italy around 1990 with the work of Ferrarini et al. (1991, 1992, 1995). The systems trialled were continuous and have clear similarities to those that were already being used for wastewater clarification (e.g. Figure 6). There appears to have been good uptake of this technology in some countries, but the uptake in Australia was very limited, with only one winery seeming to have installed a system (Falkenberg 1997). At the time a lot of installations appear to have used air for flotation in order to hyperoxidise musts, instead of the nitrogen that now dominates wine industry flotation (at least in Australia). The dosing of processing aids like gelatine and bentonite was also a key aspect of the new process, in contrast with the Australian centrifugation-flotation process that was not quite so reliant on perfect flocculation because it had a centrifugation step as well.

Large continuous flotation systems are cheaper than centrifuges, but still reasonably expensive. Apparently to make the process more affordable, systems were also sold without the continuous separation basin, with existing winery tanks being used for separation. As a next step to reduce cost, the large tank saturator was also removed, and small mobile units were developed in which gas and processing aids were injected during pumping between valves on the same winery tank (Figure 7). More than one full pump-over volume is generally used to try and counteract the inferior gas-liquid contacting from not using a large saturator. It could be argued that this arrangement is less sophisticated than the flotation systems that had been used in the wine industry 20 years earlier; however, they are a true wine industry adaptation of flotation. These systems allow many small batches to be processed (not a consideration in water treatment), cause no extra product movements compared with juice settling and, importantly, systems are relatively cheap, facilitating more rapid adoption. Interestingly, after some significant adoption of these recirculation flotation pumps, many large Australian wineries are now installing continuous flotation systems similar to those introduced to the wine industry around 1990. While these continuous systems are relatively expensive, have a large hold-up volume and are less flexible, they can be more efficient when large volumes of the same juice need to be clarified because they are more automated and centralise float lees accumulation for reprocessing.

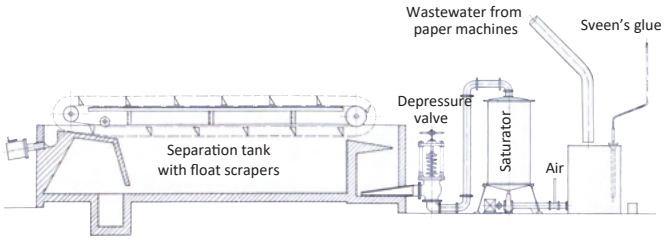


Figure 5. Sveen-Pedersen flotation cell (adapted from Brecht and Scheufelen 1938)

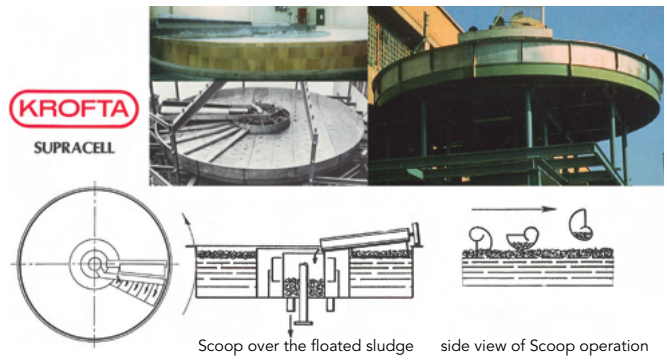


Figure 6. High capacity shallow circular flotation separation basin, c. 1970s (Krofta, supplied)

Figure 7. Mobile recirculation flotation pump (Juclas, supplied)

Flotation is already an effective process but perhaps it may be improved further in the future. For any technology development to be successful, it would have to be continuous and have a much smaller separation basin than existing continuous systems. It would also need to be able to handle intermittent flow such that it could be attached directly to the outlet of a batch press, clarifying the juice as it was produced and sending it directly to the fermenter. Technology that can achieve this has not yet been demonstrated.

Jameson flotation cells (Figure 8) have sometimes been advocated as a technology that should be adopted by the wine industry. Jameson cells were developed in Australia in the 1980s for the mining industry and have been very successful. Bubbles for flotation are created in the downcomers as the feed is jetted in, entraining air and vigorously mixing it in. Atkinson et al. (1993) reports that Jameson cells produce much smaller bubbles than traditional mechanical dispersion flotation cells. However, while no explicit comparisons exist, it seems unlikely that this technology produces as small and consistent bubbles as dissolved gas flotation, where gas is dissolved under pressure and then released from solution. Therefore, the clarification performance with a Jameson cell is likely to be lower and/or the juice occlusion in the float lees higher than with current wine industry systems.

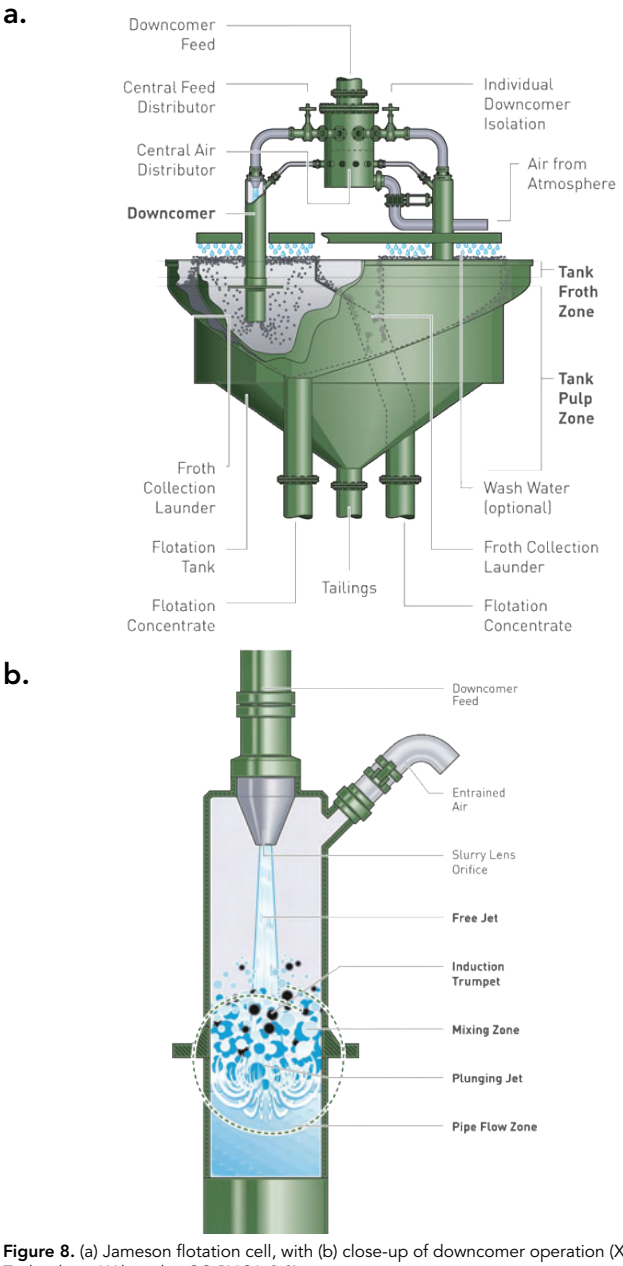


Figure 8. (a) Jameson flotation cell, with (b) close-up of downcomer operation (Xstrata Technology, Wikipedia, CC-BY-SA-3.0)

In-tank fermentation monitoring – an opportunity

Only one Australian winery currently uses in-tank sensors to monitor the conversion of sugar to ethanol during fermentation (Figure 9). It is instead standard practice to regularly manually collect samples and measure their density with a laboratory hydrometer or density meter. The low uptake of in-tank sensors for monitoring fermentation progress is similar in other wine-producing countries.

While there are some technical challenges to measuring fermentation progress in-tank (e.g. sensor fouling), the real barrier to adoption is price. The seasonal nature of wine production means that many tanks are needed to vinify grapes in the short time available and the cost of fitting all these tanks with sophisticated instrumentation is not insignificant. It is sometimes reasoned that it is cheaper just to get a vintage casual to collect samples and for them to be tested in a laboratory, since samples are needed for regular sensory analysis during fermentation anyway. However, an alternative argument is that an in-tank sensor is more than just a substitute for a manually collected sample later analysed in a laboratory. If ferment progress is measured in-tank it can feed into process control to optimise each fermentation (e.g. temperature, nutrients and agitation). If data are measured and recorded automatically this is also likely to better facilitate continual improvement. Ideally, wineries would have set programs for different types of fermentation with appropriate control parameters surrounding at least fermentation speed and temperature for different stages of the ferment (instead of just having a current temperature setting for the tank, which is common). At the end of vintage, the data could be reviewed and programs continually refined year after year in conjunction with sensory and chemical data. This strategy would likely be most useful in large wineries.

The concept of in-tank fermentation progress sensors is not new. Many different techniques have been trialled and adopted to a limited extent in wine and beer production:

- Pressure transducers to monitor ferment density were one of the first techniques to be used. In this approach two pressure diaphragms connected to a transducer or to two separate pressure transducers are installed, allowing the product density to be calculated based on the difference in pressure. Moller (1975) and later Cumberland et al. (1984) investigated this technique in breweries and similar techniques have since also been trialled to a limited extent in wineries.
- Tuning-fork-style density sensors have also received some recent attention (Endress+Hauser 2014; Zimberoff 2016). These calculate density based on the resonant frequency of the liquid (Emerson 2018).
- Coriolis flow meters can also be used for analysis of density using similar principles, but during pump-overs or using sample loops (Emerson 2015).

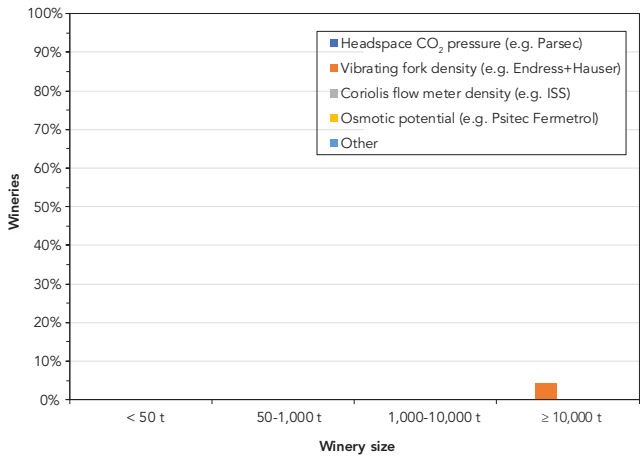


Figure 9. In-tank fermentation progress sensor use by Australian wineries in 2016

- Another approach to monitoring ferment progress has been to constantly measure the flow rate of gas (principally carbon dioxide) coming out of the fermenter. The sugar concentration/liquid density can then be back-calculated based on the stoichiometry of the fermentation reaction and the initial sugar level. In a forerunner to this approach, Saller (1958) used a device that monitored the carbon dioxide flow rate and controlled cooling to maintain a constant fermentation rate. Modern wine industry incarnations assessing carbon dioxide flow rate sold by Vivelys and Parsec appear to have their roots in French research during the late 1980s and early 1990s (El Haloui et al. 1988; Sablayrolles and Barre 1989; Bely et al. 1990; Sablayrolles 2009). While carbon dioxide flow rate can theoretically be used to back-calculate density, a major use of these systems seems to be for timing additions of oxygen to ferments to help avoid sluggish or stuck ferments (for example, oxygen addition at the time of peak carbon dioxide flow rate). Breweries have also used carbon dioxide flow rate as a means of tracking fermentation (Daoud et al. 1989; Daoud and Searle 1990; Stassi et al. 1987, 1991). A major advantage of ferment monitoring by carbon dioxide flow rate is that the sensor is not in direct contact with the liquid or ferment solids; however, it will not work if the tank/lid is opened and the initial sugar level needs to be known.
- Other in-tank sensors that have been trialled in the wine industry include osmotic potential sensors (Abbott 2016) and in-tank refractometers (VinPilot 2019). Refractometers are widely used in the wine industry for assessing juice sugar content, but during fermentation the measurement is complicated by the contribution of ethanol to refractive index. This can, however, be approximately corrected for based on the known initial sugar content (i.e. when there was no ethanol), fermentation stoichiometry and known relationships for the impact of sugar and ethanol on refractive index.

In addition to the above techniques, methods for directly assessing yeast health and nutrient/aeration requirements beyond what is possible from just tracking the fermentation speed may also be useful. Redox probes are one technique that has been trialled (Boulton 2016; Killeen et al. 2018; Wilson 2018). Another approach has been to measure the hydrogen sulfide concentration in the gas from the fermenter, using relatively cheap electrochemical gas sensors (AEB's Ctrl-Ferm). These sorts of techniques may prove important to the successful adoption of other fermentation progress sensors, because if winemakers still need to perform sensory analysis once or twice a day on ferments to determine nutrient additions and these same samples could be tested for density in the laboratory, then the argument against installing in-tank sensors is stronger. For high-end products, winemakers will likely always still want to taste the wine as a check, but in large wineries with large batch sizes where the technology would be most applicable, tasting as regularly as is currently performed is probably not necessary and could be limited to only when a problem is identified by sensors.

Breweries have also used other technologies to monitor yeast, particularly in relation to pitching control. In-line turbidity measurement before and after yeast dosage has been quite widely used in breweries (Boulton and Quain 2006; Kunze 2014). A problem with techniques like turbidity measurement for monitoring yeast is that they do not distinguish between viable and non-viable yeast cells. However, an alternative technique has been developed that detects only viable cells, based on their dielectric properties, and it appears that this may have had some commercial success (Harris et al. 1987; Boulton et al. 1989; Carvell 1997; Boulton and Quain 2006; Aber 2020).

In-tank colour/phenolic/tannin measurements may also be of value for red ferments to control decisions about fermenter mixing regimes, but this is not currently practised. Shrake et al. (2014) developed one system with a sample loop to analyse ferments using UV/Vis spectroscopy. The system provided valuable data; however, it worked based on light transmission through a 100 µm flow cell and therefore needed an in-line pre-filtration system. Unfortunately, the need for sample filtration means that this style of system is less likely to be adopted by wineries. The need for sample clarification has long been a major practical problem for immediate phenolic/colour measurements needed for in-line or at-line process control and has likely contributed to very low adoption levels of phenolic/colour measurements during fermentation. One interesting development that has achieved some commercial uptake is voltammetry using disposable electrodes, which requires no sample clarification (Lagarde-Pascal et al. 2019). However, the disposable electrodes mean that this is still a manual at-line rather than an in-line technique. Another approach that is being developed is a UV/Vis spectrometer that uses an 'integrating sphere' to separate scattered and absorbed light and which can therefore be used with turbid samples (Darby et al. 2016, 2019).

Continuous processes in the wine industry

Continuous processes are generally seen by engineers as being preferable to batch processes. Among other advantages, they usually have a smaller footprint and lower operating costs; however, there are some important aspects to consider in the adoption of a continuous process:

- What is the hold-up volume of the continuous process?
- How long does it take to start up and reach steady-state?
- If it is an operation that can currently be performed in many tanks simultaneously, would adopting a continuous process with a single piece of equipment create a process bottleneck?
- What is the impact on wine quality?
- Does it involve purchase of an additional piece of equipment?
- Is it appropriate across the range of different products being made?

The answers to some of these questions can make continuous processes not as easily applicable to wineries as they are in other industries. However, there have been many efforts at continuous processes in the wine industry because of the potential benefits.

An early example of continuous winery equipment was the continuous press. Batch basket presses were labour intensive and a typical process bottleneck. To address this, many different types of continuous press were developed in France in the late 19th century (Ferrouillat 1894). The continuous screw press (e.g. Figure 10) quickly became the most popular continuous press design. Continuous screw presses are still used today in wineries following many improvements; for example, more hygienic materials, improved feeding systems, larger screw diameters, lower speeds and better automation. Even with these

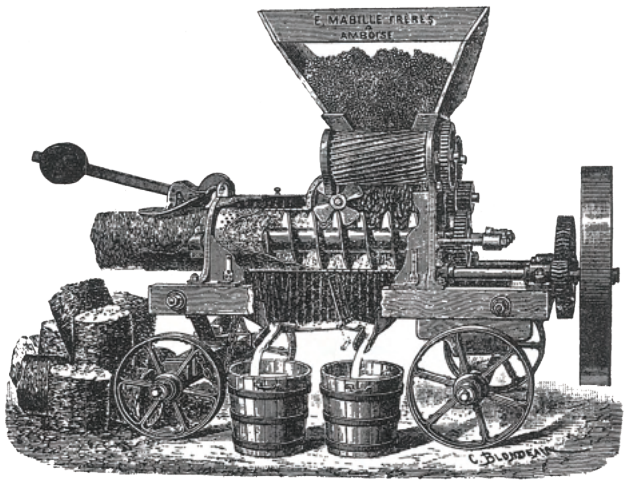


Figure 10. Continuous press, c. 1890s (Ferrouillat 1894)

improvements, continuous screw presses generally produce juice with higher solids levels than batch press designs. The advent of large automated axial filling membrane presses that produce juice with low solids levels has gradually led to the decline in use of continuous screw presses; however, they remain an important part of pressing operations in many large wineries around the world. While superior to earlier batch processes, membrane presses are still slow and there is therefore intermittent interest in continuous alternatives like decanter centrifuges (Nordestgaard 2015).

One fascinating continuous process that has been used in the wine industry, but which is now almost extinct, is continuous fermentation. This was a prominent technology in France in the 1960s and 1970s. One of the earliest systematic attempts at continuous wine fermentation was performed by Semichon (1926). Fresh juice was added to fermenting juice containing around 4% alcohol. This alcohol facilitated the selection of *Saccharomyces* yeast over other species (sometimes referred to as the 'Super 4' principal) and the continued addition of fresh juice also served to cool the ferment. A conically bottomed tank was used to allow for yeast removal. Juice removed from the tank at 4% alcohol completed the remainder of its fermentation in other tanks. For red wines, drained juice was put through the process and then added back to the skins. The first commercial implementation of continuous wine fermentation was by Victor Cremaschi in Argentina in the 1940s (Nègre 1949; Willig 1950). Cremaschi's continuous fermenter (Figure 11) used the 'Super 4' principal, but also incorporated a means to manage skins. The automatic removal of skins was a key consideration in this and many later designs of continuous wine fermenter, because the standard practice at the time of digging skins out of fermenters was labour-intensive and there were risks of carbon dioxide asphyxiation. The largest adoption of continuous fermentation was ultimately in Southern France (Ladousse 1962; Nègre 1967; Peynaud and Guimberteau 1967; Fages-Bonnery 1968; Roubert 1970). Continuous fermenters lack the flexibility of batch fermenters since large volumes over multiple days are mixed in the same tank. Bacterial contamination is also a risk given the large volume of wine and long use of each tank. There were also debates about how cost-effective these devices really were. Claims that continuous fermenters greatly reduced the overall winery tank capacity needed were contested by others since the often only partially fermented wines from these devices still needed to be stored in other tanks to complete fermentation. Continuous fermenters ultimately fell from favour. The availability of improved designs of batch fermenter that facilitated easy skin removal and that were built from steel and stainless steel likely also contributed to the decline of continuous fermenters.

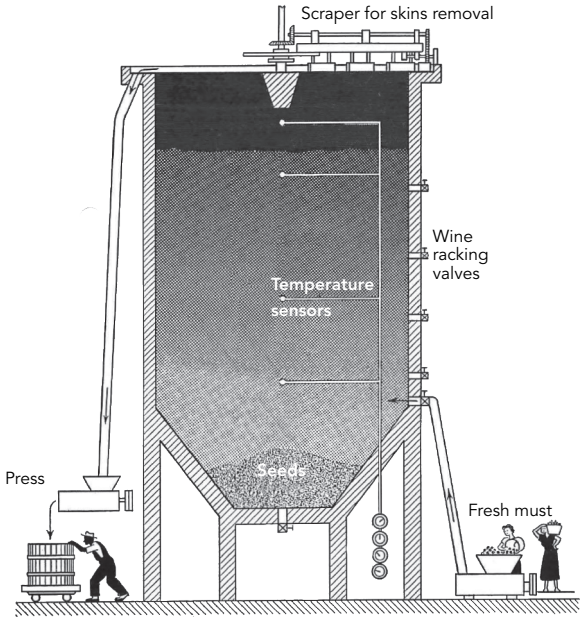


Figure 11. Cremaschi continuous fermenter (adapted from Anon. 1953)

As already mentioned, winery technology choices are heavily affected by the seasonal nature of wine production, and this also applies to the use of continuous processes. Attempts have been made to try to 'de-vintage' wine production. For example, in the late 1970s large quantities of juice used to be stored heavily sulfited and at low pH and used for year-round fermentations (after de-sulfiting and pH adjustment) for bag-in-box wine production. Continuous fermentation would have coupled well with this process since fermenters could have been run for many months and even years without stopping, but this did not happen (Potter 1984). The method of storing and processing juice in this manner, always controversial, fell out of favour in the 1980s.

Continuous fermentation is more easily applicable to sparkling wine production since it could be performed all year round using base wine, a much more stable feedstock than juice. Continuous sparkling wine production was pioneered in the Soviet Union (Amerine 1959) and it may have been quite widely used there. Continuous fermentation has also been used in beer production, which, like sparkling wine production and unlike still wine production, can easily be performed all year round. Continuous beer fermentation was pioneered in New Zealand by Morton Coutts in the 1950s (Campbell 2017) and for a long time it was used to produce most of the beer in New Zealand. Its use in New Zealand is much lower than it once was, but at least one brewery in New Zealand still uses this approach. Continuous fermentation has also been used for periods by other breweries around the world but has since been abandoned (Bud 1989). Interestingly, at the time when the technique was widely adopted in New Zealand there were some restrictive building regulations and taxation arrangements that made it desirable to minimise plant footprint and beer volume on-site, which further contributed to the merit of the technology (Kennedy 1996).

Another area of wine production where continuous processes are often proposed is cold stabilisation; for example, continuous tartrate contact and electrodialysis systems. These technologies were first used in the late 1960s (Caputi 1967; Vialatte 1979) and exist in improved forms today. Both techniques can work, but the economics can be difficult to justify (Low et al. 2008) for wineries that already have refrigeration and insulated jacketed tanks to manage ferments that can be used for cold stabilisation outside vintage. While slow, this arrangement gives the ability to cold stabilise many batches at the same time, whereas adopting a single piece of equipment might create a process bottleneck.

It should also be noted that the line between what is a continuous process and what is a batch process can be somewhat blurred. For example, multiple batch presses used in sequence can process a continuous intake of grapes. Even processes like continuous fermentation were not generally continuously fed with fresh grapes and wine and skins continuously removed. Instead, enough wine was removed each day so that there was space to add that day's grapes.

Volume measurement – is there a better option than a dip tape?

Most wineries currently measure the volume of liquid in tanks using a tape measure with a floating weight on the end (Figure 12). The ullaged distance from the surface of the wine to the top of the tank is measured and the corresponding volume of liquid in the tank is read from a table. This technique is relatively cheap, simple and hygienic. However, it requires somebody to go above the tank to perform the measurement, relies on them performing it accurately and it is not a live measurement. Small differences in level can make quite a big

difference in volume measurement (e.g. a 2 cm dip error in a 5 m diameter tank is a 400 L error). Another potential source of error in this and most of the other techniques discussed below is any inaccuracies in the tank dip tables, since tanks that are nominally the same often have slightly different volumes.

External tubes next to a graduated scale are another basic level measurement technique that has sometimes been employed by wineries (Figure 13). While not requiring access to the top of the tank, the level would be difficult to view on taller tanks, and it is likely a less hygienic solution than a dip since there is a thin tube containing wine that is at risk of not being properly cleaned.

Hydrostatic pressure at the bottom of tanks has also been used to measure levels in winery tanks. Both mechanical pressure gauges and electronic pressure sensors have been employed (Figure 14). An advantage of electronic sensors is that they can be connected to a Supervisory Control and Data Acquisition (SCADA) system and monitored remotely. Measurement errors increase with height. For example, in the electronic pressure sensor shown, the error in pressure measurement is ±0.2%, so assuming a constant and known liquid density, at 2 m height the error is ±4 mm, while at 10 m it is ±20 mm. A major disadvantage of level and volume measurement based on hydrostatic pressure is that the results are dependent on density, which can vary with product type and temperature. For example, a density difference of 0.4% between dry red and dry white wine would regularly be encountered (40 mm for a 10 m liquid level), and, more significantly, sweet and fortified wines can often be 7% more dense than dry wines (700 mm for a 10 m liquid level). This issue might necessitate having a second pressure transducer on the same tank so that the real density can be calculated based on the difference in hydrostatic pressure between the transducers (similar to using pressure transducers to monitor ferment progress).

Radar is another technique for level measurement (Figure 15). This works based on the time of flight of a radar pulse reflected off the surface of the liquid. Radar should generally be more accurate than hydrostatic pressure transducers and the result is not dependent on liquid density. The device shown has an error of ±1 mm across most of its range, increasing up to ±4 mm right next to the sensor. These devices are already used to a small extent in wineries, mainly for sparkling wine pressure tanks, where it is not possible to access the inside of the tank to make a manual dip measurement.

Trials have not been performed by the authors using these technologies, but based on discussions with suppliers it seems likely that they could be very useful. Electronic level sensors will be more expensive than dip measurements in the short term. While the cost would be significant, it is likely to be only around 5% of the cost of a 250 kL tank and less for larger tanks and large multi-tank installations (the exact costs would vary depending on the specific circumstances). The installation position would need to be carefully considered to ensure that systems collect the correct data and do not get in the way of other operations or create cleaning problems.

More sensors would lead to some different skill requirements in wineries; for example, likely more instrumentation maintenance staff and less basic labour. At some point, individual sensors will inevitably give incorrect readings and some clever system design is likely to be required to identify and manage these issues. For example, automatic cross-checking between levels measured in feed and product tanks and flow meters during transfers.

The live nature of automated level measurements is likely to provide greater centralised process oversight and can ultimately facilitate greater process automation for product movements. As a basic example, some wineries that installed electronic level sensors many years ago, and have them integrated with the SCADA, have commented how useful they are for tracking jobs and scheduling which tanks the next batch should go into during the peak of vintage.

Figure 12. A dip tape used for level measurement. Photo credit: AWRI

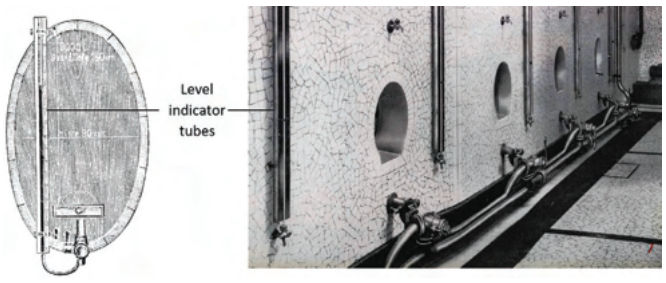


Figure 13. External level indicator tubes (Meißner 1920; Gasquet c. 1950s)

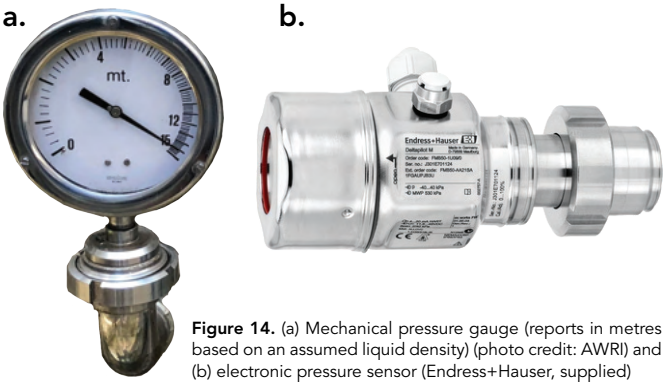


Figure 14. (a) Mechanical pressure gauge (reports in metres based on an assumed liquid density) (photo credit: AWRI) and (b) electronic pressure sensor (Endress+Hauser, supplied)

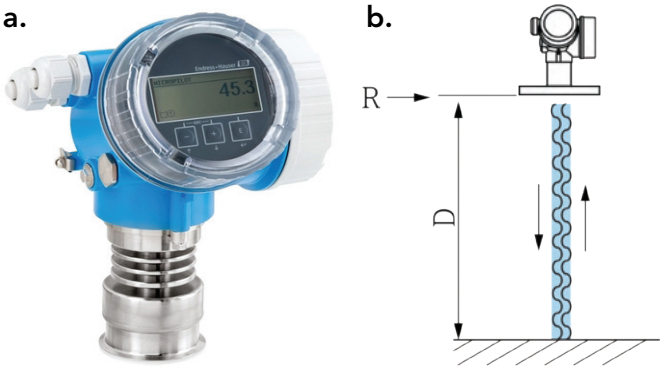


Figure 15. (a) Radar level measurement sensor (80 GHz with a narrow beam) and (b) radar measurement principle (Endress+Hauser, supplied)

Eliminating hoses and automating product movements

Hoses are widely used in wineries because they facilitate the movement of product between any two points. They are a trip hazard, require manual handling and their use is a barrier to improved winery automation (e.g. they are problematic to 'pig').

Some old winery design catalogues (e.g. Daubron 1931; Gasquet c. 1950s) contain fascinating examples of wineries with very few hoses. These wineries had pipework that went all the way to tanks fitted with multi-way valves (Figure 13) and used centralised distribution boards (e.g. Daubron's 'Centralisateur', Figure 16). One driver in these designs was the need to use fixed steam-powered pumps; they probably fell out of favour following the advent of electrification and mobile electric pumps, and because of issues with hygiene and metal leaching.

However, in some respect these designs are more advanced than many modern wineries despite the much more limited technology available at the time of their construction. They should serve as some inspiration for designers of modern automated wineries. Designers now have at their disposal stainless steel, hygienic pumps and valves, and computers.

Pigging would likely form a part of a modern automated winery. Pigging uses mobile plugs (pigs) to clean, inspect or push products through pipelines (Figure 17). Advanced automated pigging systems are already used at some wineries for key fixed transfer lines, particularly in botting facilities for key transfer lines between the winery and bottling tanks, between bottling tanks and bottling lines, and on some winery must lines. The use of pigging could potentially be expanded in wineries to all stages of production. Pigging loops around tank farms might be used in addition to the point-to-point systems that are now most common. Increased use of pigging would be expensive but would allow significant process automation and would help with reducing winery water use.

There are other technologies that may also assist with automation, beyond the electronic level sensors discussed and flow meters that are already common in wineries (electromagnetic flow meters are common, but more accurate Coriolis flow meters may be useful in some applications). For example, equipment using electrical impedance spectroscopy to automatically detect interfaces between different liquid types and stop a pump is now commercially available (Figure 18; Cozbel 2015; Pellenc 2019) and cheaper but less sophisticated electrical conductivity and turbidity sensors may also be useful for interface detection in some applications.

In-line dissolved gas management using membrane contactors

One newer technology that is starting to gain traction in the wine sector is membrane contactors for dissolved gas adjustment (Figure 19). When combined with appropriate control systems these can be used to adjust carbon dioxide levels up or down to a set level, while simultaneously removing some oxygen, all in the same pass. They are a viable alternative to sparging for gas adjustment in the later stages of wine production and potentially allow for looser winery carbon dioxide specifications with adjustments being made automatically during bottling. Membrane contactors can be used for both minor adjustments to carbon dioxide levels and for full carbonation. The 'bubbleless' method of gas addition can also allow for carbonation at warmer temperatures than might currently be practised (Nordestgaard 2018).

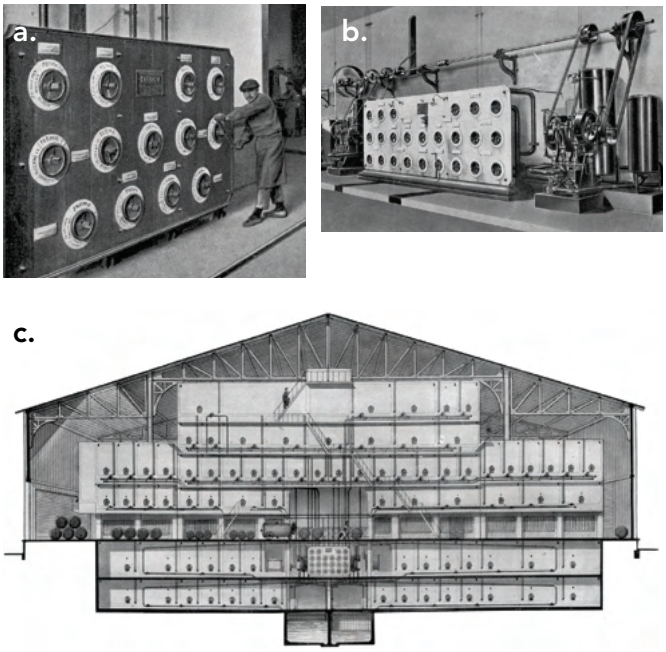


Figure 16. (a,b) Centralisateur distribution boards and (c) a winery built around this principle (adapted from Daubron 1931)

Figure 17. Illustration of a pigging system (Hygienic Pigging Solutions, supplied)

Figure 18. Smart Glass system for interface detection: (a) key components, (b) example implementation (Pera-Pellenc, supplied)

Conclusions

This article has outlined a range of technologies that have been used in wineries, including some that have become very successful (such as cross-flow filtration and flotation) and others where adoption has been lower. Something that stands out, even in large wineries, is that many practices are still very manual. The costs for some of the more automated approaches discussed in this article may be higher in the short term, but they may also be a path to continued improvements in quality and cost reduction in the longer term.

Acknowledgements

Grape and wine producers who filled out the AWRI Vineyard and Winery Practices Survey and met for discussions are thanked for their assistance, as are grape and wine associations that helped with survey promotion. AWRI colleagues who assisted with the survey project are also thanked, particularly Ella Robinson, Maria Calabrese, Assoc. Prof. Paul Petrie and Con Simos. Vinitech-Sifel sponsored a survey prize of a trip to its equipment trade show in Bordeaux and this is kindly acknowledged. The authors also appreciate the information provided by equipment suppliers. Leon Deans, Luke Wilson, Alan Hoey and Darrell Fabian are thanked for useful discussions prior to the conference. This work is supported by Australia's grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster in Adelaide.

Disclaimer

Readers should undertake their own specific investigations before purchasing equipment or making major process changes. This article should not be interpreted as an endorsement of any of the products described. Manufacturers should be consulted on correct operational conditions for their equipment.

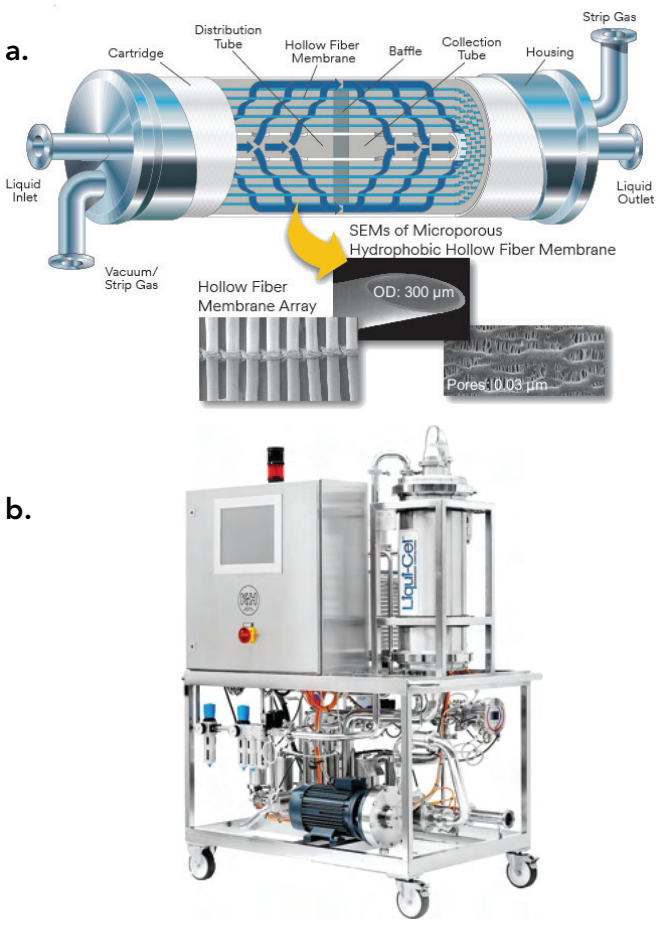


Figure 19. Membrane contactor: (a) module, (b) automated dissolved gas management system incorporating a membrane contactor module (3M and K+H, supplied)

References

Abbott, T. (2016) Using sensors to monitor sugar levels during fermentation. Urrbrae, SA: The Australian Wine Research Institute Ltd: https://s3.amazonaws.com/wea-website-files/2016+SA+Presentation+McLaren+Vale/AWRI+-+Tadro+Abbott+Ferment+Monitoring+presentation.pdf

Aber (2020) Compact Yeast Monitors. Aberystwyth, UK: Aber Instruments Ltd: https://www.aberinstruments.com/productscategories/view/online-compact-yeast-monitors

Amerine, M.A. (1959) Continuous flow production of still and sparkling wines. Wines and Vines 40(6): 41–42.

Anon. (1953) Prácticas modernas en la gran industria del vino. Revista de la Cámara Argentina de Comercio: p. 109.

Atkinson, B.W.; Conway, C.J.; Jameson, G.J. (1993) Fundamentals of Jameson Cell operation including size-yield response. Proceedings of the Sixth Australian Coal Preparation Conference, Mackay, 6-9 September: https://www.jamesoncell.com/en/downloads/TechnicalPapers/Fundamentals-of-Jameson-Cell-Operation-including-Size-Yield-Respone-Atkinson-Conway-Jameson.pdf

Bely, M.; Sablayrolles, J.M.; Barre, P. (1990) Description of alcoholic fermentation kinetics: Its variability and significance. Am. J. Enol. Vitic. 41: 319–324.

BHP (2015) Making history, History of BHP, The Potter Dell Pratt Process: https://www.youtube.com/watch?v=H5g4tRtq2mg

Boulton, C.A.; Maryan, P.S.; Loveridge, D.; Kell, D.B. (1989) the application of a novel biomass sensor to the control of yeast pitching rate. European Brewing Convention. Proceedings of the 22nd Congress, Zurich: 653–661.

Boulton, C.; Quain, D. (2006) Brewing yeast and fermentation. Oxford, UK: Blackwell Science Ltd.

Boulton, R. (2016) Controlling redox potential during wine fermentations. 9 December: RAVE: https://lecture.ucanr.edu/Mediasite/Play/0bcd2d15875c466cac572b08297450921d

Boulton, R.; Green, G. (1977) Field testing of the WEMCO juice clarifier. Wine Industry Technical Seminar, 3 December, Monterey, California.

Brecht, W.; Scheufelen, K. (1938) Untersuchungen eines Flotationsstoffängers nach Sveen-Pedersen. Papier-Fabrikant 15: 121–129, 16: 136–140.

Bud, R. (1989) Biotechnology and the chemical engineer: a case study in the history of continuous brewing. Int. Ind. Biotechnol. 9(6): 17–20.

Campbell, S.L. (2017) The continuous brewing of beer. DB Breweries Ltd: https://nzic.org.nz/app/uploads/2017/10/6A.pdf

Caputi, A. (1967) Wine stabilization by electrodialysis. Report to Wine Institute Technical Advisory Committee Meeting, 8 December.

Carvell, J.P. (1997) Developments in on-line monitoring of viable yeast in the brewery process. Paper presented at the Institute of Brewing (Asia Pacific Section) Regional Technical Symposia, April.

Chan, A.L. (1984) Juice storage alternatives – clarification and refrigeration. Lee, T.H. (ed.) Proceedings of the 5th Australian wine industry technical conference, Perth, WA, 29 November – 1 December 1983. Urrbrae, SA: The Australian Wine Research Institute: 317–330.

Cozbel, M. (2015) Nouveaux débouchés pour les capteurs innovants d'Inozy: https://objectif-languedoc-roussillon.latribune.fr/entreprises/agroalimentaire/2015-11-27/l-innovation-de-la-societe-inozy-lui-ouvre-des-perspectives.html

Cumberland, W.G.; MacDonald, D.M.; Skinner, E.D. (1984) Automated fermenter control at Moosehead Breweries Limited. MBAA Technical Quarterly 21(1): 39–44.

Daoud, I.; Dyson, R.; Irvine, J.; Cuthbertson, R.C. (1989) Practical experiences of on-line monitoring of evolved CO_2 from production fermenters. European Brewing Convention. Proceedings of the 22nd congress, Zurich: 323–330.

Daoud, I.S.; Searle, B.A. (1990) On-line monitoring of brewery fermentation by measurement of CO_2 evolution rate. J. Inst. Brew. 96: 297–302.

Darby, B.L.; Auguié, B.; Meyer, M.; Pantoja, A.E.; Le Ru, E.C. (2016) Modified optical absorption of molecules on metallic nanoparticles at sub-monolayer coverage. Nat. Photonics 10: 40–45

Darby, B.; Setford, P.; Robinson, A.; Miles, J. (2019) The CloudSpec - A new spectroscopic tool for analysis of unfiltered ferments. Poster presented at the 17th Australian wine industry technical conference, 21-24 July, Adelaide, SA: https://awitc.com.au/wp-content/uploads/2019/07/163-MARAMALABS_POSTER_FINAL.pdf

Daubron (1931) Brochure de références.

Edzwald, J.K.; Haarhoff, J. (2011) Dissolved air flotation for water clarification. McGraw Hill Professional: 352 p.

El Haloui, N.; Picque, D.; Corrieu, G. (1988) Alcoholic fermentation in winemaking: On-line measurement of density and carbon dioxide evolution. J. Food Eng. 8: 17–30.

Emerson (2015) Coriolis flow meter theory of operation: https://www.youtube.com/watch?v=31jYXlnu-hU

Emerson (2018) Liquid density meter from Emerson micro motion: https://www.youtube.com/watch?v=0d5XG8l6g2g

Endress+Hauser (2014) Density measurement in wine fermentation process: https://portal.endress.com/wa001/dla/5001070/7403/000/00/CS01410F00EN0114.pdf

Fages-Bonnery, A. (1968) Procédés de vinification continue. Fermentations et vinifications. Proceedings of 2nd Symposium International d'Œnologie, Bordeaux-Cognac, 13-17 June 1967. INRA Volume 2: 553–583.

Falkenberg, W. (1997) Juice clarification by flotation. Allen, M.; Leske, P.; Baldwin, G. (eds) Proceedings of ASVO Seminar - Advances in juice clarification and yeast inoculation, 15 August 1996, Melbourne, Vic.: 8–10.

Ferrarini, R.; Zironi, R.; Buiatti, S. (1991) Prime esperienze di applicazione della flottazione nei processi di chiarifica ed illimpidimento dei mosti d'uva. Vignevini 18: 29–32.

Ferrarini, R.; Zironi, R.; Celotti, E.; Buiatti, S. (1992) Prémiers résultats de l'application de la flottation dans la clarification des moûts de raisin. Rev. Française d'Œnologie 32: 29–42.

Ferrarini, R.; Celotti, E.; Zironi, R.; Buiatti, S. (1995) Recent advances in the process of flotation applied to the clarification of grape musts. J. Wine Res. 6(1): 19–33.

Ferrouillat, P. (1894) Les pressoirs continus. Rev. de Vitic. 1(25): 597–601, 1(27): 645–654, 1(28): 671–674, 2(29): 14–17, 2(30): 33–38, 2(32): 91–93.

Fuerstenau, D.W. (2007) A century of developments in the chemistry of flotation processing. Fuerstenau, M.C.; Jameson, G.; Yoon, R.-H. (eds) Froth flotation - a century of innovation. Society for Mining, Metallurgy and Exploration, Inc.: 3–64.

Gasquet (c. 1950s) Équipement et installations de cuvieres pour liquides alimentaires. Notice 1137.

Gibson, R.L. (1986) Cross flow membrane technology for the wine industry. Aust. Grapegrower Winemaker 268: 17–23.

Harris, C.M.; Todd, R.W.; Bungard, S.J.; Lovitt, R.W.; Morris, J.G.; Kell, D.B. (1987) Dielectric permittivity of microbial suspensions at radio frequencies: a novel method for the real-time estimation of microbial biomass. Enzyme Microb. Technol. 9: 181–186.

Hsu, J.C.; Heatherbell, D.A.; Flores, J.H.; Watson, B.T. (1987) Heat-unstable proteins in grape juice and wine. II. Characterisation and removal by ultrafiltration. Am. J. Enol. Vitic. 38: 17–22.

Institut Technique de la Vigne et du Vin [ITV] (1985) Ultrafiltration et microfiltration tangentielle en œnologie. Proceedings of the conference held 23-24 January.

Kennedy, M.J. (1996) The World's first brewery exclusively designed to use continuous fermentation: Biotechnology history made in New Zealand. Aust. Biotechnol. 6(1): 13–18.

Killeen, D.J.; Boulton, R.; Knoesen, A. (2018) Advanced monitoring and control of redox potential in wine fermentation. Am. J. Enol. Vitic. 69: 394–399.

Klinger, L.L. (1958) What you should know about flotation saveall design and operation. Paper Trade Journal, 1 September: 26–31.

Kunze, W. (2014) Technology Brewing and Malting. 5th revised English ed. (translated by Pratt, S.) VLB Berlin: 960 p.

Ladousse, G. (1962) Vinification continue et automatique. Vignes et Vins 115: 11–13.

Lagarde-Pascal, C.; Charpentier, E.; Diéval, J.-B.; Vidal, S. (2019) Méthode électrochimique pour la mesure en temps réel des polyphénols au cours de la vinification. Rev. des Œnologues 173: 41–44.

Low, L.L.; O'Neill, B.; Ford, C.; Godden, J.; Gishen, M.; Colby, C. (2008) Economic evaluation of alternative technologies for tartrate stabilisation of wines. Int. J. Food Sci. Tech. 43: 1202–1216.

Meißner, R. (1920) Technische betriebskontrolle im weinfach: Handbuch für betriebsleiter im weinfach, weinhändler, küfermeister, weingutsbesitzer und sonstige interessenten. Stuttgart: E. Ulmer.

Miller, G.C.; Amon, J.M.; Gibson, R.L.; Simpson, R.F. (1985) Loss of wine aroma attributable to protein stabilization with bentonite or ultrafiltration. Aust. Grapegrower Winemaker 256: 46–50.

Milliken, F.R. (1962) Introduction. Fuerstenau, D.W. (ed.) Froth flotation 50th anniversary volume. New York: American Institute of Mining, Metallurgical, and Petroleum Engineers, Inc.: 1–3.

Moller, N.C. (1975) Continuous measurement of wort/beer extract in a fermenter. MBAA Technical Quarterly 12(1): 41–45.

Nègre, E. (1949) Procédé original de fermentation continue. Le Progrès Agr. Et Vit. 132: 313–325.

Nègre, E. (1967) Le point actuel sur la vinification continue. Le Progrès Agr. Et Vit. 84: 511–524.

Nordestgaard, S. (2015) SIMEI 2015 – Wine, olive oil and decanters. Aust. N.Z. Grapegrower Winemaker 624: 66–68: https://www.awri.com.au/wp-content/uploads/2019/02/Nordestgaard2016-SIMEI.pdf

Nordestgaard, S. (2018) Gains in speed, labour and gas consumption for winemakers. Aust. N.Z. Grapegrower Winemaker 648: 61–67: https://www.awri.com.au/wp-content/uploads/2018/01/Nordestgaard2018-gasmanagement.pdf

Nordestgaard, S. (2019) AWRI Vineyard & Winery Practices Survey, May: www.awri.com.au/survey

Nordestgaard, S. (2020) Wine History Posters: https://wea.org.au/archives/wine-history-posters

Pedersen, N. (1921) Process for separating solid particles from suspension. US1376459.

Pedersen, N.; Sveen, K. (1930) Process of separating particles in aqueous suspensions. CA305759.

Pellenc (2019) Smart Glass – wireless sensor for pump/device control: https://www.youtube.com/watch?v=QPiTHaiFbWc

Peynaud, E.; Guimberteau, G. (1967) Vinification continue mise au point oenologique. Connaissance de la Vigne et du Vin 3: 128–157.

Potter, R.A. (1984) The Brimstone process - past, present and future - Part I. Lee, T.H. (ed.) Proceedings of the 5th Australian wine industry technical conference, Perth, WA, 29 November – 1 December 1983. Urrbrae, SA: The Australian Wine Research Institute: 293-298.

Rankine, B.C. (1996) Evolution of the modern Australian wine industry: a personal appraisal. Adelaide, SA: Ryan Publications: 192 p.

Roubert, J. (1970) In: Équipement viticole. Vignes et Vins 196: 9–23.

Sablayrolles, J.M.; Barre, P. (1989) Pilotage automatique de la temperature de fermentation en conditions œnologiques. Sci. Aliments 9(2): 239–251.

Sablayrolles, J.M. (2009) Control of alcoholic fermentation in winemaking: Current situation and prospect. Food Res. Int. 42: 418–424.

Saller, W. (1958) Control of cold fermentation. Am. J. Enol. Vitic. 9: 41–48.

Semichon, L. (1926) Nouveau procede de vinification par fermentation continue. Rev. de Vitic. 65(1671): 21–27, (1672): 41–43, (1673): 53–59, (1674): 71–79.

Shammas, N.K.; Bennett, G.F. (2010) Principles of air flotation technology. Wang, L.K.; Shammas, N.K.; Selke, W.A.; Aulenbach, D.B. (eds) Flotation technology. Handbook of environmental engineering, Volume 12. New York: Humana Press: 1–47.

Shrake, N.L.; 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. Am. J. Enol. Vitic. 65(4): 463–470.

Stassi, P.; Rice, J.F.; Munroe, J.H.; Chicoye, E. (1987) Use of CO_2 evolution rate for the study and control of fermentation. MBAA Tech. Q. 24: 44–50.

Stassi, P.; Goetzke, G.P.; Fehring, J.F. (1991) Evaluation of an insertion thermal mass flowmeter to monitor CO_2 evolution rate in plant scale fermentations. MBAA Tech. Q. 28: 84–88.

Truscott, S.J. (1923) A text-book of ore dressing. London: Macmillan and Co.

Vialatte, G. (1979) Stabilisation des vins en continu vis à vis du bitartrate de potassium. Rev. Française d'Œnologie 16(73): 67–71

VinPilot (2019) VinPilot Brix: https://vinpilot.com/en/vinpilot-brix-en

Wang, L.K.; Fahey, E.M.; Wu, Z. (2005) Dissolved air flotation. Wang, L.K.; Hung, Y.-T.; Shammas, N.K. (eds) Handbook of environmental engineering, Volume 3. New York: Humana Press: 431–499.

Willig, M. (1950) Wine now made continuously. Food Ind. 22: 1184–1185.

Wilson, L. (2018) Practical oxygen management in the winery: https://s3.amazonaws.com/wea-website-files/2018_WineEng_SA/Yalumba+-+Luke+Wilson+-+Practical+Oygen+Management+in+the+Winery+-+Oxygen+Management+Forum.pdf

Wucherpfennig, K. (1978) Possibilities of applying pressure-filtration through membranes (ultra, and hyper-filtration) to drink production. Lemperle, E.; Frank, J. (eds) Fifth international oenological symposium, 13-15 February, Auckland, N.Z.: 93–113.

Zimberoff, L. (2016) Napa's fermenting your wine with submarine technology. Wired: https://www.wired.com/2016/01/how-to-make-wine

New techniques and technologies for wine protein stabilisation

J.M. McRae

The Australian Wine Research Institute, Urrbrae, SA. Current address: The University of Adelaide, Adelaide, SA
jacqui.mcrae@adelaide.edu.au

Abstract

Producing protein-stable or 'heat-stable' wines that remain clear and bright from packaging to consumption remains a key area of interest for winemakers and is therefore also an important research topic. Bentonite addition remains the most effective and widely used approach to ensure protein stability of wines, although many alternatives are currently in development. Enzyme additions, flash pasteurisation and novel protein adsorbents are just some of the latest approaches. To better prevent protein haze, it is also important to have an accurate test for protein stability, which has traditionally been achieved with a heat test. Recently the heat test has been modified to improve its accuracy and reproducibility. Accurately predicting protein instability and effective use of protein-removal technologies is the most cost-effective and efficient way of preventing protein haze formation in wines.

Introduction

Protein instability is a major concern for winemakers. If a conventional wine is sold that later becomes hazy, consumers are likely to consider the haze to be a fault and may not re-purchase the product, resulting in brand damage.

Protein haze in wine is caused by pathogenesis-related proteins that are extracted from grape skins during winemaking. These grape proteins have robust, rounded structures and are stable at a pH range of 4.5-6 and temperature up to 35°C (depending on exposure time). However, after long-term exposure to low pH conditions (such as in wine) or short-term exposure to high temperatures, the proteins unfold and aggregate, eventually forming particles large enough to produce a visible haze (Van Sluyter et al. 2015). Many wine components contribute to haze formation, including phenolics, sulfates and metal ions, and therefore there is no direct relationship between protein concentration and the amount of haze formed (McRae et al. 2018b). The best strategy for preventing protein haze is to remove the haze-forming proteins.

The most widely used method for removing proteins from wine is to add bentonite, a clay that is globally available and comparatively inexpensive. Bentonite binds to wine proteins through cation exchange and the proteins settle out of the wine with the bentonite lees, which are then removed. The main concern with using bentonite is that when the bentonite lees are removed from wine, they can also trap a substantial proportion (around 10%) of the wine itself. Some of the lost wine can be recovered through processes such as rotary drum vacuum filtration, but there is generally an associated quality downgrade. Overall, the losses to the global winemaking industry due to bentonite use have been estimated at more than $1 billion per year (Majewski et al. 2011). Adding the optimal dose of bentonite during ferment (Pocock et al. 2011) and using a centrifuge to recover the wine from the bentonite lees reduces wine loss but this is not feasible in all wineries.

The cost associated with bentonite use is the main driver for research into viable alternatives to bentonite. This paper highlights some of the more promising research outcomes for preventing protein haze formation in wine without bentonite.

Novel strategies for preventing protein haze

Enzymes

The idea of using enzymes to remove wine proteins has been around for many years but has only recently shown much promise. Pathogenesis-related proteins are inherently resistant to enzymes due to their spherical, stable structures. These features, combined with the low pH of wine, make the search for a naturally occurring enzyme that is effective against grape proteins near impossible. However, when grape proteins are heated, they unfold, and this makes them much more susceptible to enzymatic degradation. Aspergillopepsin enzymes are active at the temperature range at which grape proteins unfold and therefore heating grape juice in the presence of these enzymes prior to ferment can produce heat-stable wine (Marangon et al. 2012). These enzymes are approved for use in wines sold in Australia and in most export markets and are a viable alternative to bentonite (Godden and Guy 2015).

Some winemakers may be concerned that the heating step required to activate the enzymes and unfold the grape proteins (75°C for one minute) might have a negative effect on the sensory profile of treated wine. To investigate this possibility, heating trials were conducted on Semillon and Sauvignon Blanc juice, prior to fermentation. For both varieties, no significant difference was found between the sensory profiles of wines produced using heat-treated juice and that of the control wines produced from non-heated juice (McRae et al. 2019). These results are comparable with previous trials and further suggest that short-term heating of grape juice has no adverse sensory effects on the wine made from the heated juice.

Heating grape juice at 75°C for one minute can also reduce the protein concentration in wine by around half (McRae et al. 2019). This reduction can be enough to heat-stabilise some wines or otherwise substantially reduce bentonite use in other wines without enzyme addition. Short-term heating of juice alone may therefore be another viable strategy for producing heat-stable wines with minimal lees production.

Carrageenan

Carrageenan is an effective alternative to bentonite that is commercially available, widely used in other food and beverage industries and is permitted in many wine export markets. It comes from red seaweed and is therefore also a renewable natural product. Recent trials have shown that wines produced after addition of carrageenan at the juice, ferment or wine stages of production are protein stable and have higher intensities of flavours and aromas than wines treated with bentonite. This suggests that carrageenan is more selective than bentonite in removing wine proteins without also removing desirable wine sensory compounds (Ratnayake et al. 2019), making it another promising potential alternative to bentonite.

Membranes

The idea of removing wine proteins using a membrane is very attractive and has been around for many years. The process would ideally involve transferring protein-unstable wine through a membrane to give protein-stable, clear and bright wines. Yet this concept is very difficult to turn into reality. The main difficulty is in producing a membrane that selectively removes protein from wine without removing other wine components and ensuring that there is no protein breakthrough. This is particularly important because such a membrane might be used on a bottling line where there is no margin for error if the membrane starts to allow proteins to pass through into the bottled wine.

A recent collaboration between the University of Adelaide and VA Filtration is investigating new strategies for using membrane technology to heat stabilise wines and is already yielding some promising results. It is likely that this work will lead to the development of new membrane technology for wine protein stabilisation in coming years.

Grape seed powder

Grape seeds are a readily available and perpetually renewable resource that can be sourced directly from white grape marc. Grape seeds contain high concentrations of polyphenols that readily bind to proteins. After seeds are roasted (180°C, 10 minutes) and powdered, they can be added to grape juice to bind haze-forming proteins. The juice is then racked and fermented, producing clear, bright and protein-stable wine (Romanini et al. 2020). This approach has demonstrated very promising results on a laboratory scale and warrants further research to assess any impacts on wine sensory properties.

Magnetic nanoparticles

Mierczynska-Vasilev et al. (2019a) developed a bentonite alternative based on magnetic nanoparticles coated to make them selective for wine proteins. The particles can be added to protein-unstable wine and, after a short interaction time, an external magnet is used to attract the protein-rich particles to the bottom of the tank or to an in-line trap, leaving protein-stable wine and minimal lees. This exceptionally promising technology has demonstrated efficacy at laboratory scale and further work will investigate the commercial feasibility at larger scale.

Zeolites

Zeolites work in a similar way to bentonite but settle more efficiently, reducing the amount of wine lost as lees compared with bentonite addition. Zeolites can also potentially be repurposed for improving soil quality for agriculture after use in wine, making them a more sustainable technology for wine protein stabilisation (Mierczynska-Vasilev et al. 2019b).

Predicting protein haze

Getting the best out of bentonite or a selected bentonite alternative requires an accurate test to determine the dose of the protein-removal agent needed to render a wine protein-stable. This is best achieved using a haze-inducing test such as a heat test. Recent investigations into heat test conditions have determined that optimal results are gained by using consistent heating and cooling times of two hours at 80°C followed by three hours at 20°C. If the difference in haze formed in samples before and after heating is <2.0 NTU, a wine is considered to be protein-stable (McRae et al. 2018a). These test conditions allow for rapid, reproducible and accurate results for protein stability in wines.

Conclusions

Bentonite remains the most effective, commercially available strategy for producing heat-stable wines and is most effective when added during fermentation to reduce bentonite lees and in conjunction with a centrifuge to recover as much wine as possible. New alternatives to bentonite are showing great promise and different technologies are likely to become available in the short, medium and long term.

References

Godden, P.; Guy, S. (2015) Wines heat stabilised with Aspergillopepsin enzymes are now accepted by major Export Markets. AWRI Technical Review No. 218: October.

Majewski, P.; Barbalet, A.; Waters, E. (2011) $1 billion hidden cost of bentonite fining. Aust. N.Z. Grapegrower Winemaker 569: 58–62.

Marangon, M.; Van Sluyter, S.C.; Robinson, E.M.C.; Muhlack, R.A.; Holt, H.E.; Haynes, P.A.; Godden, P.W.; Smith, P.A.; Waters, E.J. (2012) Degradation of white wine haze proteins by Aspergillopepsin I and II during juice flash pasteurization. Food Chem. 135: 1157–1165.

McRae, J.M.; Barricklow, V.; Pocock, K.F.; Smith, P.A. (2018a) Predicting protein haze formation in white wines. Aust. J. Grape Wine Res. 24: 504–511.

McRae, J.M.; Schulkin, A.; Dambergs, R.G.; Smith, P.A. (2018b) Effects of white wine composition on protein haze potential. Aust. J. Grape Wine Res. 24: 498–503.

McRae, J.M.; Godden, P.W.; Romanini, E. (2019) Effect of juice heating on the sensory profile of wine. AWRI Technical Review No. 242: October.

Mierczynska-Vasilev, A.; Mierczynski, P.; Maniukiewicz, W.; Visalakshan, R.M.; Vasilev, K.; Smith, P.A. (2019a) Magnetic separation technology: Functional group efficiency in the removal of haze-forming proteins from wines. Food Chem. 275: 154–160.

Mierczynska-Vasilev, A.; Wahono, S.K.; Smith, P.A.; Bindon, K.; Vasilev, K. (2019b) Using Zeolites to Protein Stabilize White Wines. ACS Sustainable Chem. Eng. 7: 12240–12247.

Pocock, K.; Salazar, F.N.; Waters, E.J. (2011) The effect of bentonite fining at different stages of white winemaking on protein stability. Aust. J. Grape Wine Res. 17 280–284.

Ratnayake, S.; Stockdale, V.; Grafton, S.; Munro, P.; Robinson, A.L.; Pearson, W.; McRae, J.M.; Bacic, A. (2019) Carrageenans as heat stabilisers of white wine. Aust. J. Grape Wine Res. 25: 439–450.

Romanini, E.; McRae, J.; Colangelo, D.; Lambri, M. (2020) First trials to assess the feasibility of grape seed powder (GSP) as a novel and sustainable bentonite alternative. Food Chem. 305: doi.org/10.1016/j.foodchem.2019.125484

Van Sluyter, S.C.; McRae, J.M.; Falconer, R.J.; Smith, P.A.; Bacic, A.; Waters, E.J.; Marangon, M. (2015) Wine protein haze: mechanisms of formation and advances in prevention. J. Agric. Food Chem. 63: 4020–4030.

New techniques and technologies for cold stabilisation of wine

R.C. Runnebaum

Department of Viticulture and Enology, University of California, Davis, USA
Department of Chemical Engineering, University of California, Davis, USA
rcrunnebaum@ucdavis.edu

Abstract

Alternative methods of stabilising wines from bitartrate precipitation are of continued interest because of trade-offs associated with available processes. Cold stabilisation can be one of the most costly wine processes as a result of its energy use, wine loss, treatment time and subsequent water use for cleaning. The use of a fluidised bed crystalliser (FBC) addresses these important metrics to the benefit of wineries. In addition, this approach uses identical chemical and physical interactions of seeded crystallisation to minimise unintended differences in wine composition compared with the traditional approach. A FBC has been designed and constructed to generate data at pilot scale that will complement data collected at bench scale to develop predictive models for efficient wine treatment. The controlled crystal growth on seeded crystals of potassium bitartrate in the FBC minimises wine loss and reduces water and energy used in removing bitartrate from tank surfaces, which can be maintained at storage temperatures. Heat exchangers minimise energy use and loss by contacting treated wine exiting the FBC with untreated wine entering the FBC. In-line sensors confirm stability of treated wines in real time, which enables wine to either be immediately returned with minimal mixing to the original tank or be directly transferred to bottling operations. The development and modelling of the FBC provides another important alternative to stabilising wines from bitartrate precipitation, with an approach that minimises energy and water usage, wine loss and treatment time, while also minimising unintended differences in wine composition.

Introduction

Technologies for cold stabilisation that prevent potassium bitartrate (KHT) crystallisation and precipitation in the bottle are needed because of the consequences, from a consumer standpoint, when KHT crystals are observed in the bottle (Salamone and Oberholster 2015). Consequences can be amplified if observation of KHT crystals expands beyond individual consumers to impact distribution channels as well. While the ideal solution might be continued informing of the consumer that these crystals of potassium bitartrate are harmless and do not impact wine quality, it is important to recognise that it is very difficult to change consumer perceptions. Cold stabilisation and other stabilisation techniques are likely to be best addressed, therefore, by treatment within the winery. During this stage of post-fermentation winemaking, one can still control the product prior to bottling and what will eventually be presented to the consumers on the shelf. One approach to treatment, which has interest from a number of wineries in California, has the overarching goal of identifying stabilisation techniques to enable wine treatment on the time-scale of minutes to hours without any additions, and with minimal usage of water and energy.

Understanding of the physical and chemical conditions that lead to KHT instability is important because it can help to inform decision-making with respect to why one might want to choose a certain treatment technique over another. The basis for the instability also helps one to appreciate the challenges of controlling or impacting the conditions before the grapes arrive at the winery. One reason that this issue arises is that the most prevalent cation in grape berries is potassium (K^+), which is readily transported into the grape berries through proton/cation exchange (Boulton 1980a). This exchange also has important implications for the pH of the resulting wines (Boulton 1980c, b). There are actions that can be taken in the vineyard to limit the exchange of potassium, such as crop level, soil moisture and harvesting timing, while other influences on extent of exchange are not easily altered, such as the age of the vine (Boulton 1980a). The organic acid anion is that of tartaric acid, which is the most prevalent organic acid in winegrapes. The bitartrate form (HT^-) is the most prevalent form of the two anions from tartaric acid under wine pH conditions. The solubility of KHT decreases from the initial aqueous conditions of grape juice to when ethanol is present in wine (Balakian and Berg 1968). The need for cold stabilisation arises, therefore, because potassium bitartrate is relatively soluble in an aqueous sugar solution, and as ethanol is formed, the solubility decreases. Storage conditions, such as low temperature, can further decrease solubility limits. When high concentrations of both potassium and bitartrate occur in wines, conditions can be created where the solubility limit might be exceeded and subsequently KHT crystallisation and crystals can be observed.

One challenge is predicting KHT instability, especially kinetics. Treatment decisions are often based on equilibrium measurements (Coulter et al. 2015) such as a conductivity test (Bolan 1996), as shown in Figure 1. Crystallisation kinetics are difficult to predict, especially when crystals will be formed in the bottle once the wine goes into the marketplace. In general, crystal formation is difficult to predict across a variety of wines because of the variation in concen-

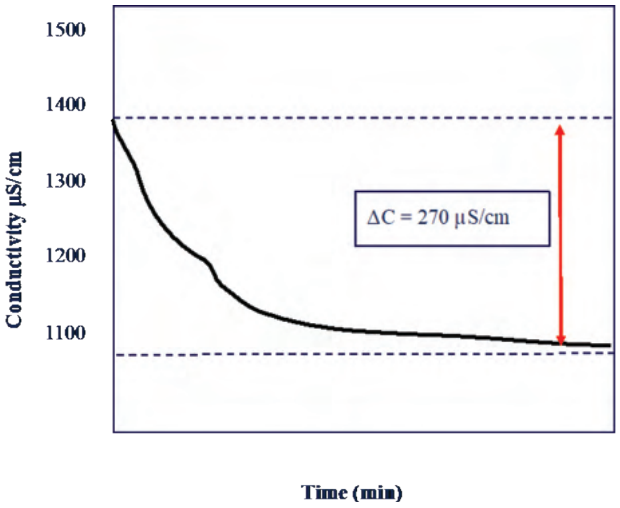


Figure 1. The use of change in conductivity to determine the likelihood of KHT instability at a particular temperature. Adapted from Hirzel (2008)

trations of potassium and bitartrate across the many wines produced (Dunsford and Boulton 1981b, a). To complicate things even further, the importance of pH on the bitartrate form of tartaric acid impacts KHT solubility. By shifting the pH to form less of the bitartrate anion, one can change the solubility of potassium bitartrate in wine, even when the overall concentration of tartaric acid remains unchanged. The presence of, and interactions with, other molecules found in wine, such as anthocyanins (Balakian and Berg 1968), also impacts the ability of a wine to have a high holding capacity for potassium bitartrate. Because the time-scale of instability is difficult to predict, one typically makes an equilibrium measurement, such as with the UCD Conductivity Test by measuring a change in conductivity. Other measures exist for evaluating the potential formation of KHT crystals, but these typically require a time-scale in the order of hours to days; in contrast, the UCD Conductivity Test is a measurement that tests the potential for instability in about 20 minutes. Lastly, because wines differ in their concentrations of potassium and bitartrate, pH and holding capacity, each wine should be tested to assess its potential for developing a KHT instability.

The multi-step process of crystal formation, which requires both nucleation and crystal growth, also influences potassium bitartrate instability (Geankoplis 1993a). The concentration of KHT in the wine needs to be supersaturated at the storage temperature for nucleation and crystallisation to occur. A wine might be stable at shelf or room temperature and not be unstable until it is stored at a lower temperature for some period of time. Nucleation is an unpredictable step, where the organisation of a few molecules begin to crystallise. Once a small crystal has formed, another crystallisation process—crystal growth—can start. In this instance, crystal growth occurs by the diffusion of potassium bitartrate to the surface of the crystal for surface integration. Mathematical equations, developed for other crystallisation processes (Geankoplis 1993a), can be used to model this process in wine. These mechanisms are based on an understanding of physical and chemical processes similar to those in many other systems and industries, and the wine industry is able to apply that knowledge for wine treatment.

Nucleation and crystal growth are understood to be functions of factors such as particle size, agitation and surface area of the crystals (Geankoplis 1993b). By understanding these relationships developed in other application areas, this knowledge can be applied to wine treatment (Bolan 1996). Agitation minimises concentration gradients and accelerates diffusion of potassium bitartrate to the crystal surface (Dunsford and Boulton 1981a). Introducing crystals for a laboratory test or into a winery treatment is based on insights from these types of relationships. Use of crystal powders, for example, enhances the surface area of these crystals. The practice of additions is also based on interfering with the processes of nucleation or crystallisation. An understanding of nucleation and crystal growth has been used as the basis for the current types of tests and treatments, especially seeded crystallisation, additions and the UCD Conductivity Test.

Approaches to resolve cold stabilisation and potassium bitartrate precipitation can be categorised by how they stabilise the wine and how the treatment is implemented, as shown in Figure 2. Approaches can be categorised as either removing potassium bitartrate or as adding a compound that will interfere with either nucleation or crystal growth. In the latter category the interference can be with either potassium or bitartrate, or both. These processes can be available as batch or in-tank treatments, and opportunities exist for developing and using these types of treatments in a more continuous operation, or at least continuously for a single tank. In addition to these technical aspects of stabilisation, a number of other considerations are quite important across all types of treatments. In the future, demand for better understanding and transparency of what is being added into foods will likely increase. In California, there is interest in minimising energy usage—as the cost of energy is increasing, especially at certain times of the day or different periods of the year. There is also widespread interest in minimising or reducing water usage—the effective use of water is related to wine loss. In a number of regions in California there are strong correlations between irrigated water and the juice volume taken into the winery: for every gallon of white wine that is lost or downgraded in quality, one can likely correlate that volume with a significant amount of water used for irrigation. For those with an interest in minimising additions and downstream processing, one can take advantage of continuous processing operations (originating from other chemical engineering processing applications).

The traditional approach to cold stabilisation is the use of seeded crystallisation and the need for chilling tanks to around 0°C for several weeks. The seeding helps to eliminate the need for nucleation of crystals and thus facilitates crystallisation. The use of chilling tanks for cold stabilisation presents challenges especially because it is inefficient in terms of both energy use and the deployment of winery assets. This traditional approach also results in some amount of wine loss and requires the use of chemicals and water for removing crystals from tank walls. This additional use of energy and chemicals has implications for energy use as well as disposal of wastewater. As a result, an interest in wine additions, as well as new approaches to potassium bitartrate removal, has developed over the past several decades as alternatives to this traditional cold stabilisation practice.

Additions that stabilise K^+ or HT^- (or interfere with crystal formation)

Over the past several decades, compounds have been identified or developed to prevent or delay the formation of KHT crystals in wine. The list of compounds includes metatartaric acid, carboxymethylcellulose (CMC), mannoproteins, and, more recently, potassium polyaspartate. The chemical and physical interactions between these compounds, and either potassium or bitartrate, impact nuclei formation, crystal growth, or both. These compounds, in some instances, reduce the overall rates of crystallisation and thus can be effective over the typical time-scale that one might store a bottle. Many winemakers find additions to be limiting because their presence can have a sensory impact, sometimes desirable or undesirable. Furthermore, compounds such as CMC and metatartaric acid can lead to other wine instabilities that depend upon the time-scale in bottle and storage conditions (Coulter et al. 2015; Guise et al. 2014; Sommer et al. 2016). More recently, there has been a continued effort to develop other compounds that are more stable over longer time

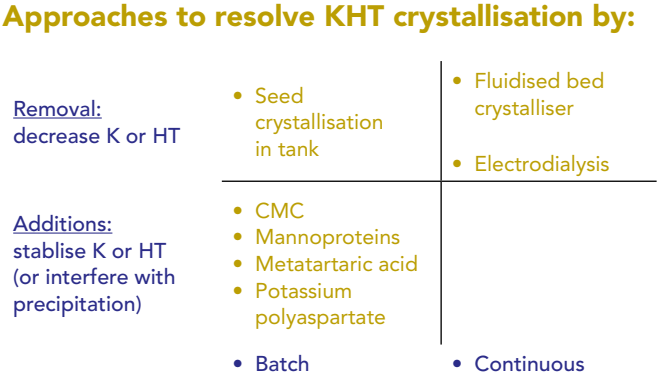


Figure 2. Approaches to resolving KHT crystallisation by either removal of K or HT or by addition of stabilising compounds. Technologies for decreasing K or HT concentrations are categorised by either batch or continuous processing. Additions are typically introduced in batch processing to each tank but can be introduced continuously as well.

periods. One of the compounds that has been undergoing approval and recently become available is potassium polyaspartate (Bosso et al. 2015; Canuti et al. 2019). This polymer is able to stabilise the wine from KHT crystallisation, at least based on the data that is available publicly, over the time-scale of 6 to 12 months (Canuti et al. 2019). At this point, more research and experimentation is needed with white wines, as well as red wines, to better understand when additions—especially potassium polyaspartate—are most useful to winemakers.

Technologies that decrease K^+ or HT^- concentrations

Opportunity exists for the continued development and deployment of technologies that can be available as a continuous process for the treatment of individual or multiple tanks. One approach available in various markets is electrodialysis, and another that is undergoing research at UC Davis is fluidised bed crystallisers (FBC). These approaches remove excess potassium and tartrate that would be responsible for the instability under shipping or storage conditions. Both options can take advantage of their continuous processing design, which could minimise tank transfers to reduce water usage or enable direct-to-bottling treatments.

For electrodialysis, the physical and chemical impact of this approach is the removal of cations and anions by applying an electric field across an ion selective membrane (Gonçalves et al. 2003). The use of ion selective membranes is only selective for either positively or negatively charged ions, as shown in Figure 1 of Gonçalves et al. (2003), but not necessarily selective for a particular ion such as potassium or bitartrate. One of the implementation challenges wineries can encounter with electrodialysis is the initial investment of the unit. Mobile electrodialysis units are being made available in some markets to eliminate this upfront capital cost. While it is useful to be able to apply technologies that have been developed for other industries, such as electrodialysis, the direct application in this instance can be relatively water-intensive and so this becomes an important consideration. Vendors of this technology seem to be aware of this limitation and are actively working to minimise water usage. For a number of wineries in California, water usage is becoming a more important consideration, and this is also likely to be the case in Australia.

Lastly, fluidised beds and crystallisers have been used in other industries. One example of fluidised beds is in their use for fluidised catalytic cracking (FCC) for the production of many important small chemicals. We can apply the fundamental understanding established in commercialising these approaches to develop knowledge of the physical chemical dynamics for wine treatment (Geankoplis 1993b, a). During operation, the velocity for fluidisation of the crystals is countered by terminal settling velocity of the crystals to result in crystals that are suspended in the liquid as the liquid passes through the column. In the instance of using a fluidised bed crystalliser (FBC), potassium bitartrate crystals (not a powder) are loosely packed into a cylindrical column. By introducing a large quantity of crystals into the column, one can generate very high surface areas and short treatment time. A short contact time can be beneficial in facilitating only removal of cation and anion species that comprise the crystals added to the column (Bolan 1996). In addition, only wine within the column is cooled. One can recover energy by using heat exchangers before and after the column. Initial attempts, which have yet to be optimised, have shown the ability to reduce energy consumption by 44% in bench-scale experiments (Hirzel 2008). Figure 3 shows the beginning of the bed being fluidised at pilot-scale operation. Again, the understanding of the physical phenomenon in chemical processes for fluidisation can be applied to the treatment of wine. This approach to cold stabilisation can complement the use of bench-scale trials so that treatment time can be determined for each wine.

Figure 3. Beginning operation of a fluidised bed crystalliser for wine treatment at pilot scale

Conclusion

In summary, cold stabilisation treatments will continue to be needed for the near future. Understanding nucleation and crystal growth can be used to help inform the choice of treatment for different wines and different tiers of wine that potentially need treatment. In addition, an understanding of the physical and chemical processes, such as crystallisation and separations, can be used to develop technologies for this treatment. Options may include additions that can be relatively easy or techniques that enable wine treatment on the time-scale of minutes to hours without any additions, and with minimal usage of water and energy.

Acknowledgements

Funding for this work has been provided by Treasury Wine Estates. I would like to thank the Conference Planning Committee for the invitation to speak at the 17th Australian Wine Industry Technical Conference.

References

Balakian, S.; Berg, H.W. (1968) The role of polyphenols in the behavior of potassium bitartrate in red wines. Am. J. Enol. Vitic. 19: 91–100.

Bolan, R.E. (1996) Development of a fluidized–bed crystallizer for wine treatment. Master Thesis: Davis, CA: University of California.

Bosso, A.; Panero, L.; Petrozziello, M.; Sollazzo, M.; Asproudi, A.; Motta, S.; Guaita, M. (2015) Use of polyaspartate as inhibitor of tartaric precipitations in wines. Food Chem. 185: 1–6.

Boulton, R. (1980a) A hypothesis for the presence, activity, and role of potassium/hydrogen, adenosine triphosphatases in grapevines. Am. J. Enol. Vitic. 31(3): 283–287.

Boulton, R. (1980b) The general relationship between potassium, sodium and pH in grape juice and wine. Am. J. Enol. Vitic. 31(2): 2–6.

Boulton, R. (1980c) The relationship between total acidity, titratable acidity and pH in wine. Am. J. Enol. Vitic. 31(1): 76–80.

Canuti, V.; Cappelli, S.; Picchi, M.; Zanoni, B.; Domizio, P. (2019) Effects of high temperatures on the efficacy of potassium polyaspartate for tartaric stabilization in wines. Am. J. Enol. Vitic. 70(3): 332–337.

Coulter, A.D.; Holdstock, M.G.; Cowey, G.D.; Simos, C.A.; Smith, P.A.; Wilkes, E.N. (2015) Potassium bitartrate crystallisation in wine and its inhibition. Aust. J. Grape Wine Res. 21: 627–641.

Dunsford, P.; Boulton, R. (1981a) The kinetics of potassium bitartrate crystallization from table wines. I. Effect of particle size, particle surface area and agitation. Am. J. Enol. Vitic. 32(2): 100–105.

Dunsford, P.; Boulton, R. (1981b) The kinetics of potassium bitartrate crystallization from table wines. II. Effect of temperature and cultivar. Am. J. Enol. Vitic. 32(2): 106–110.

Geankoplis, C.J. (1993a) Liquid-Liquid and Fluid-Solid Separation Processes. In: Transport Processes and Unit Operations. 3rd ed. Prentice-Hall, Inc.: 697–753.

Geankoplis, C.J. (1993b) Principles of Monentum Transfer and Applications. In: Transport Processes and Unit Operations 3rd ed. Prentice-Hall, Inc.: 114–213.

Gonçalves, F.; Fernandes, C.; Cameira dos Santos, P.; De Pinho, M.N. (2003) Wine tartaric stabilization by electrodialysis and its assessment by the saturation temperature. J. Food Eng. 59(2–3): 229–235.

Guise, R.; Filipe-Ribeiro, L.; Nascimento, D.; Bessa, O.; Nunes, F.M.; Cosme, F. (2014) Comparison between different types of carboxylmethylcellulose and other oenological additives used for white wine tartaric stabilization. Food Chem. 156: 250–257.

Hirzel, D.R. (2008) The Development of a Fluidized Bed for Crystallizing Potassium Bitartrate from Wine. Thesis. Davis, CA: University of California.

Salamone, P.; Oberholster, A. (2015) Non-subtractive approach to potassium tartrate stabilization. Wines & Vines 96(2): 67–74.

Sommer, S.; Dickescheid, C.; Harbertson, J.F.; Fischer, U.; Cohen, S.D. (2016) Rationale for Haze Formation after Carboxymethyl Cellulose (CMC) Addition to Red Wine. J. Agric. Food Chem. 64(36): 6879–6887.

Winemaking with high Baume juice: optimising fermentation and extraction

K.A. Bindon[1], B. Teng[1,2], P.A. Smith[1,3], D. Espinase Nandorfy[1], M. Solomon[1], P.R. Petrie[1,4]

[1]The Australian Wine Research Institute, Urrbrae, SA
[2]College of Science, Shantou University, Guangdong, P.R. China
[3]Wine Australia, Adelaide, SA
[4]South Australian Research and Development Institute, Urrbrae, SA
keren.bindon@awri.com.au

Portions of this article were previously published in *Wine and Viticulture Journal* and are reproduced here with permission from the publisher, Winetitles (Bindon et al. 2019).

Abstract

To help manage winemaking with grapes and juice at high sugar concentrations, Food Standards Australia and New Zealand (FSANZ) in 2016 changed the regulations to allow the limited addition of water to high sugar musts and juice by Australian producers. In line with these changes in legislation, a project was initiated to address the question as to whether the extent of water addition, or the method used (e.g. direct addition versus run-off/replace) affects wine composition and sensory properties in Shiraz. Wines were made from Shiraz grapes (Nuriootpa, Barossa Valley, SA, Australia) harvested at 13.5, 14.5 and 15.5°Baume. Water was added to additional batches of the 15.5°Baume fruit either by direct addition or using a run-off/replace approach. The water addition treatments aimed to approximate the alcohol concentrations of wines made from the earlier harvests. The wines made from the fruit that was harvested earlier (13.5 and 14.5°Baume) were generally of a lighter style, with low colour and tannin concentration and 'red fruit' aroma and flavour as the defining sensory attributes. Undiluted wines made from the 15.5°Baume harvest had the highest colour and tannin concentration, and were characterised by the attributes 'dark fruit' aroma/flavour, 'hotness', 'viscosity', 'astringency' and 'opacity'. The water addition treatments were found to reduce wine tannin concentration and colour density compared to undiluted wines harvested at the same maturity regardless of the water addition method used (direct addition versus run-off/replace) or quantity of water added. However, tannin concentration and colour were consistently higher in diluted wines (from 15.5°Baume) than in wines made from the 13.5 and 14.5°Baume harvests. Sensorially, the water addition treatments reduced all the key attributes: 'dark fruit' aroma/flavour, 'astringency', 'viscosity' and 'opacity', with a notable reduction of the alcohol-driven attribute, 'hotness'. Not surprisingly, the run-off/replace treatment with the smallest water addition (15.5 to 14.5°Baume) was the closest sensorially to the 15.5°Baume control. Higher water addition levels also increased 'cooked vegetable' and 'drain' attributes in the wines, suggesting possible negative effects of water addition.

Introduction

Over the last two decades grapegrowers and winemakers have observed that their fruit is ripening earlier and over a shorter period (Petrie and Sadras 2017). This can lead to a lack of resources to harvest fruit in the required time frame and the intake of fruit at higher sugar levels than are ideal. Problems which may arise from this are that fermentations may 'stick' before all the sugars have been fermented, or that wines with undesirably high alcohol are produced. In United States wineries, water addition to must is legal and widely accepted as a method to facilitate fermentation. Recognising that water addition was legal in other countries, and to address the above-mentioned problems in dealing with high Baume musts, Australian regulations were revised late in 2016 allowing for techniques to dilute high sugar musts (with water) to limit the risk of 'stuck' fermentations (FSANZ 2016) that do not complete alcoholic fermentation. According to the new legislation, water may be added to must to dilute it down to 13.5°Baume, but cannot be added to finished wine. The rationale behind these changes was to reduce the chances of problems arising during fermentation and to help the industry resolve the logistical problems caused by compressed vintage periods.

In response to the change in FSANZ regulations, the AWRI launched a Shiraz winemaking trial in the 2017 vintage, to investigate the impact of juice run-off and water additions on wine chemistry and sensory parameters. Previous work had been conducted with Cabernet Sauvignon grapes and showed that when different quantities of juice were run off after crushing and replaced with an equivalent volume of water, impacts on wine composition (tannin and colour) and sensory attributes were minor (Schelezki et al. 2018a, b). These were encouraging results, suggesting that quality losses from water addition might be expected to be minimal. The AWRI project took a similar approach to the published work on Cabernet Sauvignon, firstly using a run-off and replace approach to water addition, but also looking at the direct addition of water, since this was considered to be simpler, and therefore the preferred approach for the wider wine industry. Also, earlier work conducted with Cabernet Sauvignon had shown important increases in wine tannin and colour were found when wines were made from grapes at different ripeness levels between 12.9 and 14.4°Baume, but interestingly that no change in consumer preference was observed (Bindon et al. 2014). Based on these observations, a key take-home message was that must sugar (and hence wine alcohol) might be best controlled simply by reverting to harvesting at lower °Baume levels as was done historically (Godden et al. 2015). In light of this, a key focus of the AWRI water addition study was to compare harvest date (ripeness) and water addition as two approaches to modulate final wine alcohol, and to assess outcomes on phenolic extraction and wine sensory properties as potential indicators of wine quality.

Materials and methods

The Shiraz fruit was hand-harvested from a vineyard near Nuriootpa, in the Barossa Valley on three occasions, targeting 13.5, 14.5 and 15.5°Baume. Care was taken to ensure that the fruit was harvested systematically from across the block to ensure that the part of the vineyard where it was grown did not affect the final wines. The fruit harvested at 13.5 and 14.5°Baume was fermented normally, as was a portion of the fruit harvested at 15.5°Baume. The wine produced

from the 15.5°Baume harvest served as the control treatment for the study. The balance of the 15.5°Baume harvest was diluted either by direct addition of potable rainwater, or by a run-off/replace approach with the same water to achieve targeted Baume levels of 14.5 or 13.5°. The inclusion of harvests at 13.5 and 14.5°Baume in the study was to enable a comparison of the composition of diluted wines with wines of the same must sugar concentration produced naturally through earlier harvest dates. All ferments were of 45 kg of fruit and each treatment was processed in triplicate. The ferments were inoculated with a commercial yeast (EC1118) and lactic acid bacteria (VP41) on the second and third day after crushing, respectively. All ferments were maintained on skins for nine days in a 20°C room from crushing to pressing. Two acid additions were made (early in the ferment and post-pressing) to target a titratable acidity of approximately 5 g/L. Wines were assessed sensorially at the AWRI at approximately 12 months by a trained panel of 11 assessors with an average age of 49 years (± 9.5). At the time of sensory analysis, the wines were analysed for colour properties using the modified Somers assay, and total tannin using the methyl cellulose precipitation assay (Mercurio et al. 2007).

Results and discussion

The titratable acidities of the final wines were successfully managed to the relatively narrow range of 4.6–5.1 g/L and this resulted in a pH range of 3.5–3.8 across all the treatments (Table 1). The alcohol content ranged from 13.9% for the first Shiraz picked (13.5°Baume), through to 16.5% for the latest harvest (15.5°Baume). Water addition successfully reduced the alcohol content of the final wines. It was, however, less effective for the must that was diluted to a target of 13.5 °Baume, in that the alcohol concentrations of these wines were higher than those of the wines from the fruit picked at 13.5°Baume.

A strong increase in wine tannin, non-bleachable pigments (bisulfite-resistant colour) and wine colour density was found with the transition of harvest date from 13.5 to 15.5°Baume (Table 1). Water addition consistently reduced wine tannin concentration, non-bleachable pigments and colour density compared to the 15.5°Baume control wines. However, it was interesting to observe that neither the method of water addition (direct addition versus run-off/replace) nor the quantity of water added had a significant impact on tannin and colour. This indicates that the reason for the reduction in phenolics extraction was not simply due to a change in the solids:must ratio (i.e. the proportion of skins and seeds to liquid in the ferment).

Sensory analysis showed that the wines could chiefly be defined by multiple attributes associated with harvest date. The wines made from the fruit at 15.5°Baume had higher 'dark fruit' aroma/flavour, 'hotness', 'viscosity', 'astringency' and 'opacity' (among other attributes), while the wines made from the 14.5 and 13.5°Baume harvests had progressively less of these attributes and higher scores for 'red fruit' aroma (Figure 1). For the treatments where water was added to the 15.5°Baume must, the effect of water addition on sensory attributes was greater as the quantity of water added increased, in contrast to the effects observed on wine phenolics (Table 1). Dilution introduced losses in the above-mentioned attributes associated with the 15.5°Baume control wine, and caused increased expression of 'red fruit', but the wines were still more similar in their sensory attributes to the control than to the wines made from the earlier harvests. For wine sensory properties, the method of dilution was important for the lower level of water addition (15.5°Baume to 14.5°Baume treatment). Here, it was found that the run-off and replace treatment was more like the control than the direct addition treatment (Figure 1). The highest level of water addition produced very similar wines, and introduced 'cooked vegetable', 'drain' and 'savoury' aromas. This suggests that possible negative attributes may result from a greater quantity

Table 1. The impact of harvest date and water addition on wine chemical parameters: data analysed by one-way ANOVA with different superscripted letters after the mean (of triplicate ferments) value indicating a significant difference using a post-hoc test

	Alcohol (%)	Titratable acidity (g/L)	pH	Tannin (mg/L)	Colour density (AU)[1]	Non-bleachable pigment (AU)
15.5°Be control	16.5 a	4.6 d	3.8 a	723 a	11.8 a	2.62 a
Run-off and replace (15.5°Be to 14.5°Be)	15.6 b	4.7 c,d	3.8 a	548 b	10.9 b	2.10 b
Direct addition (15.5°Be to 14.5°Be)	15.6 b	4.9 b,c	3.7 b	544 b	11.0 b	2.04 b
Run-off and replace (15.5°Be to 13.5°Be)	14.4 e	4.6 d	3.6 c,d	513 b	10.7 b	2.01 b
Direct addition (15.5°Be to 13.5°Be)	14.9 d	4.7 c,d	3.6 c	538 b	10.6 b	1.99 b
14.5°Be control	15.3 c	5.0 a,b	3.6 d,e	368 c	9.47 c	1.58 c
13.5°Be control	13.9 f	5.1 a	3.5 e	232 d	7.64 d	1.35 d

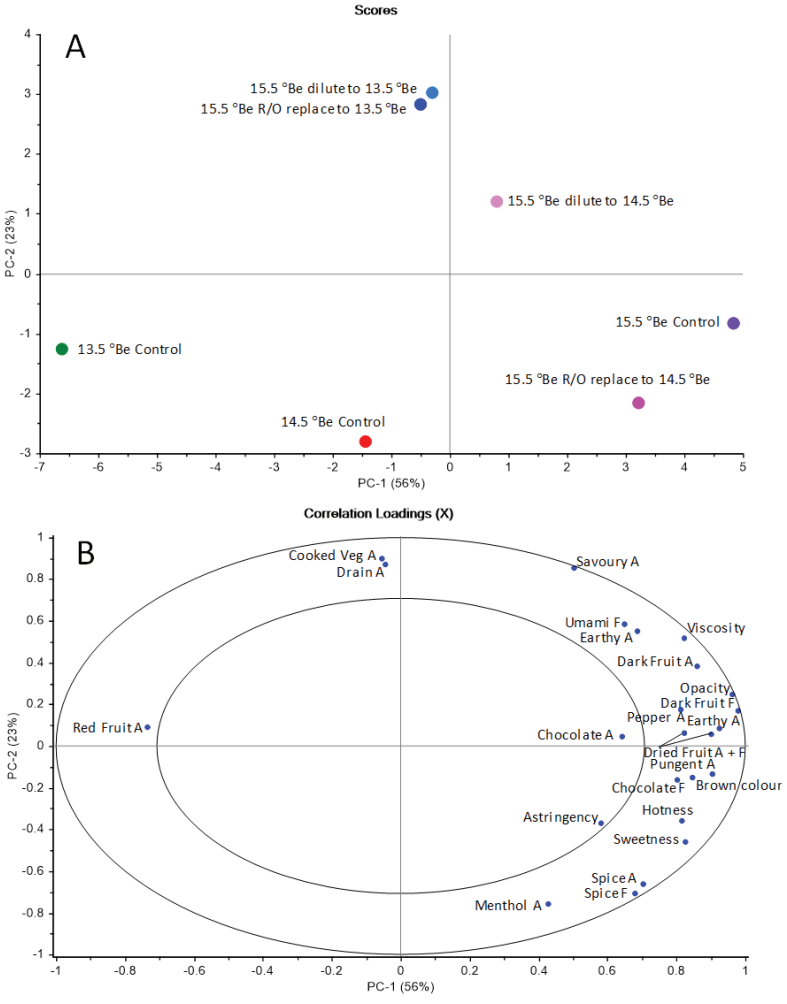


Figure 1. Principal component analysis showing (A) scores and (B) correlation loadings of significant (P<0.05) and close to significant (P<0.10) sensory attributes for the harvest time and water addition winemaking treatments. A: Aroma, F: Flavour.

of water addition to must. It is important to note that this potential issue was not previously found for Cabernet Sauvignon wines which received even greater levels of water addition (Schelezki et al. 2018b) and further investigation is needed before firm recommendations on water addition can be provided to the wine industry.

Conclusion

The addition of water to dilute the must by approximately 7.5% (15.5 diluted to 14.5°Baume) or 14% (15.5 diluted to 13.5°Baume) appears to be an effective way to manage high sugar concentrations associated with very ripe fruit. The wines made from the water-added must maintained many of the fuller-bodied and richer flavours that are more typical of the styles produced in the Barossa and other warmer climate regions (Iland et al. 2017), and were also associated with the wines made from the undiluted must at the latest harvest time. The wines made from the fruit that was harvested earlier was generally a lighter style with less colour and tannin and more 'red fruit' aromas. The mode of water addition (i.e. if juice was run off prior to the addition of the water or water was added directly) had a relatively small impact on wine phenolics, but introduced larger changes in wine sensory properties. A note of caution can be highlighted in that higher quantities of water addition may have introduced off-odours in the wines. A more moderate addition of water using a run-off and replace technique produced wines more similar to the undiluted control, and did not express off-odours.

Acknowledgements

The authors acknowledge support from John Gledhill (Winemaker at WIC Winemaking Services), John Maywald (Vineyard Manager at PIRSA research station, Nuriootpa) and Dr Marcos Bonada (Senior Research Officer - Viticulture at SARDI) for collection of maturity samples. This work was supported by Australian grapegrowers and winemakers through their investment body Wine Australia, with matching funds from the Australian Government. The Australian Wine Research Institute is a member of the Wine Innovation Cluster in Adelaide.

References

Bindon, K.; Holt, H.; Williamson, P.O.; Varela, C.; Herderich, M.; Francis, I.L. (2014) Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. Food Chem. 154: 90–101.

Bindon, K.A.; Teng, B.; Smith, P.A.; Petrie, P.R. (2019) Managing high Baume juice using dilution. Wine Vitic. J. 34(1): 36–37.

FSANZ (2016) A1119 Addition of Water to facilitate Wine Fermentation: https://www.foodstandards.gov.au/code/applications/Pages/A1119AdditionofWatertoFacilitateWineFermentation.aspx

Godden, P.; Wilkes, E.; Johnson, D. (2015) Trends in the composition of Australian wine 1984–2014. Aust. J. Grape Wine Res. 21: 741–753.

Iland, P.; Gago, P.; Caillard, A.; Dry, P. (2017) Australian wine – styles and tastes – people and places. Adelaide, SA: Patrick Iland Wine Promotions Pty Ltd.

Mercurio, M.D.; Dambergs, R.G.; Herderich, M.J.; Smith, P.A. (2007) High throughput analysis of red wine and grape phenolics adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. J. Agric. Food Chem. 55(12): 4651–4657.

Petrie, P.R.; Sadras, V.O. (2017) Practical options to manage vintage compression. Beames, K.S.; Robinson, E.M.C.; Dry, P.R.; Johnson, D. (eds) Proceedings of the 16th Australian Wine Industry Technical Conference, Adelaide, SA, 24-28 July 2016. Adelaide, SA: Australian Wine Industry Technical Conference Inc.: 63–67.

Schelezki, O.J.; Smith, P.A.; Hranilovic, A.; Bindon, K.A.; Jeffery, D.W. (2018a) Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on polysaccharide and tannin content and composition. Food Chem. 244: 50–59.

Schelezki, O.J.; Šuklje, K.; Boss, P.K.; Jeffery, D.W. (2018b) Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. Food Chem. 259: 196–206.

Social conscience

Rev T. Costello AO

Former CEO of World Vision Australia, Melbourne, Vic.
icmi@icmi.com.au

Abstract

What is the intersection of social ethics and reputation with economic interests or the business model of the wine industry? There are a range of issues we might discuss in terms of corporate responsibility; however, the biggest is the community's heightened awareness of the impact of excessive drinking on health, domestic violence, mental health and addiction. Some argue that the normalisation of drinking as essential to fun delivers the wrong message to children and so we have seen the banning of alcohol sponsorship in sporting events and on television. It is ironic to me that now you cannot advertise wine on the nightly TV news but sports-betting advertisements are deemed to be fine. But the social questions are: How does a beautiful and enjoyable experience in life intersect with the shadowy side when wine is abused? How do we frame wine consumption in the public square given the duality of wine's pleasure and cultural acceptance with adequate protection from social abuse? What should be the limit of state intervention and how does the burden of social responsibility get sheeted home to the wine sector? Wine is taxed differently from milk or lemonade because of its alcohol content. In economic language it is a negative externality. As a social drinker I acknowledge that and I am happy to pay the tax. But studies around the world show that when the floor price of alcohol is raised—affecting mostly cask wine which is the cheapest with the greatest damage—the result is significantly less social damage. The health sector has less problem with the bottled wine industry because those who buy a bottle over $12 are less likely to get drunk or, if they do get drunk, to then cause damage. But the health industry has huge problems with cheap cask wine. While environmental, water, bottling, packaging and sustainable land-use practices are in the mix, the social impact of abuse is still the big question mark hovering over the sector. What are the ways forward?

What is the intersection of social ethics and reputation with economic interests or the business model of the wine industry?

The traditional way we have found to answer this question is by the concept of a social licence. Although untested in corporate law, a social licence imagines a fourth stakeholder beyond the usual three of the shareholder, customer and employee and adds another stakeholder – the community. Although it remains unclear what exactly this means or what it requires, there is a widespread acceptance that it exists and must be nourished. We know that because the communal winds can very quickly change, and social licences can be lost (like with the tobacco industry) or questioned (like the role of poker machines in communities and now the coal mining sector whose licence is currently under threat).

It remains a somewhat nebulous licence, but I sensed a watershed moment to embrace it at the time of the Boxing Day Tsunami in 2006. The ANZ Bank gave World Vision some $500,000 for its response and was roundly criticised by Phillip Curry from the Australian Shareholders' Association. He said to the bank, 'This is illegitimate as your job is to make a profit and return it as a dividend to shareholders and we the shareholder will decide if we want to be charitable. You cannot give away our profit.' The CEO of ANZ said, 'Yes that is how we corporates once thought but something has changed. The community is a stakeholder and they are distressed by the tsunami and expect a bank, as part of their social licence, to respond to community expectations.' This has never been tested in law but is widely embedded in corporate practice.

Profits made within a community need to resonate with that community's values and priorities and, therefore, be at least partially deployed to give back to that community as a way of legitimating the social licence to operate. In concrete terms this may be the rural community where vineyards employ and seek the betterment of regional life through communal goods, or it may be the wider Australian community where scarce water in the driest continent and sustainable practice further social equity and ecological harmony. Expressions of the social licence come under various nomenclatures such as triple bottom line accounting, corporate social responsibility or shared value but they illustrate the acceptance of the concept of a social licence.

But is a social licence really a new concept?

The economist and philosopher Adam Smith said self-interest was the key to capitalism. Smith wondered whether a society populated by self-seekers was consistent with the general good. He assumed that we do not owe our bread, beer and meat to the benevolence of the baker, brewer and butcher but to their self-interest. But he worried about where self-interest might lead and posited that there was an 'invisible hand' which would balance out our individual egos. We wrongly think he just meant the market. Actually, he meant providence—this divine invisible hand marvellously uses our narrow self-interest nonetheless to create a common good. He argued that since the richest man cannot consume all his harvest, his surplus traded in the market feeds others and a good is created by providence to benefit the poor or hungry. He believed human beings are simultaneously 'self-regarding and other-regarding'.

In *The Theory of the Moral Sentiments*, written before his better-known *The Wealth of Nations*, Smith argued that the foundation of a good society, a virtuous society, is moral sympathy. Or as we'd say today, empathy. It is in interfacing with the needs in our community that a business practises empathy and implements its social licence.

According to Smith, the foundation for a good society is a strong sense of connection to and responsibility for one another. This is a social licence, although bowdlerised by interpreters of Smith to think it is only the market's invisible hand efficiently allocating preferences and distributing resources.

Markets will create wealth, but they will not create a good society or virtue without being 'other regarding' or attending to what we call today a social licence and practising corporate empathy.

Today we need to be proactive about doing that because a social licence can vanish. Henry Ford said, 'A business that makes nothing but money is a poor kind of business'. Two of the giants of capitalism agree that we cannot be driven merely by the accumulation of wealth.

Businesses do have a responsibility to contribute—to proactively promote—good societal values, especially for the most vulnerable.

Another implication of a social licence is the distinction between goods and commodities. A good is based on experiential values and a commodity on exchange values. Wine can be a good insofar as it enhances hospitality, culture and relationships. It provides pleasurable social good that enhances life. If it only has an exchange value (profit) and is only regarded as a commodity with a price, then one day someone may add up the social cost measured against the economic benefits and decide it is too high a price. This is why the focus on maintaining small producers in rural communities—who employ locals, support local sporting teams and give back, in the face of the trend for concentration in the market—goes to social licence. The free market with a commodities mentality does not protect rural communities. The big players with ghost brands unconnected to smaller communities may lower price, but at what cost?

A good as opposed to a commodity resonates with the French notion of 'terrior' where the soil, sunshine, skilled management of the vine and sensitive cultivation of inputs is a good. What happens to this sense of pride and purpose with mechanised bigger farms in the hands of bigger companies pushing the grape price down to serve the bulk market? I understand that the national body Australian Grape & Wine is seeking to find a solution to iniquitous grape pricing and the loss of this good.

Grapes take a lot of water to grow. It is why drip irrigation replacing sprinklers is a good and not just an efficiency on this dry continent. Sustainability is core to a social licence.

Implications for the wine industry

There are a range of issues we might discuss in terms of corporate responsibility; however, the biggest is the community's heightened awareness of the impact of excessive drinking on health, domestic violence, mental health and addiction. Some argue that the normalisation of drinking as essential to fun delivers the wrong message to children and so we have seen the banning of alcohol sponsorship in sporting events and on television. It is ironic to me that now you cannot advertise wine on the nightly TV news, but sports-betting advertisements are deemed to be fine. But the social questions are: How does a beautiful and enjoyable experience in life intersect with the shadowy side when wine is abused? How do we frame wine consumption in the public square given the duality of wine's pleasure and cultural acceptance with adequate protection from social abuse? What should be the limit of state intervention and how does the burden of social responsibility get sheeted home to the wine sector?

Wine is taxed differently from milk or lemonade because of its alcohol content. In economic language it is a negative externality. As a social drinker I acknowledge that, and I am happy to pay the tax. But studies around the world show that when the floor price of alcohol is raised—affecting mostly cask wine which is the cheapest with the greatest damage—the result is significantly less social damage. The health sector has less problem with the bottled wine industry because those who buy a bottle over say $12 are less likely to get drunk or, if they do get drunk, to then cause damage. But the health industry has huge problems with cheap cask wine. A four-litre cask reduces the cost of a standard drink to ~30 cents and we know the abuse this leads to, particularly in indigenous communities. Such questions cannot be avoided when we think of the sector's social licence to operate.

While environmental, water, bottling, packaging and sustainable land-use practices are in the mix, the social impact of abuse is still the big question mark hovering over the sector. What are the ways forward? A deeper narrative built on a deeper purpose.

Unilever has noticed that among their extraordinary range of brands those that are doing best in profit are its 'for-purpose' brands. Indeed, its for-purpose brands grew 70% faster than its normal for-profit brands. Such results mean that Unilever has now decided that by 2022 all their brands will be for-purpose. And the purpose does not have to even be too lofty! Take the brand Dove, which does not even have a certified supply chain but nonetheless is seen as a values-based or for-purpose product because the narrative wrapped around it of being body positive and accepting and inclusive of all shapes.

Now the cynics are right to suspect that there may be a fine line between selling a vacuum cleaner that is organic and a cheaper non-organic one, but the evidence is clear that the bottom line is better when companies tell the story of what the deeper purpose or contribution of the product represents.

Values-based for-profits are now a challenge to sacrificial giving to charities. Millennials (those under 35) are giving less to charity but channel their charitable impulse into selective purchasing of products that 'do good'. It represents a win for them in consuming for inclusive values and social or environmental ends. Their charitable giving is subsumed by their shopping for the good, not just the price.

What is the for-purpose story that the wine industry can tell? The answer lies in thinking deeply about its social licence to operate.

The consumer of the future

K.D. Suter

Global Directions, Sydney, NSW
keith.suter@bigpond.com

Abstract

This paper is about the challenge of change and the need for our industry to take a broader view of what is happening and will cover three matters. First, it examines two ways of categorising consumers: based on 'generations' and then based on expenditure patterns. Second, it identifies key factors in shaping future consumer behaviour: economic and technological change, health considerations, rise of the 'experience economy', and environmental considerations. Finally, it provides a technique for thinking about the future so as to reduce the risk of being taken by surprise. This is the management technique of scenario planning.

Different 'generations'

There have always been tensions between people of different ages. William Shakespeare, for example, wrote a famous play about young lovers who did not share the political priorities of their respective families: Romeo and Juliet. A difference between 16th century Italy and today is the 18th century Industrial Revolution's emphasis on the growth of consumerism and the need to encourage people to spend. For example, from the late 1950s young people had wealth (for the first time in history) and so a 'generation gap' was identified, illustrated by different tastes in music.

The marketing dimension has been joined by changing demographics (Suter 2018). First, there has been an increase in life expectancy. We have gained as much life expectancy in the last century as in the previous 5,000 years; an increase of about 25 years. About 5,100 years ago, people lived on average for 25 years. In 1900 the figure had crept up to 50. Now life expectancy is around at least 75 years.

This change can be seen in the various phases of ageing. Traditionally a person had three stages: young, middle aged and then getting to ready to die. Now there are four stages: (i) childhood (ii) maturity (iii) well aged (the new 'third age' with perhaps one third of a life spent in retirement) and (iv) the compression of morbidity (whereby a person's body declines quickly). Never before has any society had so many older people; there are no precedents to guide us. The first Australian to live to 120 is already alive and she is probably currently in her 60s (unfortunately we do not know who she is and so we cannot warn her).

Second, there is increased health expectancy. Growing older does not necessarily mean feeling older ('60 is the new 50'). Average incapacity-free life expectancy is rising faster than average life expectancy overall, and so people are not only living longer but they are also living more healthily.

Many people are taking better care of their health and so reducing lifestyle risks (such as smoking). There is also the rise of the 'counter-ageing society': older people refuse to act as though they are 'old'. This means that today's older people are much 'younger' than their parents were when their parents were at their age (assuming the parents managed to live that long) (Buzan and Keene 1996).

Finally, there is the growth in human enhancement technology. Human enhancement technology as such is not completely new; for example, the invention of spectacles and hearing aids. Now far more technological progress is underway, either (i) to restore an impaired function (such as eyesight) or (ii) to raise the function to a level considered to be 'beyond the norm' for humans.

Examples include the use of cognitive-enhancing drugs to improve memory and concentration, the use of hearing aids and retinal implants to improve sensory perception, and the use of bionic limbs to restore mobility. These developments will, among other things, enable older workers and people with disabilities to stay in the workforce for longer and broaden their potential opportunities for work.

All these developments have—for good or ill—economic implications. This paper will deal with the issue of marketing to different generations.

Demographic slicing and dicing

The traditional ways of categorising populations are on gender, race, religion and class. Using 'age' means looking at the different generations. Schools do this on a regular basis by dividing students into years. The argument is that the different generations have different life experiences and so look at the world differently.[1] They therefore have different 'age paradigms'/world views which then flow through into a variety of manifestations, such as memberships of organisations (Sladek 2011).

Here are the different generations:

- *Depression* (born up to 1945): people who lived through the Great Depression and World War II, who remain haunted by the risk of poverty until they die.
- *Boomers* (1946-66): few people were born in the Great Depression and World War II; the war ended in 1945 and the soldiers made up for lost time; they expect to have increased life expectancy (they exercise, stopped smoking, have a better diet); their parents (Depression Generation) may still be alive but as they die they will provide the greatest transfer of inter-generational wealth in history (the Boomers are impatient for the bequests: 'inheritance impatience').
- *Generation X* (1967-76): social innovators: we now have single, professional, educated women, who are the beneficiaries of the increased educational attention in schools; they are more focused on their careers; they no longer need marriage as the escape route from parents and a way of moving up in society. The males and females are very individualistic; both parents (if they had them) worked outside the home and so they raised themselves; they are flexible – able to cope with blended families and their parents' partners; they grew up in the wreckage of their parents' marriages; they are more tolerant than their parents (and certainly far more than their grandparents).

[1]Different age groups 'see' the world differently because they have had different experiences. UK novelist Ian McEwan, for example, writes about the British generation who lived during World War II: they "…would have fought, or suffered, in the war and known death on an unusual scale, and would not been able to believe that a drift into irrelevance was the reward for all the sacrifice". Baby Boomers by contrast have not known that sacrifice and have accepted Britain's decline as a normal fact of life. (McEwan 2008)

- *Generation Y* 'Millennials' (1977–96): children of the boom times: they want it all and they want it now. They were born into wealthy families; they have grown up in an era of full employment and so treat the economic boom (which began in Australia around 1992) as the norm; they are environmentally conscious; females take the academic and career advances made by women as a 'given' and so nothing special; girls expect to have brilliant careers. They are alienated from much of the mainstream mass media (which are seen as Boomer-controlled and anti-youth). They will be 50 per cent of the workforce by 2022.
- *Generation Z* (1997–): 'evolving consumers': we are still to learn more about them. They do not know of a time without mobiles and tablets. They are highly mobile workers, eager to seek out new opportunities: 'move in, move out, move on'.

It is worth noting that these are marketing terms rather than precise scientific ones. There is much disagreement about them, and they are vulnerable to the differing perceptions of the users.

Categorisation by expenditure

From a business point of view, categorisation based on age tells us little about consumer behaviour. The level of income may not necessarily be much of a guide either. It is possible that 'income' does not equal 'spending'; some people may prefer to save money for a rainy day while others seek a more adventurous life.

This point of view comes from Ross Honeywill and Verity Byth (Honeywill and Byth 2006). Beginning around 1991 (they argue) there was the creation of the Information Age and the rise of the Knowledge Economy (Moore's Law is dealt with below). The NEO (New Economic Order) emerged from this era of work. NEOs are socially progressive, metropolitan-based high spenders, who are early adopters of new technology. The NEOs are about 24 per cent of the adult population but 54 per cent of the discretionary spending. 45 per cent of NEOs are women; 55 per cent are men. Fifty per cent of all Australians with a university degree are NEOs. NEOs define themselves by their interests and abilities. NEOs need a workplace culture that recognises talent and imagination. NEOs are attracted to knowledge jobs because they involve the decoding of complexity; they like to be put to the test.

By contrast, 'Traditionals' represent about 50 per cent of the adult population. They have low spending ambitions. They are attracted to the 'deal'. Brands are a shortcut to certainty and a badge of belonging. They are slower to adopt new technology. They are located across all the age groups but are particularly well represented in the 50+. They are comfortable with established institutions, more willing to accept instructions from authority figures, and define themselves by their career.

The residue of the population are 'Evolvers', who most likely will end up as NEOs.

To sum up, this is yet another way of thinking about consumers. There is clearly a diversity of viewpoints. The rest of this paper shifts the discussion to looking at how we can understand what might affect consumer behaviour.

Thinking about the future

There are three broad ways of thinking about the future: prediction (Silver 2012), preferred and possible.

Prediction

Prediction means extrapolating current trends out into the future. This is the most common form of thinking about the future. Lines on graphs, for example, will often reveal a pattern. People make 'predictions' every day and take this for granted, for example, by making arrangements to have dinner with someone the following evening.

Economic predictions are perhaps the most widespread—and most criticised—branch of forecasting covered by the mass media. Studies of the Global Financial Crisis (GFC) which hit most of the Western world in 2008 have revealed the extent of the failure of extremely well-paid financiers to predict the future (Lowenstein 2010).

Accurate prediction has underpinned human development. Being able to predict the rise and fall of the Middle Eastern rivers, for example, was a turning point in the evolution of civilisation, as historian David Gress has pointed out:

> Both the early high cultures, Egypt and Mesopotamia, arose along great rivers and depended for survival on being able to predict and control the seasonal variations in water flow. Without accurate knowledge and without the technology of irrigation, organised society was impossible. Centralized, autocratic power was necessary to codify this knowledge and maintain the technology. (Gress 1998)

One of the greatest predictions made last century which is having a huge impact this century is Moore's Law. Gordon Moore, a founder of Intel, on 19 April 1965 speculated on the increasing power of computers: every 18 months (sometimes noted as 24) it will be possible to double the number of transistor circuits etched on a computer chip, and halve in price the cost each of chip.

In 1981, French writer Jean-Jacques Servan-Schreiber was an early convert to the power of Moore's Law and the microprocessor revolution: 'The rapid decline of the price of microcomputers, their increasingly smaller size, their general accessibility to non-specialised users, should lead to general expansion'. He went on to talk about the new era that will come from the linkages between the computer and the telephone, all of which seemed revolutionary at the time but now three decades later we take for granted (Servan-Schreiber 1981).

Management writers Philip Evans and Thomas Wurster (both associated with the Boston Consulting Group) have warned organisations and companies that increasing computer power will transform business:

> This law, or its equivalent, has prevailed for the past 50 years. In the judgment of some of the world's leading experts, it is likely to prevail for the next fifty years. Moore's Law implies a tenfold increase in memory and processing power every five years, a hundredfold every ten years, a thousandfold every fifteen. This is the most dramatic rate of sustained technical progress in history. (Evans and Wurster 2000)

Therefore, there is a risk of economic and social disruption. Some jobs will be lost, some may be created, and many people will get angry at the disruption—as we saw with the 2016 US presidential election. Driverless vehicles, for example, will in themselves create major changes, with (among other things) the disappearance of humans to drive trucks and the highway cafés which look after the drivers (Suter 2017).

Finally, there has been a great improvement in the capacity for prediction because of the rise of super computers and their 'super number crunching'. Along with Moore's Law there is also Kryder's Law, first proposed by Mark Kryder, the Chief Technology Officer of hard drive manufacturer Seagate Technology. He successfully noticed that the storage capacity of hard drives has been doubling every two years. Storage capacity has increased, and the cost has come down (Ayres 2007). This permits extensive 'data-mining': collecting and high-speed analysing of information. For example, Princeton-based economist Orley Ashenfelter loves wine but instead of the 'swishing and spitting' approach of wine gurus, he has developed a computer program to predict how good a wine will be well ahead of the actual years of consumption (Ayres 2007).

Preferred futures

A 'preferred' future is where a person or organisation has a desired vision towards which they work. For example, when President John F Kennedy took office in January 1961, he knew there was a need for a bold vision to revive American spirits which had been dampened by all the Soviet space 'firsts', such as the 1957 Sputnik. Then came Yuri Gagarin's heroic trip on 12 April 1961 and it seemed that the Soviet space lead was invincible. On 25 May 1961, Kennedy addressed a joint session of Congress in which he laid out his vision of putting a man on the moon and returning him safely before the end of the decade. This was achieved in July 1969, 50 years ago—virtually on the anniversary of this conference! With a 'preferred' future we move from what is currently being suggested by prevailing trends ('prediction') to what we would like to see happen.

In my workshops I use the management best-seller *Blue Ocean Strategy* (Kim and Mauborgne 2005). The authors claim that most strategy work is based on 'red ocean' thinking—imagine blood in the water from all the struggles—whereby firms are competing against each other. They offer a whole new approach: instead of trying to beat the competition, go elsewhere.

Kim and Mauborgne (2005) provide an Australian case study: Yellow Tail wine (Griffith, NSW). Many Americans don't drink wine; they have the money but not the 'culture' (they put it more politely). Yellow Tail is designed for the American market and it sells well. Yellow Tail has created a new market space for wine (Lim and Mauborgne 2005). There is a similar story with De Bortoli wines which has created specific wines for the Chinese market (Australian Business Foundation 2009).

Possible futures

Possible futures are what *could* happen. They are not necessarily being currently suggested (via prediction) and they may not necessarily be what one would like to see happen (via preferred futures). The signs of possible change may be there—but one is simply not 'seeing' them. Unfortunately, in all walks of life, there is a tendency to get into a 'comfort zone' and to mix with a narrow range of people.

Scenario planning is not so much about getting the future right—as to avoid getting it wrong. Done properly it reduces the risk of being taken by surprise. As Clem Sunter has pointed out:

> A critical thing to remember is that a scenario is a story of what can happen. It is not a forecast of what is going to happen. The problem with forecasting is that we so often are deceived into forecasting our wishes and desires. I have seldom come across a strategic plan which goes against the ambitions of the CEO. (Sunter 1996)

A popular word in scenario planning methodology is 'paradigm'. The classical Greek word *paradeigma* meant model, framework, pattern or example (Clarke and Clegg 1998). The word entered the common parlance with Thomas Kuhn's classic book *Structure of Scientific Revolutions* (Kuhn 1962). He challenged the then common viewpoint that scientific progress 'advanced' via one neat step at a time, with scientists, so to speak, standing on the shoulders of their predecessors. Kuhn argued that there is in fact no steady accumulation of scientific knowledge. Instead, each theory is a revolutionary break from the previous theory, resulting eventually in the arbitrary replacement of one way of viewing knowledge with another view. Kuhn was attempting to explain change specifically in the natural sciences. The word has since been used (or misused) extensively in virtually all the other disciplines.

The key point is that 'paradigm' is now the central term in scenario planning. The term means both (a) a set of beliefs and assumptions about how each person/organisation 'sees' the world and (b) a filtering device which is the window through which each person/organisation sees the world. Once a paradigm is commonly accepted it lingers and becomes the new 'reality'.

Scenario planning technique

1. Work out the basic issue. Scenario planning is done in response to the perception that there is a 'problem' to be solved. It is important that the right initial 'question' be identified.

2. Understand the organisation that has commissioned the scenario planning. What is the 'official vision' of the organisation? How does the organisation perceive its business? Why has it decided on that 'problem' to be investigated? What is the 'official perception' of the future (namely the line laid down by the board or CEO)? How do they see that future changing? What are their hopes and fears? What is its future strategy? What are its stated values?

3. Work out the driving forces. The forces can be broadly grouped into five areas under the acronym STEEP:

- *Social* – for example: what are the demographic changes? Australians have gained as much life expectancy in the past century as in the previous 5,000 years: what can they expect in this century? What are the changing expectations that people have? Will wine continue to be see seen as a healthy drink?
- *Technological* – for example: how will the genome project (mapping the body's DNA structure) impact on medical research? What could be the impact of Moore's Law in IT? We are reducing the need for meat to make hamburgers ('vegie-burgers'); could we reduce the need for grapes to make wine?
- *Economic* – for example: how will the economy go? Will the gap between rich and poor Australians increase? What will be the impact of the rising giants like India and China? How will changing income levels shape wine consumption patterns?
- *Environmental* – for example: how will climate change affect Australia? What old diseases will reappear? How will notions of 'environmental responsibility' shape future wine consumption patterns? Will Australia's changing climate affect the cultivation of grapes?
- *Political* – for example: will there be an increase in ethnic tensions? What about the risks of terrorism? Will China and the USA go to war? Will concern about alcohol-fuelled violence create a backlash against wine consumption?

4. Rank the key factors in order of importance to determine the most important two. Form a cross: '+' (a Cartesian coordinate system, with the two most salient driving forces as the X- and Y-axes). The two axes cross each other at their mid-points, thereby creating four quadrants. These will be the basis of the four scenarios, with the end of each axis having a 'high' and a 'low'. The maximum number of scenarios is best kept at four because it gets a bit too complicated to go beyond that number in terms of easily recalling the scenarios and making use of them: these are (up to) four different 'worlds'. Avoid just creating three because the client is tempted to go for the 'middle' one as the most moderate. The purpose of the exercise is to encourage the client to re-perceive their future: they need to be challenged (and not comforted).

5. Work out the scenario logic. The drivers are then used as the axes along which the eventual scenarios will differ. These are four different 'worlds'. Create four plausible scenarios. In other words, for one 'world' think through what the future of wine consumption would look like if there were both high social change and high economic growth.

6. Make the scenarios come alive. Each scenario needs to be compelling. There has to be sufficient detail in each story to make it easy to follow. A scenario may be uncomfortable, but it needs to be believable. Each scenario should have a memorable name. Conversations with outside experts will be useful here. These are people who are

outside the current scenario planning project who may have different perceptions from what the scenario planning team may be thinking. They are acknowledged experts in a particular field—but not the one under examination for the scenario planning project. They help guard against 'group think' and narrow perceptions. They can also suggest new matters to examine. Two questions are put to them: (a) is each draft scenario plausible? (b) is there something we have overlooked?

7. Identify the leading indicators. The future will determine which scenario was 'right' in the sense that it was closest to what actually happens. It is important to have indications as quickly as possible of which scenario is coming into play.

8. Work out the implications of the scenarios. We now return to the original problem identified by the organisation. What do the scenarios mean for the organisation? What are the implications for the organisation's current strategy? What contingency plans need to be in place? What is Plan B? What are the options for the stakeholders?

9. Do not argue over the value of each scenario: don't try to pick winners. If probabilities are assigned to the scenarios, then this has become a 'prediction' project rather than a 'possibility' one. There should not be arguments over which scenario is more likely than the others. Each scenario has to be equally plausible. Future events will tell you which scenario was 'right'. Meanwhile, one scenario may seem more 'preferable' than the others. But scenario planning is not about creating 'preferred' futures. People are welcome to create 'preferred futures' (particularly after having their perceptions expanded by a scenario planning exercise)—but that is a separate project. Creating a preferred future is not scenario planning.

10. Strategic conversation. This, in effect, represents 'part two' of the process. This is the 'downstream' work: getting the word out to staff (and/or volunteers). An organisation learns through its network of interconnecting conversations and exchange of ideas between individuals.

The implication here is that the company/organisation has to 'own' the document. This is not just a matter for external consultants to devise the document and then move off to the next project elsewhere. The staff/volunteers have to be fully conversant with it and looking for the warning signs. It has to be embedded within the culture. This is not, then, an obscure document in a ring-bound folder that is only examined once a year.

People need to 'live' within each scenario and become fully familiar with it. They will then be well positioned to gauge which of the scenarios is coming into play and have the contingency plans ready.

If the scenarios are commissioned by a large organisation, then they should be discussed at the various levels of it so that staff can think through what each scenario means for their own area of work. The scenarios may represent a new world for them and so it is necessary to get their reactions.

Change often begins at the margins and so junior staff (or volunteers in not-for-profit organisations) may be best placed to detect it first. By contrast, the heads of companies/organisations may have a psychological bias in maintaining the status quo which they know and feel comfortable with, for example, they may be close to retirement and so they do not want to be challenged by potential events over the horizon.

Scenario planning challenges the 'super-specialisation'[2] of university academics. Academics do well partly through having to learn more and more about a particular topic. Scenario planning requires a different type of mindset: being able to see the connections across subjects, rather than delving deeper and deeper into one of them. Ideally one needs to be exposed to a variety of different paradigms, rather than just collecting facts. Businesses and other organisations also become super-specialised and narrowly focused, especially with a short-term financial outlook. They have difficulty seeing the 'big picture'.

An example of scenario planning

Most scenario planning is done as 'commercial in confidence' and so is not revealed to the general public. It is, after all, a business technique and most businesses say little about how they operate except when they want to make announcements to boost their share price or public image (or defend them). Government departments are even more reluctant to share their inner workings.

Clem Sunter provides one famous example of the value of scenario thinking. In the early 1980s South Africa Clem Sunter, then working for the country's largest corporation, created scenarios on South Africa's future. South Africa was under the apartheid regime which seemed destined to stay in place indefinitely.

Sunter toured the country speaking of two scenarios: the 'high road' and the 'low road'. The 'high road' was a story of the release of Nelson Mandela (then the world's longest-serving political prisoner), the creation of a multi-racial electorate and Mandela's election as the first black President. His white audiences were outraged.

Sunter would then explain the 'low road' scenario as a story of the country falling into increasing sporadic violence, continued international isolation, a white exodus to safer countries and a generally grim future. This encouraged his white audiences to ask for more information on the 'high road' scenario.

In March 1989, Frederik de Klerk was elected President. Max Hastings was the editor of conservative *The Daily Telegraph* (London) and recalled the mood of those years in his memoirs. Few observers, including his own journalist in South Africa, anticipated just what would follow because no one expected de Klerk to be any different from his predecessors. But on 2 February 1990 de Klerk suddenly lifted the 33-year ban on the African National Congress and invited Mandela to join him in negotiations towards a constitution which would grant the vote to the country's African majority. This drama was occurring around the time of the ending of the Cold War. Hastings concluded his survey of that 1990-91 period:

> Which of our generation would have dared to predict, even twenty years ago, that we should see within own lifetimes, an end to the Cold War, the collapse of the Soviet Empire, and a relatively peaceful transition to black majority rule in South Africa? Much of the business of newspapers is to purvey tales of disappointment, failure, tragedy. How intoxicating it was, that for a season, we found ourselves bearers of historic and happy tidings on two of the greatest issues that faced the world in the second half of the twentieth century. (Hastings 2002)

I have my own footnote to this story. In 2001 I was a guest of Annette Liu, then the Vice President of Taiwan (the most senior woman elected in 5,000 years of Chinese history) at her seminar of Nobel Peace Prize winners in Taipei. Frederik de Klerk was one of the Nobel participants. He knew nothing of my professional interest in scenario planning. But quite spontaneously, while explaining how he was able to manage the transfer of power to black majority rule, paid tribute specifically to Clem Sunter who had given the scenario talks in the 1980s and had created the political opportunity for de Klerk to make his historic reforms. Sunter had, so to speak, helped white South Africans 'to think about the unthinkable'.

Using scenario planning to assist the wine industry

Scenario planning encourages people to be alive to possibilities. It

[2]The eye-catching phrase 'super-specialisation' was used a few times by Barry Jones in his presentation dealing with the problems of scientists communicating with each other, let alone the general public and politicians, made at the Royal Society of NSW (2011).

widens the scope of finding options and alerts people to potential risks (to see what currently cannot be seen). For example, some of today's problems have arisen from a lack of strategic action that ought to have been taken years ago, and so the problems have been exacerbated by the elapse of time. Scenario planning helps us to reflect on the question: what are we doing today that will haunt us tomorrow? The creation of contingency plans in itself gives a greater sense of self-confidence that it is possible to weather the storm.

There are various 'layers' of planning and each requires a different approach by a person/organisation, with each being synchronised with the other layers:

- scenario: the overall setting of the wine industry
- strategic: the organisation's own plans for the next 3-5 years within that setting
- operational: annual
- business: annual

Here are some examples of 'questions' to provoke a scenario planning exercise:

- Under what circumstances could there be an increase in wine consumption? For example, new markets are created. Could the industry cater for the increased demand?
- Under what circumstances could there be a reduction in wine consumption? For example, economic dislocation brought on by robotics and the loss of jobs.
- Could an increasingly health-conscious country suddenly decide to avoid wines?
- Could healthcare providers penalise alcohol consumers?
- Could wine producers lose their social licence to operate? Could vineyards become 'stranded assets'? (Look at the speed with which the coal industry has been confronted by hostility). Could wine become the new 'tobacco'?

Scenario reasoning is a particular way of thinking about the future. It does not argue for a particular point of view (that is left to 'preferred' futures). Instead, it encourages people to 'think about the unthinkable' in a dispassionate way.

Conclusion

The intention of this paper has been to encourage the wine industry to examine the 'big picture' of change. It began with different ways of looking at consumers. It then examined three ways of thinking about the future: prediction, preferred and possible. It concluded with an expanded examination of possible futures via the technique of scenario planning.

References

Australian Business Foundation (2009) Engaging China: The reality for Australian businesses. 10 August: North Sydney, NSW.

Ayres, I. (2007) Super Crunchers: How Anything Can be Predicted. London: John Murray: 260 p.

Buzan, T.; Keene, R. (1996) The Age Heresy: You Can Achieve More – Not Less – As You Get Older. London: Ebury: 184 p.

Clarke, T.; Clegg, S. (1998) Changing Paradigms: The Transformation of Management Knowledge for the 21st Century. London: HarperCollins: 512 p.

Evans, T.; Wurster, T.S. (2000) Blown to Bits: How the New Economics of Information Transforms Strategy. Boston: Harvard Business School: 261 p.

Gress, D. (1998) From Plato to NATO: The Idea of the West and its Opponents. New York: Free Press: 624 p.

Hastings, M. (ed.) (2002) An Inside Story of Newspapers. London: Macmillan: 398 p.

Honeywill, R.; Byth, V. (2006) NEO Power: how the new economic order is changing the way we live, work and play. Melbourne: Scribe: 252 p.

Kim, W.C.; Mauborgne, R. (2005) Blue Ocean Strategy: How to Create Uncontested Market Space and Make the Competition Irrelevant. Boston: Harvard Business School Press: 240 p.

Kuhn, T. (1962) Structure of Scientific Revolutions. Chicago: University of Chicago Press: 264 p.

Lowenstein, R. (2010) The End of Wall Street. Melbourne: Scribe: 368 p.

McEwan, I. (2008) On Chesil Beach. London: Vintage: 176 p.

Royal Society of NSW (2011) Belief and Science: the Belief/Knowledge Dilemma. 6 April; The University of Sydney, NSW and RSNSW Bulletin and Proceedings 344, p. 2: https://www.royalsoc.org.au/submit-bulletin-documents/2011-issues/45-344-april/file

Servan-Schreiber, J.J. (1981) The World Challenge. London: Collins: 302 p.

Silver, N. (2012) The Signal and the Noise: The Art and Science of Prediction. London: Allen Lane: 544 p.

Sladek, S. (2011) The End of Membership as We Know It: Building the Fortune-Flipping, Must-Have Association of the Next Century. Washington DC: ASAE: 122 p.

Sunter, C. (1996) The High Road: Where Are We Now? Cape Town, South Africa: Tafelberg.

Suter, K. (2017) The Security Implications of Driverless Vehicles. Asia Pacific Security Magazine. 11 July: https://issuu.com/apsm/docs/apsm_july_aug_2017_final_issuu/48

Suter, K. (2018) The Security Implications of an Aging Population. Australian Security Magazine. 19 September: https://australiansecuritymagazine.com.au/the-security-implications-of-an-aging-population/